SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

The non-lipid effects of selective LDL apheresis on patients with severe familial hypercholesterolemia

By Viktória Evelin Varga (MSc)

Supervisor: Mariann Harangi MD, PhD

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DOCTORAL SCHOOL OF HEALTH SCIENCES

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Supervisor: Mariann Harangi, MD, PhD

Doctoral School of Health Sciences, University of Debrecen

Head of the **Examination Committee**: Róza Ádány, MD, PhD, DSc
Members of the Examination Committee: Gábor Simonyi, MD, PhD
János Sándor, MD, PhD

The Examination takes place at the Library of Bldg. A, Department of Internal Medicine Faculty of Medicine, University of Debrecen, on July 17, 2020, at 10 a.m.

Head of the **Defense Committee**: Róza Ádány, MD, PhD, DSc
Reviewers: István Barna, MD, PhD
Beáta Tóth, PhD

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The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, on July 17, 2020, at 11 a.m

INTRODUCTION

Besides efficiency every new therapeutic method need to be safe and well tolerated by the patients. Despite of the numerous time-consuming and strictly controlled studies prior to the introduction of new drugs and therapeutic methods, new side effects, which were not included in the summary of drug characteristics, during clinical use is still common. These side effects might be unfavourable, for example the increased risk of developing type 2 diabetes mellitus during statin treatment, but they can also be favourable in supporting the beneficial effect of the drug or treatment used, regarding to the course of the disease or the development of its complications. An example is the anti-inflammatory or antioxidant effect of statins, which contributes to its cardiovascular risk-reducing efficacy.

During my doctorate work, I investigated the additional side effects beyond the lowering of LDL particles of an extracorporeal therapeutic technique, the low density lipoprotein (LDL)-apheresis used in the treatment of a rare hereditary disease. These data may help to understand the changes in the condition of patients observed during the treatment, but may also provide an insight into the patomechanism of the disease, which may help to develop new therapeutic approaches therefore improving the quality and expectancy of life of the patient population.

OBJECTIVES

We aimed to investigate the additional side effects of selective LDL apheresis treatment in patients with severe heterozygous familial hypercholesterolemia (HeFH) by:

1. Measuring serum chemerin levels before and after the first LDL apheresis treatment of the patients, the determination of the chemerin-binding capacity of the apheresis column applied during the LDL apheresis treatment and observing the long term effect of LDL apheresis treatment in one patient.
2. Measuring serum afamin levels before and after the first LDL apheresis treatment of the patients, comparing the values with a healthy control population and the determination of the afamin-binding capacity of the apheresis column applied during the apheresis treatment.
3. Measuring serum α- and γ-tocopherol levels before and after the first LDL apheresis treatment of the patients and comparing the values with a healthy control population.
4. Determination of serum LDL and HDL subfractions by gel electrophoresis
5. Measuring the levels of additional pro- and anti-atherogenic hormone-like peptides (adiponectin, leptin, vaspin, omentin-1, RBP4, PEDF, visfatin and obestatin) before and after the first LDL apheresis treatment of the patients.

PATIENTS AND METHODS

Selection of patients with severe heterozygous familial hypercholesterolemia

In our study we enrolled severe HeFH patients treated at the Department of Internal Medicine, University of Debrecen, who had LDL-C levels highly above target value, despite of the lipid lowering medication they received, therefore were treated with selective LDL apheresis treatment (DALI).

Venous blood samples were collected before and after the first DALI treatment of the patients. In one case 12 months monitoring of the chemerin levels as the effect of the DALI treatment was carried out.

For the measurements of afamin and vitamin E, healthy control subjects from the Ambulance at Building „A" of the Department of Internal Medicine of University of Debrecen, were enrolled.

We selected patients with the criteria of age between 21-70 years. After detailed information on the aspects and purpose of the study an informed consent statement were signed by the patients. The study was approved by the Regional Institutional Research Ethics Committee, Clinical Center, University of Debrecen.

