The relationship between matrix GLA protein (MGP) and carotid stenosis

Relația între proteina GLA matriceală (MGP) și stenoza carotidiană

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Abstract

Serum matrix GLA protein was correlated with vascular calcification and atherosclerosis risk factors, however the relationship with carotid stenosis was not studied so far. Objectives: to study the relationship between matrix GLA protein and carotid stenosis and its degree. Methods: 60 patients were studied, 23 males and 37 females, aged 67.25 ± 9.42 years. Each patient was submitted to an Eco Doppler carotid examination, carotid stenosis was classified as insignificant <20%, moderate 20-50%, severe >50%. In each patient, matrix GLA protein was determined using ELISA method. Results: there were significant differences of matrix GLA protein serum level between subjects with (>20%) and without carotid stenosis (22.85 ± 2.92 nmol/L vs. 19.70 ± 3.06 nmol/L, p<0.0001). The values were also correlated with stenosis degree (<50% 21.48 ± 3.19 nmol/L vs. >50% 23.46 ± 3.83 nmol/L). It was possible to establish a cut-off value for severe stenosis (cut-off value 21.5 nmol/L, AUROC 0.637, sensitivity 75%, specificity 55.8%). In turn, matrix GLA protein level concentration did not correlate with cardiovascular risk factors, no significant differences being registered in relationship with sex, hypertension (21.85 ± 3.45 nmol/L vs. 21.1 ± 2.36 nmol/L), diabetes mellitus (21.77 ± 3.49 vs. 21.74 ± 3.29 nmol/L), obesity (21.44 ± 3.82 vs. 21.87 ± 3.09 nmol/L) or smoking habit (20.91 ± 3.71 vs. 21.86 ± 3.29 nmol/L). Conclusion: serum matrix GLA protein level may be used as both arterial calcification and carotid atherosclerosis index.

Keywords: matrix GLA protein, carotid stenosis, cardiovascular risk factors

Rezumat

Nivelul seric al matrix GLA proteinei (MGP) s-a correlat cu calciferile vasculare si cu factorii de risc ai aterosclerozei, dar relația cu stenoza carotidiană nu a fost studiată până în prezent. Obiectivul studiului a fost reprezentat de investigarea relației dintre matrix GLA proteina (MGP) și prezența, respectiv gradul stenozei carotidiene. Metodă: au fost investigați 60 de pacienți, 23 bărbați și 37 femei, vârsta medie 67.25 ± 9.42 ani. Fiecăreia din pacienți i s-a efectuat o ecografie carotidiană, stenoza carotidiană fiind clasificată ca nesemnificativă

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Rezultate: au existat diferențe semnificative ale nivelurilor serice ale matrix GLA proteinei între subiecții cu stenoză >20 % (22.85 ± 2.92 nmol/L) vs. cei fără stenoză carotidiană (19.70 ± 3.06 nmol/L, p<0.0001). Valorile s-au correlat cu gradul stenozei carotidiene (<50% 21.48 ± 3.19 nmol/L vs. >50% 23.46 ± 3.83 nmol/L). A fost posibilă stabilirea unei valori cut-off pentru stenoză carotidiană severă (egală cu 21.5 nmol/L, AUROC 0.637, sensibilitate 75%, specificitate 55.8%). În schimb, valorile MGP nu s-au correlat cu factorii de risc cardiovasculari, nefiind evidențiate diferențe semnificative în relație cu sexul pacienților, cu prezența hipertensiunii arteriale, (21.85 ± 3.45 nmol/L vs. 21.1 ± 2.36 nmol/L), diabetului zaharat (21.77 ± 3.49 vs. 21.74 ± 3.29 nmol/L), obezității (21.44 ± 3.82 vs. 21.87 ± 3.09 nmol/L) fumatului (20.91 ± 3.71 vs. 21.86 ± 3.29 nmol/L). Concluzie: Nivelele serice ale MGP pot fi utilizate nu doar ca un index al calcifierilor arteriale, dar și al aterosclerozei carotidiene.

