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

Relaţia între proteina GLA matriceală (MGP) şi stenoza carotidiană

Pop Dana[1*], Sitar -Taut Adela[2], Gligor Elena[1], Bodizs Gyorgy[1], Cebanu Mirela[3], Buduru Smaranda[3], Prof. Zdrenghea Dumitru[1]

1. Department of Cardiology, Clinical Rehabilitation Hospital, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania
2. "Babes-Bolyai" University, Cluj-Napoca
3. "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania

Abstract

Serum matrix GLA protein was correlated with vascular calcification and atherosclerosis risk factors, however the relationship with carotid stenosis was not studied insofar. 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 being 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, sensibility 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 mel litus (21.77 ± 3.49 vs. 21.74 ± 3.29 nmol/L), obesi ty (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: ser um 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 corelat cu calcifierile vasculare si cu factorii de risc ai aterosclerozei, dar relaţia cu stenoza carotidiană nu a fost studiată până în prezent. ***Obiectivul*** *studiului a fost reprezentat de investigarea relaţiei dintre matrix GLA proteina (MGP) şi prezenţa, respectiv gradul stenozei carotidiene.* ***Metodă:*** *au fost investigaţi 60 de pacienţi, 23 bărbaţi şi 37 femei, vârsta medie 67.25 ± 9.42 ani. Fiecărui pacient i s-a efectuat o ecografie carotidiană, stenoza carotidiană fiind clasificată ca nesemnificativă*

[*]**Corresponding author:** Pop Dana, 46-50th Viilor Street, Cluj-Napoca, postal code 428063, Romania, Phone 0040744159933, E-mail address pop7dana@yahoo.com

<20%, moderată 20-50%, severă >50%. MGP a fost determinată la fiecare pacient utilizând metoda ELISA. ***Rezultate:*** *au existat diferenţe semnificative ale nivelelor serice ale matrix GLA proteinei între subiecţii 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 corelat 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 cutt-off pentru stenoza carotidiană severă (egală cu 21.5 nmol/L, AUROC 0.637, sensibilitate 75%, specificitate 55.8%). În schimb, valorile MGP nu s-au corelat cu factorii de risc cardiovascular, nefiind evidenţiate diferenţe semnificative în relaţie cu sexul pacienţilor, cu prezenţa hipertensiunii arteriale, (21.85 ± 3.45 nmol/L vs. 21.1 ± 2.36 nmol/L), diabetului z aharat (21.77 ± 3.49 vs. 21.74 ± 3.29 nmol/L), obez ităţii (21.44 ±3.82 vs. 21.87 ±3.09 nmol/L) fumatului (2 0.91 ±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 şi 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-

Table I. Demographic and medical characteristics of patients

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

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*.

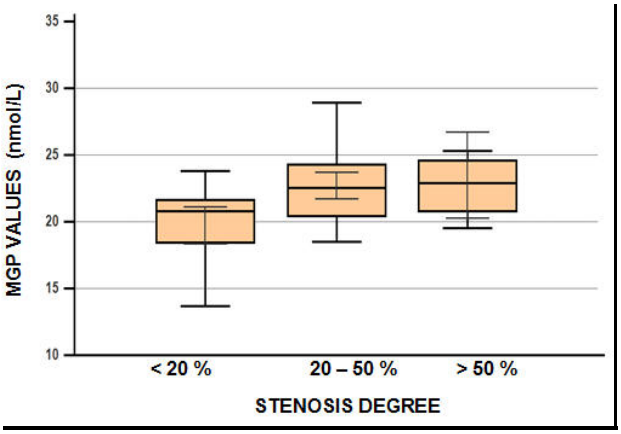
MGP mean value was of 21.75 ± 3.33 nmol/L, respectively 22.10 ± 3.33 nmol/L in females and 21.18±3.29 nmol/L in males, without significant statistic differences between the two genders.

