Association between serum cartilage oligomeric matrix protein and coronary artery calcification in maintenance hemodialysis patients

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Abstract

Background Coronary artery calcification (CAC) is common in end-stage renal disease (ESRD) patients, and the extent of CAC is closely related to cardiovascular outcomes in ESRD patients. Cartilage oligomeric matrix protein (COMP), as a component of the vascular matrix, has been found to be an inhibitor of arterial calcification in basic studies. However, there is no clinical research on the correlation between COMP and CAC in maintenance hemodialysis (MHD) patients. The aim of this study was to explore the relationship between serum COMP levels and CAC and cardiovascular events in MHD patients.

Methods Serum COMP levels were compared between 54 MHD patients and 66 healthy people. MHD patients were then divided into three groups according to the tertiles of the concentration of COMP level and were followed up for major adverse cardiac events (MACEs), which were defined as a combined end point of new onset angina pectoris, nonfatal myocardial infarction, heart failure, coronary artery revascularization, hospitalization due to angina pectoris and all-cause deaths. The CAC score was calculated based on computed tomography scans.

Results The serum COMP level in MHD patients was significantly higher than that in the general population [984.23 (248.43–1902.61) ng/mL vs. 219.01 (97.26–821.92) ng/mL, P < 0.01]. Serum COMP levels were positively correlated with CAC (r = 0.313, P = 0.021) and serum parathyroid hormone in MHD patients (r = 0.359, P < 0.01). Linear regression suggested that after adjusting for age, fasting blood glucose (Glu) and glycosylated hemoglobin (HbAlc), CAC score was an independent predictor in the final model for COMP level (β = 0.424, t = 3.130, P < 0.01). The receiver operating characteristic (ROC) curve showed that COMP ≥ 994 mg/mL had 68.0% sensitivity and 72.4% specificity for the prediction of severe CAC [area under the curve (AUC): 0.674, P = 0.030, 95% CI: 0.526–0.882]. After a median follow-up of 16 months (8–24 months), there was no difference in the incidence rate of MACEs between the upper, middle and lower serum COMP groups.

Conclusions Our study found that MHD patients have higher levels of circulating COMP than controls. The serum COMP level is positively correlated with CAC score and could be used as a biomarker of severe CAC in MHD patients. However, there is no obvious correlation between serum COMP levels and the incidence of cardiovascular events.

Keywords: Biomarker; Cartilage oligomeric matrix protein; Coronary artery calcification; Maintenance hemodialysis

1 Introduction

Cardiovascular disease is the most common cause of death in patients with end-stage renal disease (ESRD), especially patients on dialysis. Studies have elucidated that cardiovascular diseases associated with ESRD are mainly chronic heart failure and coronary and peripheral arterial diseases.[1] Vascular calcification may be the cause of high cardiovascular risk in ESRD patients, especially maintenance hemodialysis (MHD) patients. It has been reported that the prevalence of vascular calcification detected by computed tomography is over 80% in dialysis patients.[2] Due to the key function of the coronary artery, the clinical significance of coronary artery calcification (CAC) is higher than that of calcification of other sites. CAC indicates the
plaque burden of atherosclerotic lesions in the coronary artery and is closely related to the prognosis of percutaneous coronary intervention therapy outcome. The mechanism of CAC is different in MHD patients based on histological type, i.e., medial or intimal calcification. The former type occurs as a result of vascular smooth muscle cells (VSMCs) converting into osteoblast-like cells and causing local inflammation in arterial walls. In chronic kidney disease (CKD) patients, various factors, such as hyperphosphatemia, hypercalcemia and high concentrations of parathyroid hormone (PTH), initiate and accelerate the process of phenotypic transformation from VSMCs to osteoblast-like cells. The process of intimal calcification in CKD and non-CKD patients shares the same mechanism, which is a pathogenesis secondary to atherosclerosis. In fact, intimal and medial calcification complement each other during the whole process. Apart from the factors mentioned above, some calcification inhibitors have been found to regulate the calcification process. These inhibitors include osteoprotegerin, fetuin A, and matrix GLA protein (MGP). A recent study found a new factor called cartilage oligomeric matrix protein (COMP), which may also act as an inhibitor of CAC.

COMP is a 524-kDa pentameric noncollagenous glycoprotein belonging to the thrombospondin family, it acts as a matricellular protein that is widely found in the musculoskeletal and cardiovascular systems. The serum COMP level is used as a biomarker for monitoring and predicting the prognosis of rheumatoid arthritis and osteoarthritis. In the cardiovascular system, recent studies have demonstrated that COMP plays an important role in inhibiting postinjury neointima formation and vascular calcification in VSMCs, as well as maintaining the homeostasis of VSMCs. In consideration of the high prevalence of vascular calcification in MHD patients, as mentioned above, and the need for a novel biomarker to monitor calcification in MHD patients, we performed this clinical investigation of serum COMP levels in patients with MHD to assess the relationship between serum COMP concentration and CAC.