Cuvinte cheie: matrix GLA proteina, stenoza carotidiana, factori de risc cardiovasculari

Introduction

Admittedly, cardiovascular diseases are today an important cause of mortality both in developed as well as under development countries. Atherosclerosis, a multifactorial disease, is one of the main causes in the pathogenesis of such diseases. Atherosclerosis pathogenesis involves among other, inflammation, endothelial dysfunction, oxidative stress and, according to rather recent indications, vascular calcifications generating arterial stenosis [1]. Diagnosed especially following multi-slice CT, arterial calcifications are currently deemed by both ESC (European Society of Cardiology), ACC (The American College of Cardiology) and AHA (American Heart Association), important criteria in ischemic cardiomyopathy diagnosis, particularly in asymptomatic patients of intermediary risk [2, 3].

Not long ago, it was believed that calcifications represented the final stage of atherosclerosis, however, it was recently proved that they emerge as early as the first stages of the process, while various vitamin K metabolites, matrix GLA protein (MGP), leptin, osteopontin, osteoprotegerin and the RANK/RANK-L system are also involved [4-10].

The MGP, described for the first time in 1985 by Price [8] is a protein dependant on vitamin K, originally isolated in bones, which is also produced at the level of vascular smooth muscle cells (VSMC) [11]. Numerous studies have proven MGP involvement in calcification processes by several mechanisms: binding calcium ions and crystals, influencing bone protein morphogenesis and bone cell differentiation at this level, fixating various bone matrix components and influencing apoptosis [11]. Its release is influenced by various factors, like the retinoic acid, vitamin D and calcium extracellular ions and reduction of vitamin K (KH2) generation [11].

Serum matrix GLA protein was correlated with vascular calcification and risk factors for atherosclerosis; however, the relationship with carotid stenosis has not yet been investigated. Our aim was to study the relationship between matrix GLA protein, carotid stenosis and its degree.

Material and methods

Sixty patients, 23 males and 37 females aged 67.25 ± 9.42 years, admitted in 2009 in the Rehabilitation Hospital, Cardiology department, were taken into consideration. Each patient was submitted to an Eco Doppler carotid examination, carotid stenosis being classified as insignificant <20%, moderate 20-50%, severe >50%. The carotid stenosis degree was assessed using multiple parameters: color Doppler flow technique and carotid duplex ultrasound evaluation - decrease of carotid diameter, focal increases in blood flow velocity, peak systolic velocity, end diastolic velocity, spectral configuration and internal/common carotid artery ratio.

In each patient, matrix GLA protein was determined (normal values <7 nmol/L) from serum samples stored at -70°C. Serum MGP concentrations were quantified with Bio-
medica (Vienna, Austria) kit. The kit is based on the competitive ELISA principle, with antibodies against non-phosphorylated MGP coated on the microtiter plate.

Data were analyzed using MedCalc 10.3.0.0 and SPSS 16.0 (Demo Version). We calculated mean and standard deviation for normally distributed quantitative variables. Differences between quantitative variables were examined using Student’s test (independent-sample T test) and ANOVA test, while for qualitative variables, the \( \chi^2 \) test was performed. Pearson correlation was used in order to identify correlation between quantitative variables. Receiver Operating Characteristic (ROC) curve analysis and AUROC (area under Receiver Operating Characteristic) were utilized in order to identify the ability of a test (MGP) to discriminate diseased cases (with carotid stenosis) from normal cases (without carotid stenosis). A \( p \) value less than 0.05 was considered significant from statistical point of view.

## Results

The study involved 60 patients, 37 (61.7%) female and 23 (38.3%) male, of various cardiovascular pathology. Patients’ characteristics are summarized in Table I.

MGP mean value was of 21.75 ± 3.33 nmol/L, respectively 22.10 ± 3.33 nmol/L in females and 21.18 ± 3.29 nmol/L in males, without significant statistic differences between the two genders.

