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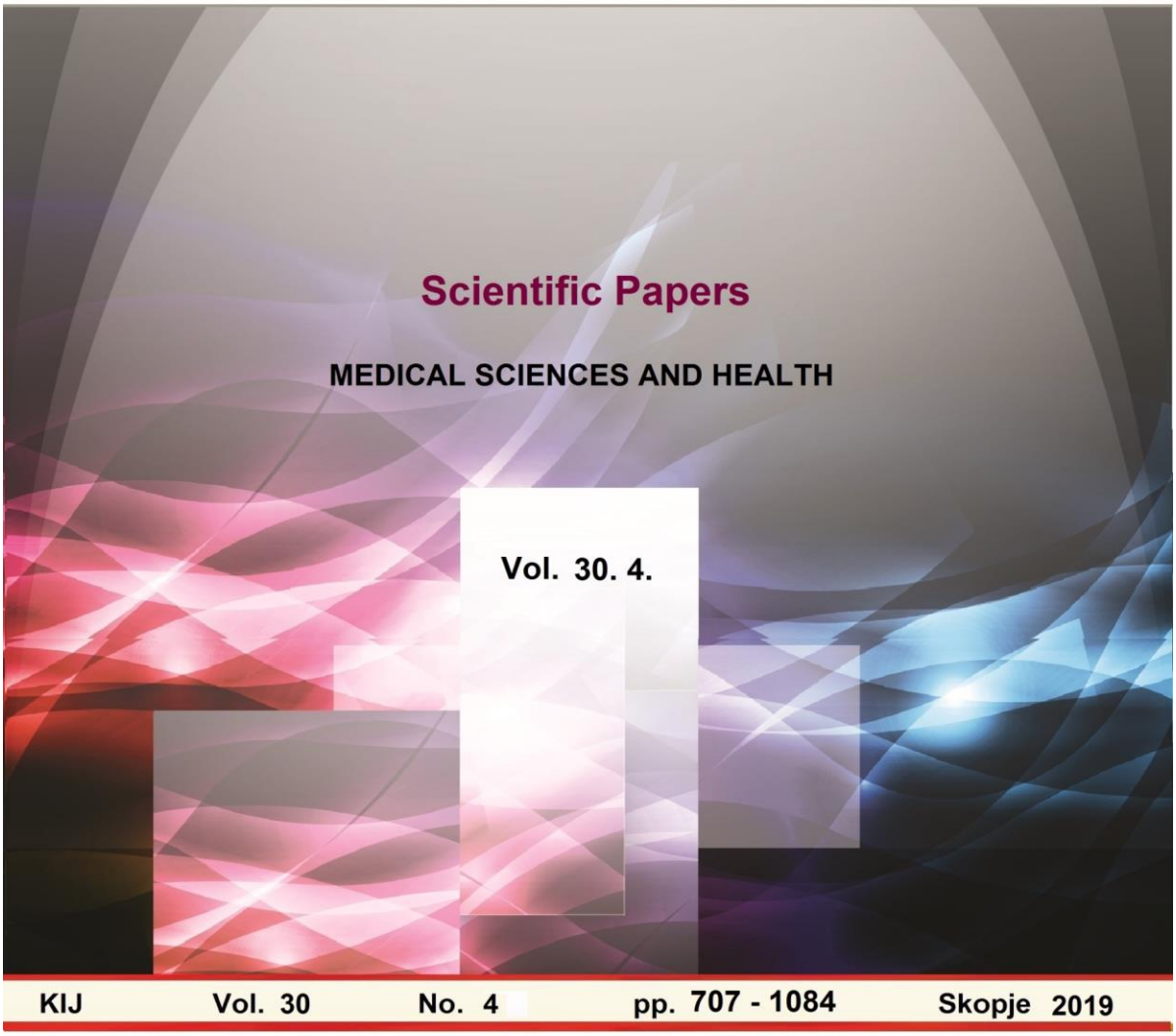

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ELECTRONEGATIVE LOW-DENSITY LIPOPROTEINS AS A CAUSE OF SECONDARY CARDIOVASCULAR DISEASES IN CHRONICALLY ILL PATIENTS – A METHOD FOR IDENTIFICATION AND MONITORING

