Evaluation of Atherosclerosis, Arterial Stiffness and Related Risk Factors in Chronic Hemodialysis Patients in Siriraj Hospital

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Background: Additional to traditional risk factors for cardiovascular disease (CVD), recent evidence demonstrates that non-traditional risk factors such as high-sensitive C-reactive protein (hsCRP), hyperhomocysteinemia and vascular calcification may cause progressive atherosclerosis in hemodialysis patients.

Objective: We aim to determine the prevalence of atherosclerosis and assess the arterial stiffness and related risk factors.

Material and Method: Common carotid artery intima-media thicknesses (CIMT), atherosclerotic plaque occurrence were determined by B-mode ultrasonography in 105 hemodialysis patients (mean age, 53 ± 15.5 years; mean dialysis duration 82 ± 59.5 months). A history of clinically significant atherosclerotic vascular disease was elicited by patient questionnaire and verified by careful patient chart review and physical examination. Cardiovascular ankle index (CAVI) was use to assess arterial stiffness. Serum biochemical marker for traditional risk factors, hsCRP and homocysteine were measured by standard method.

Results: Atherosclerotic vascular disease (defined by a history of CVD or presence of atherosclerotic plaque) was present in 79% of patients. Compared to non-atherosclerotic group, the mean CIMT and serum hsCRP in atherosclerotic group was higher (1.9 ± 0.8 mm vs. 0.8 ± 0.6 mm, p < 0.001; 6.5 ± 8.8 mg/L vs. 3.3 ± 3.5 mg/L, p = 0.03, respectively), while other biochemical markers were not significantly different, as well as the percentage of abnormal CAVI (69% vs. 54.5%, p = 0.28). CAVI was positively correlated with maximum carotid intima-media thickness (r = 0.44, p < 0.001). CAVI was also significantly greater in patients with carotid plaque (soft plaque (p < 0.05) and calcified plaque (p < 0.05)) compared with patients without carotid plaque.

Conclusion: A high prevalence of atherosclerosis and arterial stiffness was observed in hemodialysis patients. Carotid atherosclerosis is associated with an increased inflammatory marker (hsCRP). CAVI may be a useful index to assess arterial stiffness and associated with arterial intima-media thickness.

Keywords: Arterial stiffness, Pulse wave velocity, Hemodialysis, Atherosclerosis, Cardio-vascular ankle index

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Cardiovascular disease has been well recognized as the major cause of morbidity and mortality in hemodialysis (HD) patients(1,2). Undoubtedly, HD patients have many risk factors for atherogenesis including traditional risk factors such as hypertension, anemia, dyslipidemia, diabetes mellitus and smoking, and uremia-related risk factors such as fluid overload, hyperhomocysteinemia and increased oxidative stress(3). Moreover, during the last several years, infectious and inflammatory complications have been considered nontraditional risk factors of atherogenesis that recently have been received considerable attention(4,5). In several clinical studies, C-reactive protein (CRP) level is used as an objective index of inflammatory activity reflecting the generation of pro-inflammatory cytokines. It has been proven to be a strong predictor of cardiovascular disease and outcome in dialysis patients(6).

The effective and accurate methods for early
detection of atherosclerotic disease are very important because intensified lowering of risk factors strategies can be applied appropriately. Detection of subclinical phase of atherosclerotic disease can be performed by noninvasive detection of morphological and functional changes of the vasculature by measuring arterial wall thickness and pulse wave velocity (PWV), respectively. Carotid intima-media thickness (CIMT) measured by high-resolution B-mode ultrasonography has been used as a surrogate marker for atherosclerosis that well correlates with the incidence of coronary heart disease(7) and stroke(8) in nonuremic population. More recent studies also suggested that CIMT is an independent predictor of cardiovascular mortality in the hemodialysis population(9-11). Pulse wave velocity (PWV) is a non-invasive technique to quantitate arterial stiffness or distensibility of arterial wall by measuring the pulse transit time and the distance traveled between two selected arterial sites. Some evidences have shown that PWV is a useful tool to assess cardiovascular risk and risk stratification. However, classic PWV measurement varies significantly between institutions and operators because of the technical difficulty resulted in low reproducibility. It also influences by changes in blood pressure. Cardio-ankle vascular index (CAVI), a new non-invasive method of detecting arterial stiffness, has been developed and reported to be less influenced by changes in blood pressure. The purposes of the present study were: 1) to evaluate the prevalent of atherosclerosis and arterial stiffness; 2) to study the atherosclerosis and its related risk factors including inflammatory marker; 3) to evaluate the correlation of CAVI and CIMT.

