Original Article

**LDL and HDL lipoprotein subtype frequency in patients with rheumatoid arthritis before and after administration of anti–TNFα**

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**Abstract**

A higher risk of atherosclerosis and related cardiovascular disease has been observed in patients with rheumatoid arthritis. Anti-TNFα monoclonal antibodies are used for the treatment of resistant rheumatoid arthritis resulting in inhibition of TNFα binding to cellular receptors that activate disease-associated pro-inflammatory pathways. The effect of TNFα inhibition on the lipoprotein pattern may provide information on the risk of atherosclerosis in these patients. In the present study the lipoprotein profiles of patients with rheumatoid arthritis have been examined before and after treatment with anti-TNFα. 85 patients (76 women, 89%) with established RA (disease duration ≥5 years), mean age 57 ± 12 (68% with RF positive), no known cardiovascular disease, thyroid disorder and thyroid disorder’s treatment with DMARDs and not receiving biological agents as treatment, were divided into 2 groups, those who had DAS_{28} > 3.2 (43 patients) who were additionally reserved anti-TNFα biological factor for ≥12 months and those who had DAS_{28}≤3.2 (42 patients) who continued DMARD treatment and were monitored at the same time as a control group. Samples were taken at two times with a difference of ≥12 months. Separation of the lipoprotein subtypes was done by 4% polyacrylamide native gel electrophoresis. Two LDL (LDL A and LDL B) and six HDL (HDL1, HDL2a, HDL2b, HDL3a, HDL3b and HDL3c) subtypes were characterized. In patients receiving DMARDs, a decrease in the LDL-B subtype by 11.9% (p = 0.014) and an increase in HDL-3c by 2.38% (p = 0.049) were observed. In PA patients receiving anti-TNFα, a reduction in the LDL-B subtype was observed by 2.33% (p = 0.005), LDL-A by 2.33% (p = 0.0001), HDL3a by 11.63% (p = 0.072) and HDL3c by 4.65% (p = 0.035). In RA patients receiving anti-TNFα, a reduction in the LDL-B fractions was observed by 2.33% (p = 0.005), LDL-A by 2.33% (p = 0.0001), HDL3a by 11.63% (p = 0.072) and HDL3c by 4.65% (p = 0.035).

**Keywords:** lipoproteins, LDL subtypes, HDL subtypes, anti-TNFα, rheumatoid arthritis

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Introduction
The high levels of systemic inflammation have been associated with increased cardiovascular disease (CVD). Rheumatoid arthritis (RA) is a systemic autoimmune disease associated with a significantly increased of atherosclerotic risk (Charles-Schoeman et al., 2015; Charles-Schoeman et al., 2012) which remains the leading world-wide cause of morbidity and mortality (O’Neill et al., 2015). Also, lipids have been correlated with atherosclerosis. LDL and HDL levels have been characterized as the most important indicators of coronary heart disease (CHD) risk. LDL has been associated with coronary artery disease (CAD) by numerous epidemiological studies (Alabakovska et al., 2004) and large-size LDL are associated with an increased risk of CHD (Babiak and Rudel, 1987). In addition, research and clinical data have showed the anti-atherosclerotic effects of HDL via reverse cholesterol transport (O’Neil et al., 2015).

Nowadays research is focused on subtypes of lipoproteins and their role in cardiovascular diseases. According to bibliography, LDL subtypes were classified as phenotype A (≥25.5 nm) or phenotype B (<25.5 nm) and HDL subtypes as HDL1(>12.00 nm), HDL2b (9.70–12.00 nm) and HDL2a (8.80–9.69 nm), HDL3a (8.20–8.79 nm), HDL3b (7.80–8.19 nm) and HDL3c (7.20–7.79 nm) particles, as described previously (Vekic et al., 2007).