### Table I. Demographic and medical characteristics of patients

<table>
<thead>
<tr>
<th>Patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
</tr>
<tr>
<td>Females</td>
<td>37 (61.7%)</td>
</tr>
<tr>
<td>Males</td>
<td>23 (38.3%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>17 (28.3%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52 (86.7%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>38 (63.3%)</td>
</tr>
<tr>
<td>Smoking habit</td>
<td>7 (11.7%)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>41 (68.3%)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>50 (83.3%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>29 (48.3%)</td>
</tr>
<tr>
<td>Peripheral artery disease</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Stroke</td>
<td>14 (23.3%)</td>
</tr>
</tbody>
</table>

### Table II. Correlation between cardiovascular risk factors and MGP values

<table>
<thead>
<tr>
<th></th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.109</td>
<td>0.405</td>
</tr>
<tr>
<td>Weight</td>
<td>-0.009</td>
<td>0.949</td>
</tr>
<tr>
<td>Height</td>
<td>-0.117</td>
<td>0.381</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.103</td>
<td>0.441</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.129</td>
<td>0.361</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.104</td>
<td>0.460</td>
</tr>
<tr>
<td>Glycemia</td>
<td>-0.084</td>
<td>0.522</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.278*</td>
<td>0.032</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>0.220</td>
<td>0.092</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>0.036</td>
<td>0.787</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.201</td>
<td>0.124</td>
</tr>
</tbody>
</table>

* Correlation is significant at 0.05 level (2-tailed).
MGP level concentration did not correlate with cardiovascular risk factors, no significant differences being registered in relationship with sex (22.10 ± 3.33 nmol/L in females vs. 21.18 ± 3.29 nmol/L in males), hypertension (21.85 ± 3.45 nmol/L vs. 21.1 ± 2.36 nmol/L), diabetes mellitus (21.77 ± 3.49 vs. 21.74 ± 3.29 nmol/L), obesity (21.44 ± 3.82 vs. 21.87 ± 3.09 nmol/L) or smoking habit (20.91 ± 3.71 vs. 21.86 ± 3.29 nmol/L).

Concurrently, given the presence of cardiovascular risk factors (male sex, hypertension, obesity, diabetes mellitus, dyslipidemia, smoking habit – between 0 and 6 factors), the ANOVA test did not reveal any significant differences between MGP mean values according to the number of risk factors (19.80 nmol/L vs 21.52 ± 3.62 nmol/L vs 22.32 ± 3.92 nmol/L vs 21.64 ± 2.19 nmol/L vs 20.61 ± 3.82 vs. 21.25 ± 1.76 nmol/L vs 28.9 nmol/L, p = 0.360).

However, a correlation, statistically significant, with the total cholesterol value was noted (Table II).

35% of the patients exhibited insignificant carotid stenosis (<20%), 51.7% showed moderate stenosis (20-50%) and 13.13% had severe stenosis (>50%).

There were significant differences of MGP serum level between subjects with (>20%) and without carotid stenosis (22.85 ± 2.92 nmol/L vs. 19.70 ± 3.06 nmol/L, p<0.0001), and MGP values increased with stenosis degree (<20 % 19.70 ± 3.06 nmol/L, 20-50% 22.69 ± 2.71 nmol/L, >50 % 23.46 ± 3.83 nmol/L, p=0.001), as shown in Figure 1.

The values were also correlated with the stenosis degree (<50% 21.48 ± 3.19 nmol/L vs. >50% 23.46 ± 3.83 nmol/L) and it was possible to establish a cut off value for severe stenosis (cut off value 21.5 nmol/L, AUROC (area under ROC) 0.637, sensibility 75%, specificity 55.8%). Practically, a 21.5 nmol/L MGP value may differentiate with 75% sensibility and 55.8% specificity between patients with over

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**Figure 1. Relationship between stenosis degree and MGP values**

**Legend:** Box-and-whisker for mean MGP; MGP minimum and maximum values; error bars – 95% CI for mean. Data are represented for all three groups: with stenosis less than 20%, between 20-50% and greater than 50%.