Material and Method

Patients

All of hemodialysis patients in renal unit at Siriraj hospital with age of more than 18-year and dialysis duration more than 4 weeks, during September 2005 to January 2006, were enrolled in this study. Exclusion criteria included patients with bilateral vascular access surgery, arrhythmia and permanent pacemaker. All patients were clinically well and stable at the time of investigation, with no concurrent infection or active inflammation. The ethic committee approved the protocol, and all patients gave written informed consent for the study.

The definition of atherosclerosis is at least one of the following criteria: 1) patients with definite diagnosis of coronary artery disease and/or ischemic stroke and/or peripheral vascular disease; 2) patients with at least grade 1 of carotid plaque. The patients were divided into 3 subgroups as established atherosclerotic vascular disease, subclinical atherosclerosis, and non-atherosclerosis. Established atherosclerotic vascular disease was defined as a history of cardiovascular diseases and verified by patient chart review and physical examination. Subclinical atherosclerosis was defined as a presence of atherosclerotic plaque without history of cardiovascular diseases. Non-atherosclerosis was defined as absence of atherosclerotic plaque and history of cardiovascular disease. Diabetes was determined from the medical record. In the present study, we used Cardio-Ankle Vascular Index (CAVI) as a method to assess arterial stiffness. CAVI was measured 10-20 minutes after hemodialysis.

Carotid intima-media thickness measurement

Carotid intima-media thickness (CIMT) was measured by a radiologist using the standard high-resolution B-mode ultrasonography (iU22, Phillips, Bothell, WA, 98041, USA). The carotid artery was scanned bilaterally on longitudinal and transverse planes. The three points of carotid artery, proximal common carotid artery, carotid bulb and internal carotid artery were scanned. CIMT is defined as the distance from the leading edge of the first echogenic line to that of the second line. The first line represents the lumen intima interface and the second line represents the collagen containing upper layer of adventitia. Carotid plaque is recognized and categorized to four grades. Grade 0 is plaque free patients, grade 1 is the fatty plaque, grade 2 is soft plaque that has hypo-echogenic density under the fatty plaque and grade 3 is hard plaque that contains calcification within the plaque.

Cardio Ankle Vascular Index

Cardio-Ankle Vascular Index (CAVI) was measured by using a VeSara VS-100 (Fukuda Denshi Company, Tokyo, Japan). Electrocardiographic electrodes are placed on both wrists, a microphone for detecting heart sounds is placed on the sternum, and cuffs are wrapped around both arms and above both ankles. Pulse wave velocity (PWV) is obtained by dividing the vascular length (from the aortic valve to the ankle) by the time for which the pulse wave is propagated from the aortic to ankles. Intraobserver reproducibility was assessed by random measurement in 55 patients between 2 to 4 weeks. Intraclass coefficient of variation was highly significant (r = 0.90; p < 0.001).
**Biochemical analysis**

Venous blood samples in the morning after an overnight fast was performed for complete blood count, blood urea nitrogen, creatinine, calcium, phosphorus, albumin, cholesterol, triglyceride, low density lipoprotein (LDL), high density lipoprotein (HDL) and intact parathyroid hormone (iPTH). Plasma total homocysteine level was immediately sent to the laboratory centre under the appropriate temperature. The institute laboratory centre used Fluorescence-Photo Immunoassorbent assay (FPIA) technique (Automate Imx, ABBOTT Laboratories; IL, USA). High sensitive CRP (hs CRP) was performed by CardioPhase (hs CRP reagent, Dade Behirs; Newark, DE 19714 USA).