2 Methods

2.1 Study population

We recruited 54 patients who underwent or were still on MHD at the Blood Purification Center of Peking University Third Hospital. All patients underwent hemodialysis three times a week for four hours each time from January 2015 to June 2018. The exclusion criteria included obvious infection, rheumatoid arthritis, osteoarthritis, recent bone damage, tumor, moderate or severe valvular heart disease, congenital heart disease, systemic inflammatory disorders and severe organ failure except that associated with kidney failure. Another 66 healthy people who underwent a routine physical examination in Peking University Third Hospital were recruited as the control group. Participants had no history of pregnancy, mental disorder, osteoarthritis, rheumatoid arthritis, bone injury, known cardiovascular risk factors or cardiovascular diseases. There was no aortic arch calcification detected on chest radiography.

2.2 Study procedure

The serum concentration of COMP was compared between MHD patients and the control group. MHD patients were then divided into three groups according to the tertiles of the concentration of COMP level as the lower concentration group (18 cases), the medium concentration group (18 cases) and the upper concentration group (18 cases). General data were collected, and the participants were followed up for a median time of 16 months (8–24 months). No individuals were lost to follow-up. The endpoint of follow-up was new onset angina pectoris, nonfatal myocardial infarction, heart failure, hospitalization due to angina pectoris, coronary artery revascularization or all-cause deaths. Serum creatinine (Scr), albumin (ALB), total cholesterol (TC), uric acid (UA), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), fasting blood glucose (Glu), serum calcium (Ca), serum phosphorus (P), glycated hemoglobin (HbA1c), and PTH were evaluated at the central chemistry laboratory of Peking University Third Hospital. The calcium phosphate product was calculated according to Ca, P and ALB levels. Serum COMP was determined using a commercially available sandwich ELISA kit (R & D Company, USA).

2.3 Multislice computed tomography and CAC score

All of the MHD patients underwent multislice computed tomography (MSCT) in Peking University Third Hospital to quantify CAC. All MSCT examinations were performed using a 64-row scanner (General Electric, South San Francisco, California) with a protocol for prospective triggering (Snapshot Pulse, GE Healthcare). Scanning parameters for the unenhanced calcium scoring scan were as follows: 100 kV tube voltage, tube current was adjusted according to the body mass index (BMI), 0.28 s rotation time, and 2.5 mm slice thickness. CAC score measurements were individually performed by two experienced readers blinded to the patient information using CaScoring software, and then the average was used as the score. CAC was defined as a plaque of at least three contiguous pixels (area of 1.02 mm²) with a density > 130 Hounsfield units. The lesion score was calculated by multiplying the lesion area by a density factor derived...
from the maximal Hounsfield unit within the area, as described by Agatston, et al. [16] Fifty-four MHD patients were divided into two groups according to the CAC level: CAC score ≥ 400 were defined as severe CAC, and CAC score < 400 were defined as non-severe CAC. [17,18]

2.4 Statistical analysis

Continuous variables are presented as the mean ± SD, discrete data are described as median with 25%–75% range, and categorical data are summarized as frequency with percentage. The Kolmogorov-Smirnov test was used to test for normal or abnormal distribution of continuous variables. Levene’s test was used to the homogeneity test. Student’s t-test was used to compare normally distributed data. The Mann-Whitney U test was used for abnormally distribution data. The comparison among multiple groups were performed with one-way ANOVA followed by Scheffe test (equal variance assumed data) or Tamhane test (equal variance not assumed data). Proportions were compared by the chi-square test. Pearson’s correlation coefficient was used for parametric correlation, and the Spearman test was used for nonparametric correlation. Multiple linear regression analysis was used to identify factors that were independently associated with COMP level. The non-normally distributed dependent variable COMP was transformed to LnCOMP by using the natural logarithm method. Receiver operating characteristic (ROC) analysis was used to determine a cut-off value for severe CAC. Kaplan-Meier analysis was used to compare the survival rates of different groups. SPSS 21.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses. P < 0.05 (two-tailed) was considered to be statistically significant.

3 Results

3.1 Basic data and biochemical indicators

Baseline clinical characteristics and biochemical measurements are presented in Table 1. The mean age of the patients was 55.1 ± 12.3 years old, and 42 patients (77.8%) were male. The MHD patients were generally older than the participants in the control group. The percentage of males was higher among the MHD patients. BMI, UA, TGs and HbA1c were higher in the MHD group, while the levels of TC and HDL-C were lower in the MHD group than in the control group. Among the MHD patients, 41 (77.4%) had a history of hypertension, and 18 (34.0%) had diabetes.