Table II. Correlation between cardiovascular risk factors and MGP values

	MGP	
	Pearson Correlation	Sig. (2-tailed)
Age	0.109	0.405
Weight	-0.009	0.949
Height	-0.117	0.381
Body mass index	0.103	0.441
Systolic blood pressure	0.129	0.361
Diastolic blood pressure	0.104	0.460
Glycemia	-0.084	0.522
Total cholesterol	**.278***	**0.032**
LDL-cholesterol	0.220	0.092
HDL-cholesterol	0.036	0.787
Triglycerides	0.201	0.124

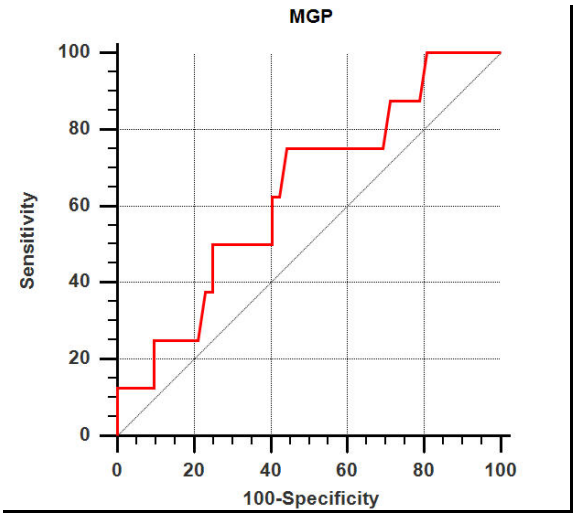
**. Correlation is significant at 0.05 level (2-tailed).*

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 –)

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, dyslipidaemia, 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

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]. Thomnsen 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

This paper has been prepared within the framework of Research Project No. 947, ID_2246/ 2009 Code, part of PN II Program funded by the Romanian Ministry of Education, Research and Innovation-The National University Research Council.

References

1. Frink RJ, Achor RW, Brown Jr AL, Kincaid OW, Brandenburg RO. Significance of calcification of the coronary arteries. Am J Cardiol. 1970;26:241-7.
2. Graham I, Atar D, Borch-Johnsen K, Boysen G, Burell G, Cifkova R et al. European Society of Cardiology (ESC); European Association for Cardiovascular Prevention and Rehabilitation (EACPR); Council on Cardiovascular Nursing; European Association for Study of Diabetes (EASD); International Diabetes Federation Europe (IDF-Europe); European Stroke Initiative (EUSI); Society of Behavioural Medicine (ISBM); European Society of Hypertension (ESH); WONCA Europe (European Society of General Practice/Family Medicine); European Heart Network (EHN); European Atherosclerosis Society (EAS). European guidelines on cardiovascular disease prevention in clinical practice: full text. Fourth Joint Task Force of the European Society of Cardiology and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of nine societies and by invited experts). Eur J Cardiovasc Prev Rehabil 2007; 14 Suppl 2:S1-113.
3. ACCF/ACR/AHA/NASCI/SAIP/SCAI/SCCT 2010 Expert Consensus Document on Coronary Computed Tomographic Angiography. Circulation 2010; 121:2509-43.
4. Libby P, Schwartz D, Brogi E, Tanaka H, Clinton SK. A cascade model for restenosis. A special case of atherosclerosis progression. Circulation 1992; 86:III47-52.
5. Yamamoto H, Imazu M, Hattori Y, Tadehara F, Yamakido M, Nakanishi T et al. Predicting angiographic narrowing > or = 50% in diameter in each of the three major arteries by amounts of calcium detected by electron beam computed tomographic scanning in patients with chest pain. Am J Cardiol 1998; 81:778-80.
6. O'Rourke RA, Brundage BH, Froelicher VF, Greenland P, Grundy SM, Hachamovitch R et al. American College of Cardiology/American Heart Association Expert Consensus Document on electron-beam computed tomography for the diagnosis and prognosis of coronary artery disease. J Am Coll Cardiol 2000; 36(1):326-40.
7. Sweatt A, Sane DC, Hutson SM, Wallin R. Matrix GLA protein (MGP) and bone morphogenetic protein-2 in aortic calcified lesions of aging rats. J Thromb Haemost 2003; 1:178-85.
8. Price PA, Faus SA, Williamson MK. Warfarin causes rapid calcification of the elastic lamellae in rat arteries and heart valves. Arterioscler Thromb Vasc Biol 1998; 18:1400 -7.
9. Lehto S, Niskanen L, Suhonen M, Ronnemaa T, Laakso M. Medial artery calcification. A neglected harbinger of cardiovascular complications in non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1996; 16:978-83.
10. Iwamoto M, Yagami K, Shapiro IM, Leboy PS, Adams SL, Pacifici M. Retinoic acid is a major regulator of chondrocyte maturation and matrix mineralization. Microsc Res Tech 1994; 28:483-91.
11. Proudfoot D, Shanahan C. Molecular mechanisms mediating vascular calcification: Role of matrix GLA protein. Nephrology 2006; 11, 455-61.
12. Farzaneh-Far A, Proudfoot D, Shanahan C, Weissberg PL. Vascular and valvar calcification: Recent advances. Heart 2001; 85: 13-17.
13. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997; 386: 78-81.
14. Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally; different roles of two GLA-containing proteins. J. Cell Biol 2004; 165: 625-30.
15. Munroe PB, Olgunturk RO, Fryns JP, Van Maldergem L, Ziereisen F, Yuksel B et al. Mutations in the gene encoding the human matrix GLA protein cause Keutel syndrome. Nat. Genet 1999; 21: 142-4.
16. Bostrom K, Tsao D, Shen S, Wang Y, Demer LL. Matrix GLA protein modulates differentiation induced by bone morphogenetic protein-2 in C3H10T1/2 cells. J. Biol. Chem 2001; 276: 14 044-52.
17. Kannel WB, Feinleib M, McNamara PM, Garrison RJ, Castelli WP. An investigation of coronary heart dis-