**Statistical Analysis**

All continuous variables were presented in mean ± SD (standard variation). Non-continuous variables were reported in median (range). Spearman rank correlation (r) was used to test the association between CAVI and CIMT. ANOVA and Kruskal Wallis test were used to compare several parameters including CAVI and carotid plaque grading by ultrasonography. We performed t-test analysis to compare two samples with atherosclerosis. A p-value of < 0.05 was considered significant.

**Results**

**Clinical characteristics**

One hundred and five patients (mean age, 53 ± 15.5 years; 42 men, 63 women) were included in the present study. Mean duration of hemodialysis was 82 ± 59.5 months (Table 1). Dialysis adequacy was evaluated from Kt/V and nPCR, the mean values are 2.1 ± 0.48 and 1.1 ± 0.4 g/Kg/day, respectively. The major etiologies of ESRD are uncertain cause (25.7%) and chronic glomerulonephritis (25.7%) (Table 2). Seventeen patients (16.2%) were known to have diabetes. Twenty two patients (21%) had ischemic heart disease and 4 patients (3%) had peripheral vascular disease. Of the study population, 21 patients (20%) were smokers or ex-smokers and 35 patients (33%) were administrated statin.

**Risk factors and inflammatory marker in atherosclerosis and non-atherosclerosis**

Patient characteristics in atherosclerosis group (included both established atherosclerosis and subclinical atherosclerosis) and non-atherosclerosis group were shown in Table 3. Patients in atherosclerosis group tend to have higher mean age (57 ± 12.9 vs. 35 ± 12.1 years, p = 0.53) and body mass index (22 ± 3.9 vs. 19 ± 2.2 kg/m², p = 0.06). Other parameters (such as diabetes, smoking, duration of HD, Kt/V, nPCR, hemoglobin, albumin, Ca, P, Ca×P product and intact parathyroid hormone level) were not significantly different in both groups. All lipid profiles were not significantly different, except that triglyceride level in atherosclerosis group was higher than in non-atherosclerosis group; however, the level in both groups was in normal range. Compared with non-atherosclerosis group, high-sensitivity CRP level in atherosclerosis groups was significantly higher (6.5 ± 8.8 vs. 3.3 ± 3.5 mg/L, p = 0.03). Homocysteine level in both groups was not significantly different (24 ± 6.8 vs. 22 ± 6.9 μmol/L, p = 0.90) but the level in both groups was significantly higher than normal control.

**Carotid intima media thickness and prevalence of atherosclerosis**

Patients were subdivided into three groups as established atherosclerosis, subclinical atherosclerosis and non-atherosclerosis group. There were 29 (28%) patients in established atherosclerosis group.
with definite diagnosis of cardiovascular disease in 22 patients, peripheral vascular disease in 4 patients and ischemic stroke in 3 patients. Overall, there were 83 (79%) atherosclerosis patients that included both the established atherosclerosis and the subclinical atherosclerosis group. The mean CIMT (the proximal common carotid artery) of all patients was $1.1 \pm 0.7$ mm (range 0.4-3.7 mm). The maximum CIMT was significantly greater at the bulb area. Compared with the non-atherosclerosis group, CIMT in the atherosclerosis group was significantly higher (CIMT, $1.9 \pm 0.7$ vs. $0.7 \pm 0.17$ mm; p < 0.001). Mean CIMT was significantly different between 3 subgroups with the established atherosclerosis subgroup having the highest CIMT at all sites as shown in Table 4.