3.2 COMP level comparison

As shown in Figure 1, the serum concentration of COMP in the MHD group was higher than that in the normal control [984.23 (248.43–1902.61) ng/mL vs. 219.01 (97.26–821.92) ng/mL], and the difference was significant (P < 0.01).

Fifty-four MHD patients were divided into two groups according to CAC level. CAC score ≥ 400 were defined as severe CAC [n = 29 (53.7%), CAC score 1550.9 (401–

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**Table 1. Clinical and biochemical characteristics of all participants.**

| Parameters            | MHD patients (n = 54) | Control group (n = 66) | P-value |
|-----------------------|-----------------------|------------------------|---------|
| Clinical characteristics |                       |                        |         |
| Age, yrs              | 55.1 ± 12.3           | 35.5 ± 9.9             | 0.000   |
| Male                  | 42 (77.8%)            | 21 (48.5%)             | 0.001   |
| Hypertension          | 41 (77.4%)            | 0.002                  |
| Diabetes              | 18 (34.0%)            | 0.002                  |
| BMI, kg/m²            | 24.2 ± 3.4            | 22.0 ± 2.6             | 0.001   |
| Biochemical characteristics |                    |                        |         |
| Scr, μmol/L           | 1098.7 ± 266.5        | 76.2 ± 12.3            | 0.000   |
| eGFR, ml/min          | 4.2 ± 1.1             | 100.7 ± 13.9           | 0.000   |
| UA, μmol/L            | 489.1 ± 92.3          | 324.9 ± 82.9           | 0.000   |
| TC, mmol/L            | 3.9 ± 0.9             | 4.3 ± 0.45             | 0.002   |
| TGs, mmol/L           | 2.6 ± 1.6             | 1.0 ± 0.3              | 0.000   |
| HDL-C, mmol/L         | 0.9 ± 0.8             | 1.4 ± 0.3              | 0.000   |
| LDL-C, mmol/L         | 2.2 ± 0.7             | 2.6 ± 0.4              | 0.320   |
| Glu, mmol/L           | 5.6 ± 2.1             | 5.0 ± 0.4              | 0.367   |
| HbA1c, %              | 6.2 ± 1.6             | 5.3 ± 0.2              | 0.032   |

Data are presented as means ± SD or n (%). BMI: body mass index; eGFR: estimated glomerular filtration rate; Glu: fasting blood glucose; HbA1c: glycosylated hemoglobin; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; MHD: maintenance hemodialysis; Scr: serum creatinine; TC: total cholesterol; TGs: triglycerides; UA: uric acid.
7929), and CAC score < 400 were defined as non-severe CAC \( n = 25 \) (46.3%), 73.5 (0–392)]. There was significant difference \( F(2, 117) = 139.0, P < 0.01 \) in COMP level among control group, non-severe calcification group and severe calcification group in MHD patients. Tamhane’s post hoc test showed that a significant difference \( P < 0.01 \) existed between control group and non-severe calcification group, control group and severe calcification group (Figure 2).

### 3.3 Relationship between COMP and other parameters

Spearman correlation analysis showed that there was a low correlation between blood COMP concentration and CAC score and PTH in MHD patients \( r = 0.313, P = 0.021; r = 0.359, P < 0.01 \). The scatter diagrams showing this relationship are shown in Figure 3 & 4. However, there was no relationship between serum COMP and other parameters, including sex, age, BMI, Scr, UA, TC, TGs, LDL-C, HDL-C, Glu, HbA1c, Ca, P, Ca and P product and dialysis duration \( P > 0.05 \).

### 3.4 Independent predictor of COMP in MHD patients

Stepwise linear regression analysis showed that after adjusting for age, Glu and HbA1c, severe CAC was the independent predictor in the final model for LnCOMP level \( \beta = 0.424, t = 3.130, P < 0.01 \).

### 3.5 Significance of COMP in the diagnosis of severe CAC

ROC analysis was used to determine a cut-off value for severe CAC (defined as a CAC score over 400). COMP ≥ 994 ng/mL had 68.0% sensitivity and 72.4% specificity to predict severe CAC \[ \text{area under curve (AUC)}: 0.674, P = 0.030, 95\% \text{CI}: 0.526–0.882 \] (Figure 5).