RA patients receive disease-modifying antirheumatic drugs (DMARDs), which reduce the signs and symptoms of the disease. While several RA patients respond to DMARDs, others need different treatments because RA symptoms remain active. The use of TNFα inhibitors in RA treatment was a revolution in treatment options of chronic inflammatory diseases (Kleinert et al., 2012). TNFα plays a central role in this inflammatory disease as this cytosine plays an extremely central role in driving inflammation (Visvanathan et al., 2010). Differences in expression of TNFα level have been observed in synovial fluid and the synovium of patients with RA. The effects of anti-TNFα monoclonal antibodies are partially dependent on synovial TNFα expression and infiltration by TNFα-producing inflammatory cells (Wijbrandts et al., 2008). Anti-TNFα binds to TNFα and inhibits its binding to the cell receptors abolishing its pro-inflammatory effect. In the present study the lipoprotein profiles of patients with rheumatoid arthritis have been examined before and after treatment with anti-TNFα (Ma and Xu, 2012).

Aim of this study is to determine the possible changes in the administration of anti-TNFα drugs to lipoprotein subtypes in patients suffering from rheumatoid arthritis. The effect of anti-TNFα on lipoprotein subtypes can be associated with cardiovascular diseases that often appear to occur in patients with rheumatoid arthritis. Thus, knowing the effect of anti-TNFα on patients’ lipidemic profile, we can determine the role of this agent in the development of atherosclerosis in patients with rheumatoid arthritis.

Methods
Patients Criteria
85 patients (76 women, 89%) with established RA (mean disease duration≥5 yrs), mean age 57yrs(SD:12yrs), without known cardiovascular disease, D/M and thyroid disorders, on sDMARD (MTX, LEF, SSZ, low dose prednisolone or combination) and naive to biologic treatment, were divided into two groups. Patients with DAS28>3.2 (43

90
Figure 1: Electrophoresis of plasma samples. The figure depicts the different subtypes of lipoproteins of eight plasma samples in a Native Polyacrylamide gel electrophoresis (PAGE) of 4% concentration.

patients), who took anti-TNFα drugs for ≥12 months were group one and patients with DAS28≤3.2 (42 patients) who continued the previous treatment with DMARDs were the control group.

Samples
Blood samples of 85 patients were collected in EDTA vacutainers and plasma was separated by centrifugation at 2000rpm. The plasma samples were stored at -80°C. Before use, the samples were diluted with electrophoresis buffer in a dilution one to four and prestained overnight with Sudan Black B, a dye which is used to stain lipoproteins, by mixing 20μl of diluted plasma and 20μl of Sudan Black Band.

Electrophoresis
Native PAGE (polyacrylamide gel) with 4% density was used for the separation of lipoproteins subtypes with a 2% stacking gel. 4% polyacrylamide gels were prepared by mixing 4880 μl H2O, 1070 μl Acrylamide mix 30% (29% Acrylamide / 1% Bis acrylamide), 2000 μl Tris 1,5M pH8,8, 80 μl APS 10% and 8 μl TEMED (Singh et al., 2008). 20μl of the pre-stained samples and 20 μl of standard solution were loaded into wells for electrophoresis. The standard solution contained a protein mixture of Thyroglobulin (Molecular weight (Mr) 669000), Ferritin (Mr 440 000), Catalase (Mr 232 000), Lactate dehydrogenase (Mr 140000) Albumin (Mr 66000). The electrophoresis took place at 4°C with TBE buffer (90mM Tris base, 80mM Boric acid, 3mM EDTA, pH 8.3). The samples were run for 2h at 100V. Then, the gels were stained in Coomasie brilliant blue for 1h and de-stained with 40% methanol and 10% acetic acid.

Statistical Method
Statistical analyses were performed using GraphPad InStat3. The results collected after electrophoresis were analyzed with a two-parameter table and their statistical significance was examined with Fisher's exact test. A p value of <0.05 was considered to indicate statistical significance.