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Abstract: Cardiovascular diseases are common in patients who already have another chronic disease -- it could be diabetes, chronic pulmonary disease, kidney disease, etc. Patients who have diabetes are two to three times more prone to develop cardiovascular disease. The risk to develop cardiovascular disease is higher in patients with chronic obstructive pulmonary disease (COPD), compared to those that do not have COPD. High cardiovascular risk has also been demonstrated in patients diagnosed with rheumatoid arthritis who have developed chronic kidney disease.
Chronic diseases lead to modification of biomolecules, including LDL (low-density lipoproteins). Therefore, in the circulation of patients with various chronic diseases are present oxidized LDL, carbamylated LDL, desialylated LDL, and glycated LDL. The main attribute of all these forms of modified LDL is their increased electronegativity, compared to non-modified LDL. In addition, the modified LDL forms are more atherogenic. Many studies have explored the involvement of electronegative LDL in atherogenesis. Electronegative LDL induce inflammatory response, and adhesion of monocytes and lymphocytes to endothelial cells, stimulating them to produce adhesive molecules and chemokines. Electronegative LDL has higher affinity to proteoglycans. This property promotes retention of electronegative LDL in the subendothelial layer. L5, the most electronegative LDL sub-fraction, at concentrations at which it circulates in the blood of chronically ill patients, causes cellular senescence, while at higher concentrations it causes apoptosis of endothelial cells. Cellular senescence may mediate endothelial dysfunction and atherogenesis.
To analyze the electronegativity of LDL we used the method of electrophoresis. First, it was necessary to isolate the LDL fraction from the serum. The isolation was successfully conducted applying three washes with heparin citrate buffer. We analyzed blood samples from a patient with diabetes, a patient with COPD, a patient with lupus erythematosus on hemodialysis, and a healthy person. In patients with different pathologies, the electrophoresis of LDL demonstrated a difference in electronegativity. The highest electronegativity was demonstrated in the patient with diabetes.
A therapy targeted to reduce the electronegative LDL would be highly beneficial to counteract the progression of cardiovascular diseases. LDL electrophoresis could be used as a method for identification of patients with electronegative LDL and monitoring of the effects of therapy.
Keywords: electronegative LDL; atherosclerosis; electrophoresis

1. INTRODUCTION

Many chronic diseases are followed by secondary developed cardiovascular disease. For example, people with diabetes are two to three times more prone to develop cardiovascular disease, compared to people without diabetes. According to the International diabetes federation, every year 14 to 47 of 1000 people with diabetes at the age of 50 to 69 years who live in high- and middle-developed countries manifest cardiovascular symptoms; 2 to 26 of 1000 have coronary disease, and 2 to 18 of 1000 have myocardial infarction (IDF – DIABETES ATLAS, Eighth Edition, 2017). Patients diagnosed with rheumatoid arthritis who have developed chronic kidney disease are also at high risk for cardiovascular disease, especially those with increased C-reactive protein (Kochi et al., 2018; Dincer et al., 2018). The risk of developing cardiovascular disease is higher in patients who have a chronic obstructive pulmonary disease (COPD), compared to those that do not have COPD (Agarwal et al., 2014). Many factors, both modifiable

and non-modifiable, are involved in the process of atherogenesis, including circulating LDL as a key player and direct atherogenic factor.

LDL and electronegative LDL

The main physiological role of LDL is transport of cholesterol to the cells, which is further used as a component of membrane systems, for biosynthesis of steroid hormones, bile acids, etc. However, LDL is particularly atherogenic because of the high level of cholesterol it carries, and the high susceptibility to oxidative modification in the subendothelial layer. In different pathological conditions, such as diabetes, chronic kidney disease and rheumatoid arthritis, various modifications of LDL occur, and LDL becomes more electronegative.

Electronegative LDL [LDL (-)] encompasses a group of LDL particles that are modified in different ways. Their common feature is higher electronegativity compared to the native LDL. Importantly, we have to consider that even the native LDL particles demonstrate variations in size (24 to 28 nm in diameter) and density (1,006 – 1,063 g/mL) (Lund-Katz et al., 1998). Moreover, a difference in electronegativity of native LDL also occurs if the level of proteins different from apoB increases (Bancells et al., 2010). As for the modified LDL, various forms may be found in the plasma, which depends on the process native LDL had undergone -- oxidation, glycation, carbamylation or desialylation.

Oxidized LDL (oxLDL) is a distinct form of LDL (-). It is considered that oxLDL appears when LDL is bound to proteoglycans in the subendothelial layer where, in absence of antioxidants, they undergo modifications such as lipid peroxidation, non-enzymatic glycation, enzymatic lipolysis and proteolysis (Lu and Gursky, 2013). Compared to the oxLDL, which is present in the plasma with approximately 0.1-0.5% of the entire LDL level, the LDL (-) fraction is present with 3-5% of the entire level of LDL, that depends on the patient's pathology (Estruch et al., 2013).