**Figure 2. AUROC (area under receiver operating characteristic) for MGP**

**Legend:** Test evaluation – MGP values ability to differentiate between patients with over 50% carotid stenosis versus those with less than 50% stenosis (the area under receiver operating characteristic for MGP- red line –)
50% carotid stenosis and those with less than 50% stenosis (Figure 2).

Discussion

Vascular calcification represents an important factor in increasing cardiovascular risk, morbidity and mortality [12]. Practically, calcium crystals build up at vascular level and at bone level as well, under the form of calcium apatite. There is much evidence on MGP’s important role in inhibiting vascular calcification in humans [11, 13, 14]. MGP was originally isolated from bone, but it was proven to exist also in kidneys, lungs, heart, cartilages and VSMC. Keutel syndrome in humans is characterized by abnormal calcification of cartilages due to some existent mutations at gene level, genes responsible for MGP synthesis [15]. Local release of MGP, especially induced by VSMC, is essential for the prevention of vascular calcification, concurrently intervening in calcium cellular homeostasis [11]. MGP is in fact a component of a complex that also contains hydroxyapatite, feritin and other proteins [16].

Within the atherosclerosis process, activation of macrophages and VSCM causes the release of many proteins - such as proteins dependant on vitamin K and MGP - that are involved in vascular calcification [17].

At present, there is little evidence on existent relations between MGP levels and artery calcifications with three times increase of this protein in animals exhibiting high calcification [8]; such increase was believed to be due to stimulation of local MGP synthesis aimed at diminishing the progression of the calcification process. The MGP increase in serum, with no concurrent increase of MGP synthesis at arteries level, did not induce the inhibition of ectopic mineralization in mice [19]. Evidence existent in humans are contradictory, thus according to various studies, given artery calcification MGP serum levels are either high or low [20, 21], such results being most likely influenced by associated morbidity as well.

Herein, we did not identify significant correlations between MGP levels and cardiovascular risk factors, except for cholesterol. Instead, other studies found correlations between MGP and HDL-cholesterol, LDL-cholesterol, respectively total cholesterol: HDL-cholesterol ratio [22, 23]. A correlation between cholesterol level over 240 mg/dL and MGP serum level was not established [22].

The increase of MGP serum level was associated to arterial walls calcification in rats [8], as well as in patients suffering from severe atherosclerosis [20]. Very high MGP concentrations were found near calcium deposits in mice and humans [24]. In humans, MGP genetic polymorphism induced an increase of both MGP synthesis and levels, being associated with the risk increase of coronary calcification occurrence [25, 26]. Thomsen et al described increased serum MGP levels in patients with ischemic heart disease (MGP being a marker of IHD characterized by intima calcification and subsequent atherosclerosis) [23]. In patients without compromised kidney function, serum MGP level depended on this glycoprotein synthesis from VSMC and subsequent binding of MGP to calcified areas within the vascular wall [23, 27]. Thus, one may speculate that artery calcifications induce MGP synthesis increase, most likely within the context of a feedback-type action attempting to discontinue the calcium accumulation by bone-like formation mechanism at atrial level [22].

In our study, MGP values were directly correlated with carotid atherosclerotic stenosis degree, which supposes by extrapolation, a direct relation between the serum level of this marker and calcifications at atheromatous plaques level. However, it is worth mentioning that it was carried on a small number of patients. The specialty literature does not include studies that approach the relation between MGP serum and carotid stenosis degree. However, a different study has proven the existence of reverse correlation between inactive uncarboxylated MGP (ucMGP) and coronary and artery calcifications (patients diagnosed with aortic stenosis) [28]. Moreover, another small extent
study indicated no direct correlation between MGP’s serum level and coronary calcification in patients with K hypovitaminosis [21].

Nonetheless, accurate mechanisms that would account for the way and occurrence context of MGP secretion increase in the circulatory system remain unknown. Further studies that would explain such mechanisms and their implications are required.

In conclusion, serum matrix GLA protein level may account for both arterial calcification and most likely, carotid atherosclerosis and its severity, independently of cardiovascular risk factors.

Acknowledgements

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