Association of fetuin-A and arterial calcification in patients with peripheral vascular disease

PhD thesis

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Introduction

Cardiovascular diseases are the leading cause of death in Hungary and the developed countries. New molecules have being identified continuously as possible regulators and markers of the pathophysiologic process of atherosclerosis and arterial calcification. Besides the classical risk factors other factors may contribute to the development of atherosclerosis. Vascular surgeons, angiologists, radiologists are familiar with the different degree of calcification of the arterial system. In specific cohorts (e.g. patients with diabetes mellitus or chronic end stage renal disease) more severe calcification is experienced with a known association to cardiovascular mortality and morbidity.

Fetuin-A is a potential regulator of extraosseal and intraarterial calcification, that may have a protective role in developing arterial calcification. The curiosity to understand the exact mechanism of fetuin-A led us to study the association of fetuin-A and arterial calcification in patients with peripheral vascular disease.

The research in different medical specialties (angiology, diabetology, vascular surgery, bone metabolism, basic

sciences) proves gathering evidence on the existence of complex and well controlled regulation system on the extraosseal calcification, that are finely tuned by stimulating and inhibitory factors.

Fetuin-A is a member of cysteine protease inhibitors. It is produced in the liver and is detected in high concentration in serum. In vitro and animal studies prove its role in the inhibition of Ca x PO_4 precipitation and regulation bone and mineral metabolism. It is also a negative acute phase glycoprotein, and an endogenous inhibitor of insulin receptor tyrosine kinase playing unclarified role in diabetes mellitus. Most of the data about the association of fetuin-A and cardiovascular calcification originates from the population of chronic uremic patients receiving dialysis. There is no unequivocal evidence that there is a link between vascular calcification and fetuin-A in patients with normal renal function.

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Objectives

1. One of our aims was to provide further evidence about the association of fetuin-A serum levels and systemic atherosclerosis and arterial calcification in nonuremic patients with advanced chronic lower limb ischemia.

2. Our other objective was to examine if serum fetuin-A levels were different in patients with atherosclerotic aortic aneurysm compared to patients with aortic aneurysm who were known to have Marfan syndrome, so these patients were likely to have aortic aneurysm without aortic calcification.

3. In our study we also measured serum levels of Hsp70 (heat shock protein) in subjects with peripheral artery disease (lower extremity and carotid artery stenosis or occlusion) and determined the possible correlation with the dimension of disease and the presence of cardiovascular risk factors (homocysteine, CRP, smoking). We aimed to investigate the association between serum Hsp70 and fetuin-a, a novel marker of arterial calcification .

Methods

Patients

We recruited 266 patients at the Department of Vascular Surgery of the Semmelweis University between 01. 01. and 31. 07. 2009. We screened all patients at the outpatient and inpatient units of the department, who suffered from chronic lower limb ischemia, carotid stenosis and aortic aneurysm of atherosclerotic origin and of Marfan syndrome and agreed to participate in the study.

Exclusion criteria were: symptoms of acute ischemia, clinical signs of infection, malignant tumour, acute or chronic liver disease, end stage renal disease, dialysis treatment, immunosuppressive therapy, any major disease or surgery in the last 6 months as stroke, myocardial infarction, vascular or cardiac surgery, percutaneous vascular interventions or other severe illnesses and operations.

The study protocol was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics.

Medical history was recorded and all patients underwent physical examination. A study questionnaire was used for

recording the relevant demographic and clinical data (age, weight, height, abdominal girth, smoking habit, medications and concomitant disease).

The traditional Fontaine classification was used to assess the clinical severity of the chronic lower extremity atherosclerotic disease (groups I, II/a, II/b, III, IV). Ankle-brachial index was calculated on the basis of Doppler ultrasound measurements.

Laboratory measurements

Fasting serum samples were used to examine standard values with conventional standardized methods performed in the core laboratory of Semmelweis University. Serum levels of fetuin-A were determined by radial immunodiffusion. Soluble Hsp70 level was measured by using enzyme-linked immunosorbent assay.

Imaging techniques

Carotid intima-media thickness was measured at three points on a plaque free area of the dorsal wall of both common carotid arteries using duplex ultrasound method and internal carotid stenosis was also determined. The overall extent of systemic atherosclerosis a calcification score was calculated after examining the vascular system at seven sites by B-mode ultrasound and transthoracic

echocardiogram: both carotid bifurcations, the infrarenal aorta, both common femoral arteries, aortic and mitral valves. Where calcification was noted, the spot was rated as 1, while sites with no calcification received 0 score. Hence the calcification range was 0-7. To assess the extent of atherosclerosis of the infrarenal aorta, iliac, femoral, popliteal and crural arteries the Bollinger score was calculated on each side based on the analysis of arteriograms (recording all stenotic lesions and occlusions).