Exclusion criteria included acute bacterial or viral infection, taking multivitamins over the last three months, chronic liver disease in the medical history or characterized by elevated liver enzymes, alcohol or drug addiction, gallstones, recent history of myocardial infarction, pregnancy and lactation, positivity for human

immunodeficiency virus, severe mental retardation (intelligence quotient: IQ<40), and patients with known cancer or antineoplastic chemotherapy were also excluded.

Sample collection and laboratory measurements

All venous blood samples were collected after 12 hours of fasting from the patients and control subjects and were also collected after each DALI treatments from the patients then sera were separated immediately. Samples were analysed by the Cobas c501 analyser (Roche Ltd, Mannheim, Germany) for carbohydrate and lipid metabolism using standard laboratory methods at the Institute of Laboratory Medicine, University of Debrecen. Total serum cholesterol and triglyceride concentrations were determined by enzymatic colorimetric method, and in the case of HDL-C and LDL-C by homogeneous enzymatic method (Roche HDL-C plus 3rd generation and Roche LDL-C plus 2nd generation). Apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB) and lipoprotein (a) (Lp (a)) were measured by immunoturbidimetry (Tina-quant Apolipoprotein A-1 ver.2, Tina-quant Apolipoprotein B ver.2 and Tin-quant Lipoprotein (a) ver.2). The measurements were performed according to instructions of the manufacturer. For further laboratory measurements, serum samples were placed at 70 °C and used within 2 months.

LDL-apheresis

Selective LDL-apheresis of the patients was carried out by DALI system (Fresenius GmbH, Germany), using DALI 750 adsorbent columns. DALI primer solution, anticoagulant citrate dextrose solution (ACD-A), blood drainage tubes and 4008 ADS hemadsorption monitor (Fresenius HemoCare Adsorber Technology GmbH, St. Wendel, Germany) were used during treatment. Before treatment adsorbent columns were washed with 3×2000 ml primer solution with 400 ml/min flow rate. The first 2 L solution contained 20000 IU heparin. During loading the adsorbents were saturated with citrate. The patient received an infusion of ACD-A during treatment. The ACD-A solution was administered at the start of treatment in a ratio of 1:20, which was reduced to 1:40 after 1500 ml of treated blood amount. During the treatment blood pressure was continuously monitored. Venous blood samples were taken before and after all treatments.

Determination of serum chemerin, visfatin, PEDF, adiponectin, omentin-1, vaspin, RBP4, leptin, obestatin és oxLDL levels

Determination of serum chemerin levels was performed by a commercially available enzyme-linked immunosorbent assay (Human Chemerin Quantikine ELISA, MN, USA) according to the recommendation of the manufacturer.

Determination of serum visfatin, PEDF, adiponectin, omentin-1 and vaspin levels was performed by commercially available sandwich enzime-linked immunosorbent assay according to

the recommendation of the manufacturer (Biovendor GmbH, Germany).

Determination of serum RBP4 and leptin levels was also carried out by a commercially available sandwich ELISA kit according to the recommendation of the manufacturer (R&D Systems, MN, USA).

Serum obestatin levels were determined by enzime immunoassay (EIA) (Yanaihara Institute Inc., Shizuoka, Japan) while oxLDL concentrations were also determined by ELISA according to the recommendation of the manufacturer (Mercodia AB, Sveden).

Determination of serum afamin level

Determination of serum afamin level and elution fractions was performed by commercially available ELISA (Afamin Human ELISA, katalógusszám: RD194428100R, BioVendor, NC, USA) according to the recommendation of the manufacturer.

