

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

**ROLE OF HOMOCYSTEINE IN
ATHEROSCLEROTIC MANIFESTATIONS OF
NATIVE CORONARY ARTERIES AND VEIN
GRAFTS**

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Debrecen, 2016

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The Examination takes place

at the training room of Institute of Pediatrics Dept. of Pediatric Haemato-Oncology, Internal Medicine bldg. „B”, Clinical Centre,

University of Debrecen on September 27, 2016, at 11 AM

Head of the Defense Committee: Csongor Kiss, MD, PhD, DSc

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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 September 27, 2016, at 1PM

1. INTRODUCTION

My research was focused on the role of homocysteine (Hcy) in atherosclerotic manifestations of native coronary arteries and venous grafts.

The coronary artery disease (CAD) and its severe manifestations and complications are a major cause of premature mortality in developed societies.

Degenerative diseases of native coronary arteries

Coronary sclerosis is a set of progressive reactions arising from chronic injuries of the endocardial surface. The damaged endothelial area is infiltrated with oxidized lipoproteins and forms a lipid-rich necrotic core and a fibrous cap. The lesion is slowly calcifying, however the marginal areas remain permanently infiltrated by inflammatory cells.

Degenerative diseases of vein grafts

Except from left anterior descending coronary artery, saphenous venous grafts are used widely for coronary artery bypass grafting (CABG) due to their number and size variety and easy accessibility or their easier preparation. But their long term patency is seriously limited. After ten years only 50-60% of the vein grafts remain functional. The vein graft atheromas are different from the native vessels' similar lesions: diffuse,

circular, contains less calcification, fibrin hat is thinner or absent, they are prone for rupture and emboli formation. According to experimental models, vein grafts have different gene regulation and gene expression mechanisms and inflammatory responses caused by inflammation.

Physiology of homocysteine

Homocysteine (Hcy) is a sulfur-containing amino acids, an intermediate product of the protein metabolism. The Hcy connects three different pathways of the amino acid metabolism: the folate and the methionine cycle, which provides C1-particules for DNA-, RNA-, and protein-, as well as glutathione synthesis. The plasma total Hcy level differs by age, sex and race. In healthy adults is between 7-14 $\mu\text{mol/L}$.

Hyperhomocysteinemia

According to our resent knowledge only $> 15\mu\text{mol/L}$ plasma Hcy has pathological role and it is listed into different cathegories of mild (15-30 $\mu\text{mol/L}$), moderate (30-100 $\mu\text{mol/L}$) and severe ($> 100 \mu\text{mol/L}$) hyperhomocysteinemia (hHcy). The homocysteine metabolism is influenced by several exogenous and endogenous factors. For the highest Hcy elevation is caused by the inherited deficiency of cystathionine-beta-synthase (CBS) enzyme that is responsible for homocysteine-metionin transformation. Other common causes of Hcy level elevation are: nutrition deficiency, lack of vitamine B₆-, vitamin B₁₂-, and folic acid, increased intake of methionine, alcoholism,

smoking, coffee consumption, permanently inactive lifestyle. Homocysteine level is also affected by certain diseases, eg. diabetes, cancer (breast-, ovary-, pancreas carcinoma, acute lymphoblastic leukemia), renal insufficiency, psoriasis, SLE, rheumatoid arthritis, hypothyroidism, certain neurological diseases (eg. dementia), medications: MTX, cyclosporine, trimethoprim, contraceptives, folic acid, B₆-, B₁₂- antagonists, anticonvulsants, thiazide diuretics, nitrogen oxides, fibrates, metformin.

The „excess” of homocysteine forms disulfide bonds, affects thrombocyte aggregation and adhesion, increases TXA₂-, but reduces prostacyclin levels and induces vascular inflammation. Changes balance of endogenous fibrinolysis by increasing resistance of fibrine clots against of fibrinolysis, increase risk of vascular thrombosis. The hHcy facilitates formation of oxidized LDL, and cholesterol esters and their deponation into endothelial lesions. Throughout incorporating into proteins Hcy generates an autoimmune response.

Hyperhomocysteinemia and cardiovascular disease, vitamin prevention trials

In the 1960s based on observations of McCully on homocysteine was clarified its relationship with atherosclerosis and thromboembolism. In 1975 he has published with Wilson the "protein theory" of atherosclerosis (between homocysteine and atherosclerosis) and about its vitamin prevention. Clinical studies performed since the middle of

1990s have verified the independent risk factor role of hHcy in cardiovascular and athero-thrombotic disorders, but the combined incidence of cardiovascular disease that was achieved by combined vitamin therapy has set contradictory results.

Most of the randomized, controlled studies have shown that reduction of plasma Hcy levels by administration of vitamin B₆-, or B₁₂- or folic acid does not improve outcomes of cardiovascular disease. The key of understanding the homocysteine paradox is clarifying its complex biochemical role.

2. OBJECTIVES

The following objectives were set up:

- 1-2. What are the features of medium to long-term manifestations of native coronary atherosclerosis and venous graft degeneration associated with homocysteine?
- 3-4. Is there a relationship between homocysteine and other risk factors?
5. Is there a possible a role of homocysteine in estimation related to coronary and vein graft disease progression in order to improve the effectivity of secondary prevention?