Statistics

Statistical analysis was performed with Prism for Windows 5.01 (GraphPad Software, San Diego, CA) and SPSS for Windows 15.0.1 (SPSS Inc., Chicago, IL) statistical software products. As many of the variables had non-Gaussian distributions nonparametric tests were applied. The Mann-Whitney's U test was performed to compare two independent groups, the Kruskal–Wallis test to compare multiple groups and Spearman's rho coefficient was calculated for correlations. Multiple logistic regression analysis was also performed. All statistical analyses were performed two-tailed and $p<0.05$ was considered as significant.

Results

1. Results of the examination of the association of serum fetuin-A and arterial calcification in patients with chronic lower extremity atherosclerosis without severe chronic renal disease

Spearman's rank correlation analysis revealed a significant negative correlation between serum fetuin-A levels and UH calcification score (r= -0.257, p= 0.018) (Fig. 1.A.) and also between fetuin-A levels and Bollinger score (r= -0.347, p= 0.035) (Fig. 1.B.).

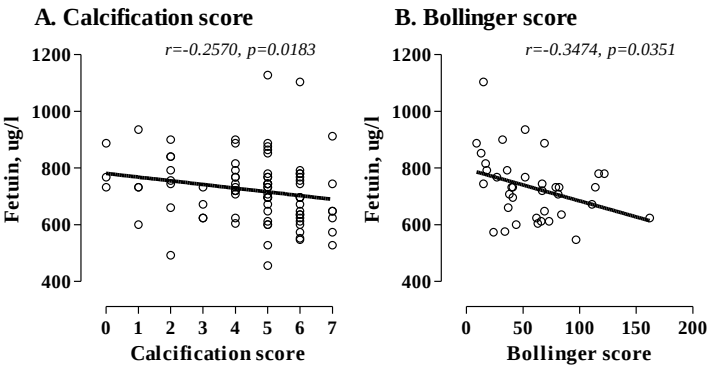


Fig. 1.: Correlation of serum fetuin-A levels with calcification and Bollinger scores. Spearman's rho and p-values are indicated.

Comparing the patients with lower fetuin-A levels (lowest tertile) to patients with higher fetuin-A in the other two tertiles, significantly higher calcification score

(CS) was observed in the lowest tertile (Fig. 2.). Patients in the lowest tertile had almost three times odds (odds ratio /OR/= 2.9683, confidence intervals /CI/: 1.21-7.26) to have higher grade CS compared with patients in the higher tertiles. Logistic regression analysis was performed to evaluate the association of fetuin-a level and calcification score. None of these models modified the statistical significance between fetuin-A levels and calcification score.

	Odds of more severe arterial calcification (CI)	p-value
unadjusted	2.9683 (1.2128-7.2645)	0.0172
Model 1	2.8894 (1.1215-7.4438)	0.0280
Model 2	3.2790 (1.1707-9.2853)	0.0239
Model 3	3.0351 (1.0533-8.7457)	0.0398

Fig. 2.: Association between fetuin-A and arterial calcification score Odds ratio and 95 % confidence intervals (CI) were obtained by logistic regression model. Model 1: Adjusted for age, gender, BMI (Body mass index), diabetes and smoking, Model 2: Model 1 + cholesterol, triglyceride, HDL (high-density lipoprotein), Model 3: Model 2 + CRP (C-reactive protein)

2. Analysis of the results of the different fetuin-A levels in patients with aortic aneurysms of different etiologies (atherosclerosis and Marfan syndrome)

The measurements of serum fetuin-A levels in patients with aortic aneurysm showed the results below: the median (IQR) of serum fetuin-A levels in patients with peripheral artery disease (PAD) were 654 μg/ml (600-756), 708 (612-780) in atherosclerotic aortic aneurysm (ASA), 756 (708-816) in Marfan syndrome and 754 (713-777) in healthy control. The difference between the two aneurysmatic groups (ASA and Marfan) was statistically significant (p= 0.0428), Figure 3. Significant differences in fetuin-A levels were also noticed between the ASA and control group, and between the Marfan and PAD group. Differences were not significant between PAD and ASA or Marfan and control groups.

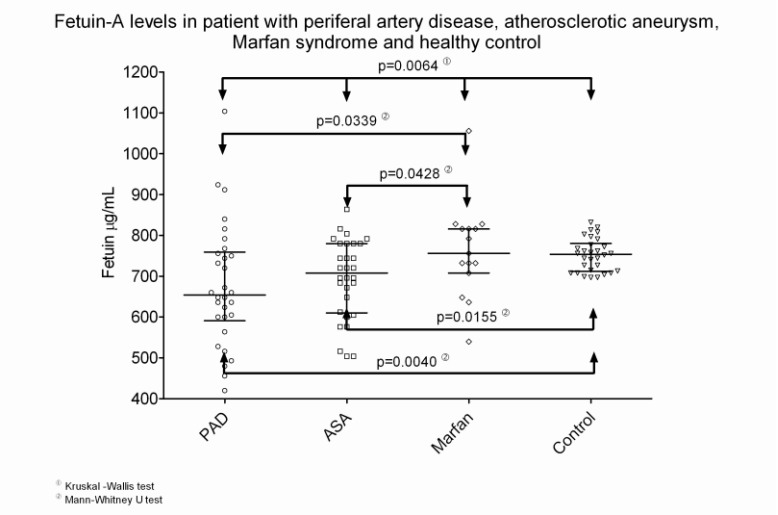
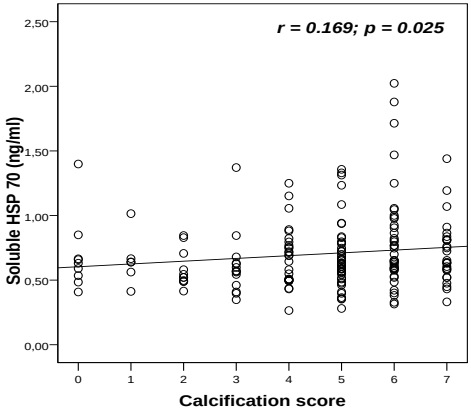