Determination of plasma α- és γ-tocopherol levels

Quantification of α- and γ-tocopherol from plasma samples was performed by gas-chromatography-mass spectrometry (GC-MS), with the modification and optimization of a previously described method in the literature. Internal standard method was used for the standard curve. Dilution series were made from α- and γ-tocopherol stock solutions (α-tocopherol, 1.0 mg/ml in methanol, certified reference material, γ-tocopherol 1.0 mg/ml in methanol,

certified reference material, Sigma-Aldrich St. Louis, MO USA). Calibration points for α-tocopherol were 3.13, 6.25, 12.5 and 25 μg/ml and for γ-tocopherol were 0.063, 0.125, 0.25 and 0.5 μg/ml. 2,2,5,7,8-Pentamethyl-6-chromanol (Sigma-Aldrich St. Louis, MO USA) in methanol was used as internal standard. Methanol was evaporated under nitrogen flow then the derivatization was completed with 130 μl Sylon™ HTP (HMDS+TMCS+Pyridine, 3:1:9, Sigma-Aldrich St. Louis, MO, USA) sylilation reagent for 30 min at 60 °C. Sylilation reagent was also evaporated under nitrogen flow then standards were dissolved in 50 μl n-hexane.

For the preparation of plasma samples 100 μl 2,2,5,7,8-Pentamethyl-6-chromanol (4 μg/ml in methanol) internal standard, 100 μl methanol and 95 μl physiological saline was added to 5μl plasma. For the extraction 1 ml n-hexane was added to the samples which were then vortexed and centrifuged. The supernatant was then pipetted into 1.5 ml sample vials and the solvent was evaporated under a stream of nitrogen. Eventually, the samples were derivatized as described above and dissolved in 50 μl n hexane at the end of sample preparation. GC-MS measurements were performed with a Finnigan Trace GC Ultra gas chromatograph connected to Polaris Q mass spectrometer (Thermo Fisher Scientific, Waltham, MA USA). Samples were manually injected onto Agilent J&W column (DB-5MS UI; 60 m x 0.25 m x 0.25 μm) using helium as carrier gas (flow rate: 1 ml/min, constant flow mode). The injection volume was 2 μl. Injection was made in splitless mode, the temperature of the injector was 260 °C throughout the measurement. The initial temperature of

the column was 150 °C for 2 minutes and then increased to 300 °C at a heating rate of 25 °C/min for a further 15 minutes. Total analysis time was 23 minutes. The mass spectrometer was in selective ion monitoring (SIM) mode. The selected ions were: α-tocopherol TMS: 237.3 m/z, γ-tocopherol TMS: 488.4 m/z, 2,2,5,7,8-Pentamethyl-6-chromanol-TMS: 292.3 m/z.

Determination of LDL and HDL subfractions

Lipoprint System (Quantimetrix Corp., Redondo Beach, CA) was used for the detection of lipoprotein subfractions. Lipoprint is an electrophoretic method which separates lipoproteins on acrylamide gel based on their size. Serum samples were added to acrylamide gel tubes and Sudan Black was applied as a lipophilic dye. After electrophoresis, lipoprotein fractions were identified by their mobility with Lipoware computer softver (Quantimetrix Corp.). Lipoprint allows the separation of 7 different LDL subfractions and 10 different HDL subfractions based on the size of the lipoproteins. The separated LDL subfractions are the large, less dense LDL-1 and LDL-2 and the small, dense LDL-3,-4,-5,-6 and -7. HDL subfractions were grouped into three major classes: large (HDL1-HDL3), intermediate (HDL4-HDL7) and small (HDL8-HDL10) HDL subfractions. The average LDL size (nm) is also calculated by the Lipoware software based on the densitogram.

Post LDL-apheresis elution of the protein fraction from DALI 750 adsorbent column and determination of chemerin concentration from the eluate

Elution of chemerin from the apheresis column was carried out by a method previously described by Dihazi et al. DALI 750 adsorbent columns were washed with phosphate-buffer saline (pH 7.4). The protein fraction elution was performed in three steps with 250 ml acetate buffers of three different pH in the following order: pH 5.0; pH 4.0; pH 3.0. Then the determination of the chemerin concentrations from the three eluate was performed by a commercially available ELISA (Human Chemerin DuoSet ELISA, R&D Systems, MN, USA) according to the recommendation of the manufacturer.

STATISTICAL ANALYSIS

Statistical calculations were performed by STATISTICA software (ver 8.0; StatSoft Inc., Tulsa, OK). The normality of data distribution was tested by Kolmogorov-Smirnov test. In the case of normal distribution data were plotted as mean ± SD (SD), for non-normal distribution data were expressed as median and lower and upper quartile values. Pre- and post-treatment results of the patients were evaluated with paired t-test. The data of the patients before treatment was compared with the data of control group using unpaired t-test. Results were considered significant at the level of $p<0.05$.