Results
The RA patients on DMARDs had a percent reduction of LDL-B subtype by 11.9% (p=0.014), but a significant increase of HDL-3c subtype by 2.38% (p=0.049). In the RA patients who took TNFα inhibitors, a prominent percent decrease of the more lipoprotein subtypes was observed. LDL-B subtype decreased by 2.33%.
Diagram 1: Lipoproteins subtype frequency pre and post anti-TNFα drugs treatment. The diagram shows the reduction of LDL-A, LDL-B, HDL3a and HDL3c subtypes after anti-TNFα treatment.

(p=0.005), LDL-A subtype decreased by 2.33% (p=0.0001), HDL3a decreased subtype by 11.63% (p=0.072) and HDL3c subtype decreased by 4.65% (p=0.035).

Conclusion
The aim of the study was to determine the effect of anti-TNFα agent on patients' lipoprotein subtypes, as it can be used as a medicine to combat the symptoms of rheumatoid arthritis. Patient plasma was studied before and after administration of anti-TNFα and changes in lipoprotein subtypes were observed. Anti-TNFα acts competitively by blocking the binding of TNFα, which is designated as an indicator of inflammation. Administration of anti-TNFα leads to the reduction of proinflammatory cytokines and chemokines that attract cells to the site of inflammation and lowering inflammation levels locally. In advance, some reports have described the effect of TNFα on lipoprotein metabolism and support that its elevated levels affect hepatic lipid metabolism, increasing de novo fatty acid synthesis and triglyceride concentration in plasma (Qin et al., 2007).

The effect of anti-TNFα on lipoproteins is unknown, so the agent of lipoprotein metabolism that is affected by its administration cannot be accurately determined. Some studies suggest that an increase of TNFα concentration reduces the lipoprotein lipase activity which plays a role in the conversion of both VLDL lipoproteins to LDL lipoproteins and HDL2 subtypes to HDL3 subtypes and vice versa (Levy et al., 2003).

Cardiovascular events associated with rheumatoid arthritis include predisposition for atherosclerosis and endothelial dysfunction, which results in coronary artery disease (CAD), stroke, congestive heart failure and peripheral arterial disease. Studies which test the effect of anti-TNFα on cardiovascular disease have led to the conclusion that the risk of cardiovascular disease is lower in patients with rheumatoid arthritis, treated with anti-TNFα. This is also consistent with the assumption that inflammation contributes to the development of cardiovascular events (Jacobsson et al., 2005).
Treatment with anti-TNFα reduces the chances of developing atherosclerosis by reducing overall concentration of LDL lipoproteins, a marker is characterized as a good prognostic indicator for the occurrence of atherosclerosis (Roman and Salmon, 2007; Georgiadis et al., 2006). LDL subtypes reduction observed and this study after the administration of anti-TNFα treatment. On the other hand, a decrease of HDL3a and HDL3c subtypes observed with the same treatment. Recent studies suggest that decrease of HDL subtypes generally lead to an increased concentration of triglyceride-rich lipoproteins in plasma and therefore increase atherosclerosis risk. Hence, HDL concentration must be considered in relation to LDL (Parhofer, 2015).

This study is the first effort to define the anti-TNFα activate on lipoproteins and thus on the progression of cardiovascular disease in patients suffering from rheumatoid arthritis. The results show that anti-TNFα administration can lower the risk of atherosclerosis as it reduces the LDL subtypes which have been linked with atherosclerosis and other cardiovascular diseases.

It should be noted that changes in lipoprotein subtypes can also be influenced by the dietary habits of patients. Alcohol, smoking, exercise, and eating habits may be related to some of the lipoprotein changes observed in this study. These factors were not defined in this study (Kotsovanis and Bei, 2003).

In conclusion, administration of anti-TNFα drugs can change the lipoprotein subtype pattern in rheumatoid arthritis patients. It can also be protective to those patients due to the reduction of LDL subtypes since LDL subtypes are linked to a higher risk of atherosclerosis.

References


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