Fig. 3.: Scatter plot diagram displays the difference of serum fetuin-A levels between different patient groups.

3. Results of our study on serum Hsp 70 levels in relationship with arterial calcification and fetuin-A n patients with peripheral arterial diseases

We found a significant relationship of soluble Hsp70 levels with homocysteine levels and calcification score are visualized with scatterplots (Fig. 4. a and b).

a



b

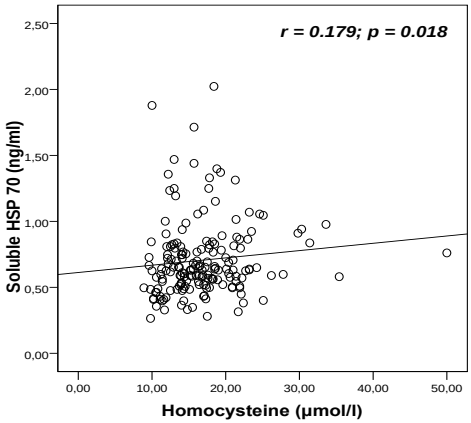


Figure 4.: Association of serum Hsp70 levels with calcification score (a) and homocysteine levels (b) in patients with peripheral artery disease and carotid stenosis.

In a univariate analysis, patients with Hsp70 level above the 75th percentile (0.7296 ng/ml) had an almost 2.2-fold risk to belong to the seriously calcified group (CS 6-7). To evaluate the association of Hsp70 level and the extent of arterial calcification, logistic regression analysis was performed. After correction for those major confounding factors the significant correlation between circulating Hsp70 and arterial calcification still remained significant (Fig. 5.).

	Odds of more severe arterial calcification Exp(B) (95.0% C.I. for EXP(B))	p-value
unadjusted	2.189 (1.156-4.144)	0.016
Model 1	2.110 (1.066-4.175)	0.032
Model 2	2.233 (1.054-4.730)	0.036
Model 3	2.403 (1.115-5.181)	0.025
Model 4	2.264 (1.021-5.020)	0.044

Fig. 5.: Association between Hsp70 and arterial calcification score
Odds ratio and 95 % confidence intervals (CI) were obtained by logistic regression model: Model 1.: Demographics: age, gender, eGFR; Model 2.: Model 1+Smoking (years); Model 3.: Model 2+CRP; Model 4.: Model 3+Homocysteine

Discussion

1. In this study we observed a negative correlation among serum fetuin-A levels and systemic arterial calcification scores in a cohort with severe chronic lower extremity atherosclerosis with normal renal function. The association was independent of age, gender, BMI, diabetes, smoking history, cholesterol, triglyceride, HDL and CRP. These findings are consistent with the hypothesis, that lower fetuin-A serum levels may be responsible for more severe arterial calcification even in the lack of uremia. These findings support the assumption, that fetuin-A may be an inhibitor of arterial calcification in human.

2. We found significantly lower serum fetuin-A levels in patients with atherosclerotic aortic aneurysms compared to those with aortic aneurysms related to Marfan syndrome. To our knowledge, this is the first study to observe the relationship of fetuin-A in patients with aortic aneurysms of different etiology. These results provide further evidence that fetuin-A may be an inhibitor of arterial calcification in atherosclerosis in patients without significant renal dysfunction independent of diabetes mellitus. We also found

significantly lower serum fetuin-A levels in the PAD group compared to patients with Marfan syndrome and in the control group which further supports the possible role of low fetuin-A levels in the pathophysiology of atherosclerosis.

3. The novel finding of the present study is that we reported a positive correlation among serum heat shock protein 70 levels and systemic arterial calcification scores in a cohort with severe chronic lower extremity atherosclerosis and severe carotid stenosis. However, no relationship was seen between soluble Hsp70 and the acute phase reactants C-reactive protein and fetuin-a. Our findings indicate that higher numbers of calcified plaques are closely correlated with higher Hsp70 levels. Further prospective studies are required to establish the possible biomarker role of Hsp70 in arterial calcification, in order to clarify whether high circulating levels of Hsp70 are a consequence of chronic atherosclerotic disease or a predisposing factor for later cardiovascular events.

Publications

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