Friedman ANOVA and Kendall's Rank Correlation were used to compare chemerin concentrations eluted with buffers of different pH.

RESULTS

Effect of selective LDL apheresis treatment on serum chemerin levels

Serum chemerin levels were significantly lower after the first apheresis treatment with a mean of 27.26% (from 82,34 ng/ml to 59,09 ng/ml). In order to exclude the effect of dilution during treatment serum chemerin/creatinine ratio was also calculated which showed a significant decrease of 16.65% after the first treatments. In one patient serum chemerin levels were measured before apheresis over 10 treatments in order to examine the long term effect of LDL-apheresis on serum chemerin levels. Chemerin levels showed decreasing tendency during the 12 months follow-up After the fifth treatment the patient requested a 3-month suspension of treatment (for family reasons). Meanwhile chemerin levels increased significantly, reaching baseline levels.

During this time, circulating chemerin levels increased significantly, reaching baseline levels prior to first treatment. Continuing the LDL apheresis treatment serum chemerin concentrations started to decrease, indicating the importance of continuous treatments to maintain a steady decrease in serum chemerin levels.

To prove that the chemerin physically binds to the adsorber column, protein fractions were eluted from the apheresis column with acetate buffer of three different pH after LDL-apheresis. Chemerin was detected in all three eluates. Significantly more proteins eluted with the higher pH buffer solutions ($p < 0.01$).

In some selected patients, LDL and HDL subfraction measurements were performed before and after LDL apheresis treatments using Lipoprint. After treatment, both LDL and HDL showed favorable quantitative and qualitative changes. The most significant of these changes is that LDL apheresis significantly reduced small dense LDL subfractions.

Effect of selective LDL apheresis on serum afamin and plasma vitamin E levels

The first treatment reduced serum afamin levels by only 9.4% on average. In parallel, HDL and ApoA1 levels decreased by 10.5 and 14.1%, respectively. Consequently, the afamin/HDL and afamin/ApoA1 ratios did not change significantly. Compared to controls. baseline afamin levels were significantly higher in HeFH patients.

We found that in the patients α- and γ-tocopherol levels were significantly decreased (by 34.1 and 32.9%) with the LDL apheresis treatment, while α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol levels were significantly increased (41.4% and 40.3%, respectively). Baseline α- and γ-tocopherol levels were significantly higher in patients compared to healthy controls. In contrast, before the treatment ratios of α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol of the patients were similar to controls.

Initial oxidized LDL level was significantly higher in patients compared to controlls. Furthermore in patients oxidized

LDL level significantly decreased (-57.4%) with the treatment. The proportion of small HDL particles decreased significantly while that of large HDL particles increased slightly, resulting in a shift towards larger HDL subfractions after apheresis treatment. The initial ratio of low HDL particle was significantly higher and the initial ratio of high HDL particle was significantly lower in HeFH patients compared to healthy controls.

Effect of selective LDL apheresis on the level of further pro- and anti-atherogenic hormone-like peptides

The effect of selective LDL apheresis on the levels of the studied pro- and anti-atherogenic hormone-like peptides varies, but is highly expressed in case of many peptides, such as omentin-1 (-75.32%) and vaspin (-58.53%).

The levels of all studied peptides decreased, but due to the high standard deviation, only visfatin, PEDF, omentin-1 and vaspin showed significant decrease.