3. METHODS

Design of study for native coronaries

A case-control study: comparing CAD patients with "negative" CAD controls based on coronary angiographies. We examined risk factors profile difference between patient and control groups by collecting data retrospectively from clinical database.

Our aim was to investigate the CAD specific risk factors between patient groups by collecting data from clinical database. The control group was formed by patients who were admitted with suspected CAD, but coronary lesions were not confirmed.

Design of study for venous grafts

Correlation evaluation between risk factors and graft status among patients who underwent CABG surgery, and received at least one saphenous vein graft. The coronary tests has been performed at least one year after heart surgery, and was based on clinical indications.

The research was carried out between 2001-2013, in a single center, at the University of Debrecen Dept. Of Cardiology in harmony with the Declaration of Helsinki and authority regulations. The research has been notified to the Institutional Ethics Commission. The involved patients received prior oral and written information about the possibility of processing data for research. They all gave their written consent, which was archived together with their clinical documentation.

Data was collected from electronic database (MedSolution) of the Institute of Cardiology Clinical Center University of Debrecen. Data of

personal identification (name, date of birth, social security number), was masked throughout the statistical analysis. The physical examination and collection of data for all patients was performed by me.

Selection of patients with native coronaries

A 1010 patients were included among those admitted to DE KK Institute of Cardiology during 2001-2002 with suspected CAD, performed a selective coronary angiogram.

Selection of patients with vein grafts

The study included 237 (two hundred thirty-seven) patients who were admitted to DE KK Institute of Cardiology during 2001-2002, followed by CABG with at least one saphenous vein graft.

Recorded data

For the patients included the following data were recorded: family history of atherosclerosis/complications, previous myocardial infarction, hyperlipidemia, smoking history and habits, high blood pressure, diabetes, carotid stenosis, peripheral arterial vascular disease, women's hormonal status. The following demographic information was recorded: age (years), sex, body weight (kg), height (meters), body mass index, vital signs, and left ventricular ejection fraction, cardiac function.

Coronary and vein graft status clarification

Selective angiography examinations and their assessment were carried by experienced cardiologists. The standard test methodology was done throughout right femoral artery approach by Judkins technic. The standard views consisted of at least 3 shots of the left coronary artery/graft branches and of two standard view shooting 12.5 frames/sec speed from the right coronary branches by Philips Integris type of x-ray machine (Inturist ViewerLite Suite v1.0, Philips, The Netherlands). Assessment of coronary artery lumen diameter narrowing consisted of determinating localisation and degree of stenoses (%). The number of grafts, their localization and graft interventions have been documented.

Coronary angiographies performed within a year after CABG have been excluded from evaluation – to separate cases due to technical error or premature graft thrombosis.

The diagnosis of SVG disease was based on independent judgement of repeat coronary angiographies by 2 expert cardiologists; SVGs were classified according to their “lumen status” (diameter stenosis; [%]) at repeat coronary angiography as “intact” with a <20% lumen diameter reduction, “narrowed” with a lumen diameter stenosis between 20-99%, and “occluded” (closed lumen). Patients having >1 SVG with different graft status at follow up were ranked according to the more

severe graft's status for classifying the patients to one of the three groups.

4. RESULTS

Results of native coronary arteries

The average plasma Hcy was elevated in all groups ($> 15 \mu\text{mol/L}$), however, women without significant coronary stenosis had lower levels of Hcy compared with men. Women but not men with CAD+MI+ had significantly elevated Hcy levels comparing to controls. Hcy levels of females were lower than of males in the absence of significant coronary stenosis. This difference disappeared in the presence of CAD. Adjusted Lp(a) levels were significantly elevated only in the CAD+MI+ group in both genders. Moderate hyperhomocysteinemia ($\text{Hcy}>15 \mu\text{mol/L}$) independently of other risk factors was associated with CAD OR: 2.27. Isolated Hcy elevation resulted in a tendency of increased risk of CAD with or without MI. In females elevated Hcy didn't increase the risk of CAD, while an OR of 2.03. Simultaneous elevation of Hcy and Lp(a) resulted in an OR of 2.05 for CAD in the total group and OR of 3.59 in females.

Results of vein grafts

Mean patient age was 57.5 ± 10.4 years. The main reason of post-CABG repeat coronary angiography was stable angina and more than 2/3 of patients showed vascular signs of SVG disease (stenosis/occlusion). Demographics, medical history, indication of repeat coronary angiography, clinical parameters or risk factors did not differ

significantly between patient groups. A significant positive correlation was found between the following parameters: CRP and SVG (luminal narrowing (%/month)), Hcy and SVG, CRP and Hcy, while a significant but negative correlation was seen between triglycerides and HDL-cholesterol. By stepwise forward multivariate linear regression analysis, only Hcy levels were associated independently with percent SVG lumen diameter stenosis: a 1 $\mu\text{mol/L}$ increase in Hcy levels was associated with a 0.066% increase in lumen diameter stenosis/month ($r = 0.752$; $p < 0.01$), based on the corresponding patient coronary angiograms. Theoretically this means that an increase of 10 $\mu\text{mol/L}$ in Hcy levels during 5 years can anticipate an 32,1% stenosis on the SVG.