ease in families. The Framingham offspring study. Am J Epidemiol 1979; 110:281- 90.
18. Luo G, Ducy P, McKee MD, Pinero GJ, Loyer E, Behringer RR et al. Spontaneous calcification of arteries and cartilage in mice lacking matrix GLA protein. Nature 1997; 386:78-81.
19. Murshed M, Schinke T, McKee MD, Karsenty G. Extracellular matrix mineralization is regulated locally; different roles of two GLA-containing proteins. J Cell Biol 2004; 165:625-30.
20. Braam LA, Dissel P, Gijsbers BL, Spronk HM, Hamulyak K, Soute BA et al. Assay for human matrix gla protein in serum: potential applications in the cardiovascular field. Arterioscler ThrombVasc Biol 2000; 20:1257- 61.
21. Jono S, Ikari Y, Vermeer C, Dissel P, Hasegawa K, Shioi A et al. Matrix GLA protein is associated with coronary artery calcification as assessed by electron-beam computed tomography. Thromb Haemost 2004; 91:790 -4.
22. O'Donnell CJ, Shea MK, Price PA, Gagnon DR, Wilson PW, Larson MG et al. Matrix GLA protein is associated with risk factors for atherosclerosis but not with coronary artery calcification. Arterioscler Thromb Vasc Biol 2006; 26:2769-74.
23. Thomsen SB, Rathcke CN, Zerahn B, Vestergaard H. Increased levels of the calcification marker matrix Gla Protein and the inflammatory markers YKL-40 and CRP in patients with type 2 diabetes and ischemic heart disease.- Cardiovascular Diabetology 2010, **9**:86.
24. Spronk HM, Soute BA, Schurgers LJ, Cleutjens JP, Thijssen HH, De Mey JG et al. Matrix GLA protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. Biochem Biophys Res Commun 2001; 289:485-90.
25. Farzaneh-Far A, Davies JD, Braam LA, Spronk HM, Proudfoot D, Chan SW et al. A polymorphism f the human matrix gamma-carboxyglutamic acid protein promoter alters binding of an activating protein-1 complex and is associated with altered transcription and serum levels. J Biol Chem 2001; 276:32466 -73.
26. Sixa I, Massy ZA. Inflammation et calcifications vasculaires Inflammation and vascular calcifications. Néphrologie & Thérapeutique 2010; 6:S13-S18.
27. Palaniswamy C, Sekhri A, Aronow WS, Kalra A, Peterson SJ. Association of warfarin use with valvular and vascular calcification: a review. Clin Cardiol. 2011 ; 34(2):74-81.
28. Cranenburg EC, Vermeer C, Koos R, Boumans ML, Hackeng TM, Bouwman FG et al. The Circulating Inactive Form of Matrix GLA Protein (ucMGP) as a Biomarker for Cardiovascular Calcification. J Vasc Res 2008; 45:427-36.