SUMMARY OF NEW RESULTS

1. Selective LDL apheresis treatment significantly lowers serum chemerin levels in severe heterozygous familial hypercholesterolemic patients after the first treatment. This decrease caused by binding of the chemerin to the adsorber column used during LDL apheresis. Examining one patient LDL apheresis efficiently decreased chemerin serum levels in a long term treatment.
2. Serum afamin level decreases slightly after the first selective LDL apheresis treatment. Initial afamin level of the patients is significantly higher compared to controls. The adsorber column used during LDL apheresis unable to bind afamin in detectable amounts.
3. Serum α- and γ-tocopherol level significantly decreases after the first LDL apheresis treatment, in contrast the ratio of α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol increases significantly. Initial α- and γ-tocopherol levels of the patients are significantly higher, while before the treatment the ratio of α- tocopherol/total cholesterol γ-tocopherol/total cholesterol similar in the patients and controls.
4. LDL apheresis treatment notably decreases the amount of smaller and denser LDL subfractions measured with Lipoprint. The proportion of small HDL particles significantly decreases while the proportion of large HDL particles slightly increases, thus shifting the proportion of HDL subfractions towards larger particles.

5. Serum levels of other hormone-like peptides (adiponectin, leptin, vaspin, omentin, RBP4, PEDF, visfatin and obestatin) also decreasing as the effect of the treatment but only visfatin, PEDF, omentin-1 and vaspin levels decreasing significantly.

DISCUSSION

In agreement with the preliminary data, selective LDL apheresis treatment significantly reduced LDL-C and Lp (a) levels in FH patients. Other pleiotropic effects of LDL apheresis that protect against atherosclerosis are known by decreasing plasma concentration of several pro-inflammatory peptide including cytokines, chemokines, adhesion factors and C-reactive protein. Although the mechanism behind these processes is not known yet, direct binding to the surface or indirect changes by gene transcription and gene translation might be a possible explanation. Dihazi et al. compared serum samples before and after LDL apheresis using proteomics. They described significant decrease of more than 70 functional peptide including peptides of the coagulation system as well as those that have adhesion, rheological and inflammatory role. They concluded that there was a strong interaction between the column and some serum proteins during treatment, while others described modified gene expression of various molecules after apheresis treatment.

Chemerin is a pro-inflammatory, therefore considered pro-atherogenic serum protein secreted by the white adipose tissue. To the best of our knowledge, the effect of LDL apheresis on serum chemerin levels has not yet been studied. In our study, we found that serum chemerin levels were significantly decreased in FH patients after selective LDL apheresis. It has also been demonstrated - however only in one patient - that continuous treatment is required to maintain permanently low serum chemerin levels. Besides lowering

LDL-C level, decreasing circulating chemerin concentration may be another beneficial effect of LDL apheresis due to the presumed pro-inflammatory and pro-atherogenic properties of chemerin. Because LDL apheresis is currently not a widely used treatment for severe hyperlipidaemia, our study was made with a limited number of patients. In the future further studies are needed to confirm the short- and long term effects of LDL apheresis on chemerin levels.

After LDL apheresis we could eluate significant amount of chemerin from the apheresis column. This supports the assumption that chemerin may physically bind to the apheresis column, although the exact mechanism of binding is unknown. There is no information about the charge of the chemerin in the literature, but we can assume that chemerin may be directly bound to the column like the ApoB100 containing positively charged lipoprotein particles. On the other hand, it is also possible that chemerin is attached to positively charged lipoprotein particles and thereby binds indirectly to the adsorber column. Further studies are needed to discover the exact mechanism of binding.

Previous studies have shown that LDL apheresis treatment involves a reduction of small, dense, especially pro atherogenic LDL subfraction and changes the subfraction pattern in a much less atherogenic direction. In our study, we found that LDL subfractions changed favourably in both volume and proportion following apheresis treatment, achieving a strong reduction in small, dense, highly atherogenic LDL subfractions. The small dense LDL subfractions are highly sensitive to oxidative modifications and

therefore they are crucial in initiating and progression of atherosclerosis. Therefore, the reduction of these subfractions in high-risk patient populations may be very important. Based on these results, the effect of LDL apheresis treatment on lipid subfractions may contribute to a beneficial reduction of chemerin levels.

Oxidative processes play a key role in the development of atherosclerosis, because through the oxidative modification of LDL, they play an important role in the vascular foam cell and plaque formation. In addition oxidative modification of HDL particles reduces the protective effect of HDL against atherosclerosis. Therefore, the role of vitamins which considered to be antioxidant, such as vitamin E, has been intensively studied for decades. Tocopherols also known as vitamin E considered to be one of the most important antioxidant of lipoproteins.