5. DISCUSSION

Results of native coronary arteries

Both Hcy and Lp(a) elevations were independently associated with CAD and/or MI only in women. The association of elevated Lp(a) with CAD and MI resulted in an OR of 1.64 for CAD and 1.89 for MI in women, respectively. The development of CAD and the onset of acute thrombosis in the coronary vessels leading to acute myocardial ischemia are very complex and multi-factorial processes, where the key players can interact with each other and one can modify another's effect.

Simultaneous elevation of Hcy and Lp(a) resulted in an OR of 3,5 for CAD in women. In atherothrombotic diseases the clotting/fibrinolytic system related factors seem to play a role more prominent in females than in males.

Results of vein grafts

The patency rate of SVGs observed throughout the 5.6-year follow up was 74.4%. Results of the univariate analysis suggested a correlation between CRP and the time proportional extent of SVG disease as well as between CRP and Hcy in SVG disease. During further evaluation by multivariate regression analysis, only plasma Hcy levels have remained to be a significant independent predictor of the time proportional extent of SVG disease. Our conclusion was that plasma Hcy is an independent prognostic factor of mid-term post-CABG graft degeneration. In our patients elevated Hcy level was a significant positive predictor of SVG outcome (graft diameter reduction (%)/month): 1µmol/L increase of plasma Hcy level was associated with 0,053%/month graft diameter reduction - theoretically this meant that +10 µmol/L increase of Hcy level may result 32.1% of lumen diameter reduction within 5 years.

The role of homocysteine in estimation of risk of CAD/ SVG disease progression and its prevention

The risk of cardiovascular disease is the result of different risk factors to be relevant in certain patient groups. Veeranna and colleagues have evaluated the role of homocysteine in two clinical trials: MESA (Multi-Ethnic Study of Atherosclerosis) and NHANES III (Third National Health and Nutrition Examination Survey) among more than 12 thousand patients. By adding homocysteine to Framingham Risk Score survey it has increased risk of cardiovascular events According to their data, at more than 20% of individuals had to be modified the earlier risk category. Ruijter and colleagues in 2008 in the British Medical Journal published their research, where more than 300 subjects were collected and analyzed the data of classic risk factors and biomarkers of cardiovascular mortality. Very elderly patients without a history of cardiovascular disease homocysteine itself could identify high risk patients, while the Framingham risk score did not. The American Heart Association previously suggested to screen homocysteine:

1. As part of homocystinuria diagnosis
2. in case of vitamin B₁₂ or folate deficiency
3. more precise calculation of cardiovascular risk

Assessing risk for patients and their relatives (<45 years) thrombotic events. Specially if classic risk factors are not present.

The European Society of Cardiology guideline for the prevention of cardiovascular disease lists homocysteine as a second-line risk factor.

6. NEW RESULTS

1. Elevated plasma homocysteine is linked with the presence of coronary artery disease and myocardial infarction independently
2. An interaction can be assumed between elevated plasma homocysteine and lipoprotein (a)
3. The simultaneous elevation of plasma homocysteine with lipoprotein (a) indicates increased two-fold risk in general, and 3,5-fold risk among women for coronary artery disease
4. In the event of increased plasma homocysteine and lipoprotein (a) younger (<55év) women have a higher risk of myocardial infarction
5. increase of homocysteine in long term (> 5 years) forecasts a remarkable degree of vein graft diameter reduction

8. PUBLICATIONS



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Registry number: DEENK/97/2016.PL
Subject: Ph.D. List of Publications

Candidate: Emília Balogh
Neptun ID: NIXMG8
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List of publications related to the dissertation

1. **Balogh, E.**, Maros, T., Daragó, A., Csapó, K., Herczegh, B., Nyul, B., Czuriga, I., Bereczky, Z., Édes, I., Kőszegi, Z.: Plasma homocysteine levels are related to medium-term venous graft degeneration in coronary artery bypass graft patients.
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Total IF of journals (all publications): 17,773

Total IF of journals (publications related to the dissertation): 4,06

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

21 April, 2016



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THANKS GIVING

I would like to express my thanks to all those who helped me perform this scientific research, as well as in the preparation of this PhD thesis: professor Dr. István Édes, professor Dr. István Czuriga, professor Dr. László Muszbek, Dr. Zsuzsanna Bereczky, Dr. Éva Katona, Dr. László Balkay, Erzsébet Ráczné Csiha, Gábor Károlyi, Balázs Nyul.

I would like to thank for all my previous colleagues at the Cardiology Institute, former Heart and Lung Clinic, Heart Surgery Department, Hemodynamic Laboratory, Clinical Laboratory Institute, Clinical Laboratory Research Department.

I would like to thank the support to my tutor, Dr. Zsolt Kőszegi and finally, with deep gratitude and love I think for my parents and my family: Without their support I could not reach my goals.