### 3.6 Relationship between CAC, COMP and prognosis in MHD patients

The 54 MHD patients were divided into three groups according to the tertiles of the concentration of COMP level as the lower concentration group (18 patients, COMP level ranging from 0 to 850 ng/mL), medium concentration group (18 patients, COMP level ranging from 851 to 1058 ng/mL) and upper concentration group (18 patients, COMP level ranging from 1059 ng/mL to higher). We followed up all 54 patients for a median time of 16 months (8–24 months). During the follow-up period, there were six major adverse cardiac events (MACEs), including three cases of acute coronary syndrome, two cases of heart failure, and one case of sudden death. One patient was excluded because
she underwent kidney transplantation. The incidence of MACEs was 11.1% (6/54). During the follow-up period, there were six main MACEs in the severe CAC group and 0 MACEs in the non-severe CAC group. Kaplan-Meier analysis showed that the survival rate of the severe CAC group was significantly lower than that of the non-severe CAC group ($P < 0.01$) (Figure 6).

The 54 patients were then divided into three groups according to COMP level tertiles. During the 16 months (8–24 months) follow-up period, there were two MACEs in each group, and Kaplan-Meier analysis showed that there was no significant difference in survival rate among the three groups ($P = 0.996$) (Figure 7).

4 Discussion

Cardiovascular disease is one of the main causes of death in ESRD patients, and making decisions for these patients is even harder due to their complicated clinical situation. The prevalence of CAC is 80% in MHD patients according to former reports, yet the results of our study showed that over 89%, i.e., 48 of the 54 MHD patients, had CAC (CAC score > 0); and 25 (46.3%) of them had severe CAC (CAC score $\geq 400$). Zhao, et al. also found that hemodialysis patients had higher levels of serum COMP than the controls; however, they used plain radiography to evaluate vascular calcification instead of a more precise method such as the computed tomography scan and calcification score method we used in our study. The results of this study also showed that serum COMP levels in MHD patients were significantly higher than those in the general population. COMP is one of the extracellular matrix proteins expressed in the musculoskeletal system, and it was used as a novel biomarker for diagnosing and prognosing rheumatoid arthritis. COMP has also been found in the cardiovascular system, especially in VSMCs and cardiomyocytes. Previous studies have shown that there is no change in serum COMP...
vascular calcification in the aortic ring environments. Bone morphogenetic protein 2 (BMP-2) is that COMP overexpression may inhibit calcification in both BMP-2 receptor. As with intimal calcification, COMP also a key element that can accelerate the VSMC phenotype switch, including a high concentration of serum PTH, hypercalcemia, and hyperphosphatemia. In addition to these factors, recent studies have found that COMP can inhibit the phenotypic switch of VSMCs. Researchers used a high Pi- or CaCl₂-environment to induce vascular calcification in the aortic ring in vivo and found that COMP overexpression may inhibit calcification in both environments. Bone morphogenetic protein 2 (BMP-2) is also a key element that can accelerate the VSMC phenotype switch, and COMP can directly block this process by binding to BMP-2 using its C terminal to compete with the BMP-2 receptor. As with intimal calcification, COMP plays a protective role in the atherogenesis and atherosclerotic calcification processes. In the atherogenesis process, macrophages switch into inflammatory and osteogenic types in the primary steps. However, macrophages also express COMP, and COMP deficiency can drive the phenotypic switch mentioned above. Moreover, COMP also inhibits inflammatory neutrophils, which also plays a pivotal role in early atherogenesis. On the other hand, COMP can inhibit vascular intima hyperplasia, thus preventing restenosis after angioplasty procedures. In conclusion, according to basic research in vitro and in vivo, COMP plays a protective role in both intimal and medial calcification processes. Because of the high prevalence of CAC in MHD patients, we assume that the higher level of serum COMP in the MHD patient group could be a compensatory reaction of the body. There was no difference in the incidence rate of cardiovascular events between the upper, medium and lower COMP groups after a median follow-up of 16 months. Although there was no significant difference in the incidence of cardiovascular events among patients in the higher, middle and lower concentration groups of serum COMP levels, we demonstrated that there is an obvious positive correlation between CAC and serum COMP level. As CKD patients, especially MHD patients, are not always able to undergo angioplasty because of their poor kidney function, serum COMP may be used as a biomarker to screen for coronary calcification in MHD patients in a more inexpensive and convenient way.

4.1 Limitations
This is a single-center study limited by a small sample size, which may restrict the generalizability of our conclusions. Further prospective studies are required to verify the effects that elevated serum COMP has on clinical prognosis in MHD patients in regard to CAC and cardiovascular events. Additionally, the follow-up duration was short; thus, we need to prolong the follow-up time to determine the prognostic value of COMP for cardiovascular events in MHD patients.

4.2 Conclusions
This study found that MHD patients had higher levels of circulating COMP than controls. Serum COMP levels were positively correlated with CAC and could be used as a biomarker of severe CAC in MHD patients. However, there is no obvious correlation between serum COMP levels and the incidence of cardiovascular events.

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