Afamin is serum protein involved in the transport of vitamin E thus its level can be important in maintaining the serum level of vitamin E. Besides this, afamin is involved in controlling the apoptosis and oxidative processes of the cells. Serum level of afamin is a marker for type 2 diabetes and metabolic changes during pregnancy. Moreover, certain so-called Wnt proteins which play an important role in regulating stem cell regeneration, bone formation, immune regulation and tumor formation bind to afamin. In addition to increased oxidative stress and accelerated atherosclerosis, FH also has a defect in bone formation, primarily a decrease in bone density, therefore examining the levels of afamin may be particularly important in this disease. The effect of LDL apheresis on serum

afamin levels has not yet been studied. In our study we found decreased serum afamin levels after LDL apheresis in FH patients, but the decrease was only 9.4 % on average. In parallel, HDL-C and ApoA1 levels also decreased by 10.5 and 14.1%, respectively. All this could indicate that the decrease in afamin is due to a decrease in HDL and ApoA1. In fact, only 13% of afamin circulates in HDL-bound form so this alone does not explain the decrease in afamin levels. However, previous studies have shown that afamin circulates predominantly in association with small, dense HDL particles. Therefore, the shift also shown by our study toward larger HDL subfractions may contribute to a decrease in afamin levels during treatment. Afamin was not detected from the eluate we eluted from the colum after treatment, thus we can conlclude that the column used during apheresis probably eliminates lipoprotein-bound afamin indirectly and no direct binding occurs on the column surface.

Afamin has been reported to bind to vitamin E via multiple binding sites in vitro and in vivo. It binds with particularly high affinity to α- and γ-tocopherol, the most important forms of vitamin E. Therefore, we assumed that the decrease of α- and γ-tocopherol after LDL apheresis described in previous studies may be caused by the decrease in afamin level. In parallel with previous studies we found that α- and γ-tocopherol level decreased significantly after LDL apheresis treatment. The ratio of α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol significantly increased since cholesterol decreased to a greater extent than tocopherol levels. The α- and γ-tocopherol levels decreased significantly more compared to

afamin level. Interestingly the decrease of α- and γ-tocopherol correlated best with the decrease of LDL-C level and not with the decrease of afamin level. Based on these results the level of afamin is not a determining factor in the serum levels of α- and γ-tocopherol. The relatively modest decrease of afamin and the marked reduction of oxidized LDL proves indirectly that the afamin does not bind to oxidatively modified LDL particles. The higher levels of α- and γ-tocopherol of patients compared to healthy controls coincide with previous studies which described increased vitamin-E levels in hyperlipidemia. In contrast, another study found that absolute levels of α-tocopherol in FH patients were similar to healthy controls, whereas α-tocopherol/total cholesterol ratio was significantly lower in FH patients. Genetic heterogeneity and different lipid lowering medication may explain the differences between different studies. Due to the high fat solubility of vitamin E, the ratio of tocopherol to total cholesterol may be a better indicator of serum antioxidant potential than total tocopherol. Elevated α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol ratio after apheresis may indicate an increase in serum antioxidant potential in FH patients.

A significant decrease in the level of oxidized LDL was observed in FH patients after LDL apheresis treatment, indicating a favourable effect of apheresis on oxidative processes. This observation is consistent with previous data in the literature. In our study, patients had even lower oxidized LDL levels than controls after the treatment. Based on the remarkable decrease of the oxidized

LDL level and the increase of the α- and γ-tocopherol/total cholesterol ratio during LDL apheresis treatment, we can conclude that in FH patients the oxidized LDL is not only poor in afamin but it has low α- and γ-tocopherol content. This suggests that despite of the higher levels of α- and γ-tocopherol in FH patients, a functional deficiency of α- and γ-tocopherol may develop in oxidized LDL subfractions.