There is a small amount of glycated LDL in the plasma of healthy people, but its quantity is significantly increased in patients with diabetes and chronic kidney disease (Bucala et al., 1994). There are also alternative ways of glycation of LDL, which are mediated by the glucose metabolites, such as glyoxal, methylglyoxal, and 3-deoxyglucosone (Rabbani and Tornalley, 2011). These compounds are highly reactive products of the Millard reaction. With non-enzymatic glycation, LDL is transformed into AGE-LDL (advanced glycation end products-LDL), which is highly atherogenic. Small and dense LDL is highly susceptible to glycation, whereas the glycated LDL is highly susceptible to oxidation (Sobal et al., 2000), which amplifies the risk of atherosclerosis (Younis et al., 2009).

Carbamylated LDL is a product of non-enzymatic modification of LDL by plasma cyanate, which is urea-derived (Kraus and Kraus, 2001). Cyanate has toxic properties, especially in its active form, isocyanic acid that causes carbamylation of proteins, thus changing their structure, function, and electronegativity. In that way, cyanate modifies enzymes, hormones, antibodies, receptors, transport molecules, and LDL. Patients that have a high level of urea in the blood, also have a high level of cyanate. Actually, when the kidney function slows down, the level of plasma cyanate increases (Kraus and Kraus, 2001).

Desialylated LDL. Carbohydrates of LDL's glycoproteins and glycolipids contain a certain amount of sialic acid at their ends, which is of importance for the physiological functions of LDL. Namely, desialylated LDL demonstrate changes in their physical and chemical properties, which are proposed as a link between the LDL from one side and the endothelial cells and macrophages' scavenger receptors from the other side (Orekhov et al., 1992). Indeed, the well-known study of Tertov and coworkers demonstrates the atherogenicity of desialylated LDL (Tertov et al., 1989; Chazov et al., 1986). Various forms of modified LDL, including desialylated LDL, have been found in patients with diabetes (Sobenin et al., 2017).

Electronegative LDL and their role in atherosclerosis

Electronegative LDL has been extensively studied, leading to discovery of some of the mechanisms of atherogenesis mediated by LDL (-). It has been demonstrated that LDL (-) induces inflammatory response through stimulation of monocytes and endothelial cells to produce cytokines. LDL (-) also mediates the adhesion of monocytes and lymphocytes to the endothelial cells, stimulating the endothelial cells to produce adhesive molecules and chemokines (Ziouzenkova et al., 2003).

L5, the most electronegative sub-fraction of LDL (-), induces secretion of metalloproteinase and expression of vascular endothelial growth factor (Tai et al., 2006). In a recent study, it has been proven that the L5 sub-fraction at concentrations at which it circulates in the blood of chronically ill patients, causes cellular senescence, while at higher concentrations it causes apoptosis of endothelial cells. Cellular senescence may mediate endothelial dysfunction and atherogenesis (Wang et al., 2018).

LDL (-) exhibits high affinity to proteoglycans. It has been postulated that modifications in the N-terminus of apoB cause this increased affinity for proteoglycans, promoting retention of LDL (-) in the subendothelial space.

Retained in the subendothelial space, LDL (-) will be highly prone to additional modifications and manifestations of inflammatory characteristics (Bancells et al., 2011).

In order to prevent secondary cardiovascular diseases in chronically ill patients, further studies of the LDL (-) are needed that will lead to development of therapies for decrease of its plasma levels. Nowadays, the alkali therapy with $NaHCO_3$ seems to be a plausible solution. Namely, it has been noticed that the alkali therapy significantly decreases the level of LDL (-) in patients with chronic kidney disease (Rizzetto et al., 2017).

Therefore, the aim of our study was to perceive the electronegativity of LDL, in order to relate some chronic diseases with secondary developed cardiovascular disease.

2. RESULTS AND DISCUSSION

The differences in electronegativity of LDL were studied using the technique of electrophoresis on agarose gel.

First, it was necessary to establish a protocol for isolation of LDL fraction from serum. The serum samples that were used for these experiments were taken from healthy persons. The efficacy of isolation was demonstrated with electrophoresis. The isolation was successfully conducted using heparin citrate buffer, and applying three washes, which yielded a well-defined LDL fraction, without impurities from other proteins (Figure 1, line 8).