During LDL apheresis, a shift towards larger HDL subfractions was observed. Changes in HDL subfractions during apheresis have been poorly studied and different methods have been used previously to measure them, therefore it is difficult to compare these results. In a previous study, it was reported that LDL apheresis reduces pre-β1-HDL levels in familial hypercholesterolemia, which may lead to transient depletion of cholesterol efflux capacity. The long-term clinical relevance of the observation is unclear. In our study, the proportion of small HDL particles before apheresis was significantly higher in FH patients, while the initial proportion of large HDL particles was significantly lower compared to healthy controls. This shift toward small HDL particles was partly corrected by apheresis treatment, indicating that apheresis may have a beneficial effect on the distribution of HDL subfractions measured by gel electrophoresis.

A limiting factor for our research is the relatively small number of individuals enrolled, which reduces the statistical power of our study. However, our results highlight the potential importance of non-lipid effects of apheresis treatment. In addition, FH patients

enrolled in the study received a combination treatment of statin and ezetimibe, which may affect α- and γ-tocopherol levels. However, it should be noted that the suspension of oral lipid lowering treatments in these severe cases is unacceptable.

After observing the effect of LDL apheresis on chemerin and afamin levels, other biologically active hormone-like peptides were also studied. Among the studied peptides there were also protective and unfavourable in terms of atherosclerosis. As expected serum level of every peptide decreased after apheresis, but the differences in degree of reduction are outstanding. The decrease was significant in the case of the pro-atherogenic visfatin and PEDF and the anti-atherogenic omentin-1 and vaspin. Since these proteins also interact with each other, forming a kind of network in the regulatory process, in the future it would be reasonable to examine them in a complex form. Our results suggest that this extracorporeal procedure which considered to be selective, may have a more significant and diverse effect in patients than previously assumed. LDL apheresis treatment beyond reducing lipid levels also modifies the proportion of adipose tissue, gastrointestinal, and hepatic bioactive proteins which may lead to further metabolic modifications.

SUMMARY

Familial hypercholesterolaemia (FH) is a congenital genetic disorder associated with significantly elevated serum low-density lipoprotein (LDL) -cholesterol (LDL-C) level. Selective LDL apheresis is an extracorporeal lipid lowering method which can significantly reduce LDL-C level. In addition to lipid lowering, LDL apheresis has other beneficial effects such as anti-atherogenic, anti-thrombotic and anti-inflammatory effects. It also reduces the level of several hormone like pro- and anti-inflammatory peptide. To date, only a few study have been made about the effect of LDL apheresis on adipokines. Chemerin is an adipokine which regulates adipogenesis, lipid- and glucose metabolism. Although the role of hormone-like peptides produced by the adipose tissue and digestive system is more evident, several data, including the change in chemerin levels as the effect of LDL apheresis is not yet known. Vitamin E is an antioxidant that is supposed to have a favourable effect in preventing and in the treatment of atherosclerosis. The best known forms of vitamin E are α- and γ-tocopherol. Human afamin is a serum glycoprotein which is known as a specific binding protein of vitamin E and therefore may be responsible for the transport of vitamin E in body fluids. Afamin predominantly expressed by the liver and then it is partly bound to apolipoprotein A1 carrying high density lipoprotein (HDL) subfraction in the circulation. Changes in afamin level as the effect of LDL apheresis has not been studied yet.

Serum level of hormone-like peptides (chemerin, leptin, adiponectin, visfatin, PEDF, omentin-1, obestatin, vaspin, RBP4),

afamin and oxidized LDL was measured by ELISA technique in the case of six patients who suffer from severe heterozygous FH before and after their first LDL apheresis (DALI) treatment. The levels of afamin and vitamin E were also measured in gender and age-matched controls. Changes in lipid levels, LDL and HDL subfractions and α- and γ-tocopherol levels were also investigated after LDL apheresis. LDL and HDL subfractions were measured by polyacrylamide gel electrophoresis (Lipoprint). Serum α- and γ-tocopherol levels were detected with gas chromatography-mass spectrometry Chemerin bound to DALI column was eluted and quantified.

Serum chemerin and oxidized LDL levels were significantly reduced (27.26% and 57.4%, respectively) after the first LDL apheresis treatment. To verify binding of the chemerin to the DALI column, the proteins were eluted with acetate buffer solutions of various pH. The first treatment reduced serum afamin levels by an average of only 9.4%. In parallel, HDL and ApoA1 levels were reduced by 10.5% and 14.1%, respectively. Initial afamin level was significantly higher in patients compared to controls. While the reduction of α- and γ-tocopherol levels was remarkable, ratio of α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol were significantly increased after LDL apheresis. Initial α- and γ-tocopherol levels were significantly higher in patients while ratio of α-tocopherol/total cholesterol and γ-tocopherol/total cholesterol were similar in patients and controls before treatment. Levels of other studied hormone-like peptides were reduced after treatment, but only

the level of visfatin, PEDF, omentin-1 and vaspin reduced significantly. LDL apheresis treatment notably reduced the levels of smaller and denser LDL subfractions. The proportion of smaller HDL particles decreased remarkably while the proportion of large HDL particles increased slightly, thus shifting the proportion of HDL subfractions towards larger particles.

Significant effect of LDL apheresis treatment on serum levels of several circulating hormone-like peptides, including chemerin, have been demonstrated. We showed that the reduction of chemerin is due to its binding to the DALI column during treatment. Our results suggest that in the circulation α- and γ-tocopherol binds primarily to lipoproteins and less to other carrier proteins, such as afamin, which is hardly reduced during apheresis treatment. These additional non-lipid effects of LDL apheresis may contribute to the reduction of cardiovascular risk in FH patients.

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LIST OF PUBLICATIONS

UNIVERSITY of DEBRECEN

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Subject: PhD Publikációs Lista

Candidate: Viktória Evelin Varga
Neptun ID: O750P3
Doctoral School: Doctoral School of Health Sciences

List of publications related to the dissertation

1. **Varga, V. E.**, Lőrincz, H., Szentpéteri, A., Juhász, L., Seres, I., Paragh, G. J., Balla, J., Paragh, G., Harangi, M.: Changes in serum afamin and vitamin E levels after selective LDL apheresis. *J. Clin. Apheresis. 33* (5), 569-575, 2018.
   DOI: http://dx.doi.org/10.1002/jca.21636
   IF: 3.088

2. **Varga, V. E.**, Lőrincz, H., Zsíros, N., Fülöp, P., Seres, I., Paragh, G., Balla, J., Harangi, M.: Impact of selective LDL apheresis on serum chemerin levels in patients with hypercholesterolemia. *Lipids Health Dis. 15* (182), 1-7, 2016.
   DOI: http://dx.doi.org/10.1186/s12944-016-0353-x
   IF: 2.073

List of other publications

3. Szentpéteri, A., Lőrincz, H., Somodi, S., **Varga, V. E.**, Paragh, G. J., Seres, I., Paragh, G., Harangi, M.: Serum obestatin level strongly correlates with lipoprotein subfractions in non-diabetic obese patients.
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7. Zsíros, N., Koncsos, P., Lőrincz, H., Seres, I., Katkó, M., Szentpéteri, A., **Varga, V. E.**, Fülöp, P., Paragh, G., Harangi, M.: Paraoxonase-1 arylesterase activity is an independent predictor of myeloperoxidase levels in overweight patients with or without cardiovascular complications.
Clin. Biochem. 49 (12), 862-867, 2016.
DOI: http://dx.doi.org/10.1016/j.clinbiochem.2016.03.011.
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8. **Varga, V. E.**, Katkó, M., Harangi, J., Balogh, I., Kapás, I., Madar, L., Seres, I., Molnár, M. J., Paragh, G., Kovács, G. G., Harangi, M.: Egy ritka, veleszületett neurodegeneratív betegség: a cerebrotendinosus xanthomatosis laboratóriumi diagnosztikája.
Orv. Hetil. 155 (21), 811-816, 2014.
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Total IF of journals (all publications): 17,618
Total IF of journals (publications related to the dissertation): 5,161

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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