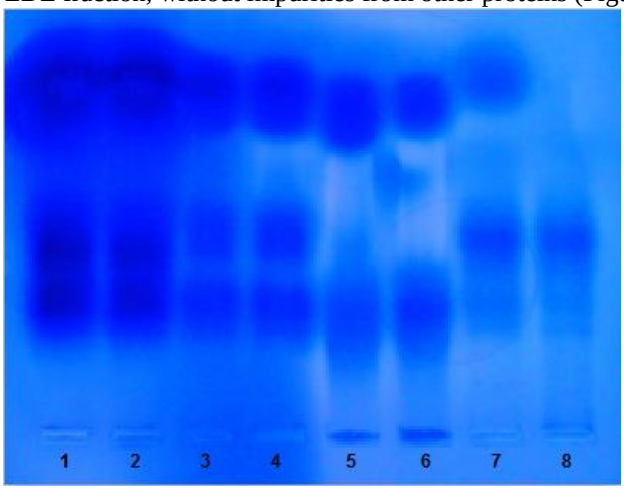

Figure 1. Three washes with heparin citrate buffer result in excellent isolation of LDL.

Positions 1, 2, 3 and 4 – whole serum; positions 5 and 6 – LDL isolated with a reagent of commercial source (polyvinyl sulphate and polyethylene glycol solution); position 7 – LDL isolated with one wash with heparin citrate buffer, solubilized with 200 mL 0.1% Triton X solution; position 8 – LDL isolated with three washes with heparin citrate buffer, solubilized with 200 mL 0.1% Triton X solution.

After establishment of the protocol for isolation of LDL, we aimed to demonstrate the differences in LDL electronegativity in various chronic diseases. For this analysis were used blood samples from a patient with diabetes, a patient with chronic obstructive pulmonary disease, a patient with lupus erythematosus on hemodialysis and a healthy person. The serum was separated from venous blood, and the LDL fraction was isolated from the serum with three washes with heparin citrate buffer. The differences in mobility of LDL fraction are shown in Figure 2. The highest electronegativity of the LDL was demonstrated in the patient with diabetes.

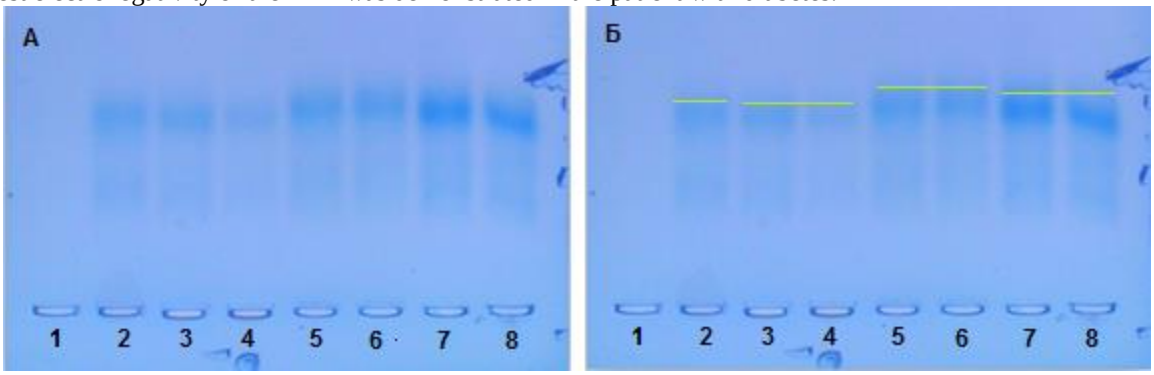

Figure 2. Electrophoretic mobility of LDL in chronically ill patients and a healthy control subject.

Position 1 – blank; position 2 – healthy person; positions 3 and 4 – patient with chronic obstructive pulmonary disease (run in duplicate); positions 5 and 6 – patient with diabetes mellitus (run in duplicate); positions 7 and 8 – patient with systemic lupus erythematosus who is on hemodialysis (run in duplicate).
(А and Б represent the same scan of the electrophoresis run; in Б are presented the fronts of migration of each sample.)

3. CONCLUSION

Chronic diseases cause modifications of LDL through several well-known mechanisms that increase LDL's electronegativity. Modified LDL gain new characteristics, which makes them more atherogenic. New treatments, targeted towards LDL (-), have potential to decrease the rate of secondary cardiovascular disease in chronically ill patients. In this context, the electrophoretic method we present here have potential to be developed in an efficient method for estimation of LDL electronegativity in patients with different pathologies. The method may also be used for monitoring of the treatments that will target the electronegative LDL in future.

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