### Table 3. Patient characteristics in atherosclerosis and non-atherosclerosis group

<table>
<thead>
<tr>
<th></th>
<th>Atherosclerosis</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>No (n = 22)</td>
<td>Yes (n = 83)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>35 ± 12.1</td>
<td>57 ± 12.9</td>
</tr>
<tr>
<td>Sex (M:F)</td>
<td>38%: 62%</td>
<td>40%: 60%</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>19 ± 2.2</td>
<td>22 ± 3.9</td>
</tr>
<tr>
<td>Duration of HD (months)</td>
<td>70 ± 45.5</td>
<td>84 ± 62.5</td>
</tr>
<tr>
<td>HD time (median, range) (hrs/wk)</td>
<td>8 (8-12)</td>
<td>8 (8-14)</td>
</tr>
<tr>
<td>Dialysis type (low: high flux)</td>
<td>10%: 90%</td>
<td>2%: 98%</td>
</tr>
<tr>
<td>Kt/V</td>
<td>2.1 ± 0.4</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>nPCR (gm/kg/day)</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>Statin (yes: no)</td>
<td>9%: 91%</td>
<td>39%: 61%</td>
</tr>
<tr>
<td>Hemoglobin (gm/dL)</td>
<td>9.5 ± 1.9</td>
<td>9.5 ± 1.7</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>168 ± 40</td>
<td>171 ± 38</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>92 ± 35</td>
<td>128 ± 86</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>92 ± 35</td>
<td>98 ± 32</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>53 ± 19</td>
<td>46 ± 15</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>8.8 ± 0.6</td>
<td>9.3 ± 0.8</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>4.9 ± 2.0</td>
<td>5.2 ± 1.7</td>
</tr>
<tr>
<td>Ca x P (mg/dL)²</td>
<td>44 ± 18.6</td>
<td>48 ± 15.9</td>
</tr>
<tr>
<td>iPTH (median, range) (pg/mL)</td>
<td>167 (48-772)</td>
<td>262 (44-4,484)</td>
</tr>
<tr>
<td>Homocysteine (mmol/dL)</td>
<td>22 ± 6.9</td>
<td>24 ± 6.8</td>
</tr>
<tr>
<td>Hs CRP (mg/L)</td>
<td>3.3 ± 3.5</td>
<td>6.5 ± 8.8</td>
</tr>
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</table>

### Table 4. The carotid intima media thickness and atherosclerosis

<table>
<thead>
<tr>
<th>Intima-media Thickness (mm)</th>
<th>Atherosclerosis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n = 22)</td>
<td>Sub clinical (n = 55)</td>
</tr>
<tr>
<td>Common carotid</td>
<td>0.7 ± 0.17</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Bulb</td>
<td>0.7 ± 0.17</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>Internal Carotid</td>
<td>0.6 ± 0.12</td>
<td>1.0 ± 0.5</td>
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**Relationship between CMIT and Cardio Ankle Vascular Index**

Overall, there were 69 patients (66%) with abnormality of CAVI. Abnormality of arterial stiffness in atherosclerosis groups was 69% and in non-atherosclerosis group was 52.4% (p = 0.28). CAVI positively correlated with CMIT ($r = 0.44; p < 0.001$) as shown in Fig. 1. The magnitude of CAVI also correlated with the severity of atherosclerosis that determined by plaque grading. Carotid plaque is categorized to four grades. Grade 0 is plaque free patients, grade 1 is the fatty plaque, grade 2 is soft plaque, and grade 3 is...
plaque with calcification. CAVI was significantly greater in carotid plaque grade 2 (p = 0.007) and grade 3 groups (p = 0.02) compared with the grade 0 group, but there were not significantly different between grade 1, grade 2 and grade 3 groups as shown in Fig. 2. There was also no significant correlation was observed between CAVI and BMI, cholesterol, triglyceride, HDL, or LDL level.

Discussion
High prevalence of atherosclerosis (79%) and arterial stiffness (66%) in hemodialysis patients was observed in the present study. High homocysteine levels were found in both atherosclerosis and non-atherosclerosis group, but the levels were not significantly different in both group, whereas hs CRP was found to be higher in atherosclerosis group. Pathogenesis of atherosclerosis in hemodialysis patients involves multiple factors which included high blood pressure as a physical factors, hyperglycemia, dyslipidemia, intact PTH and hyperhomocysteinemia as metabolic factors, and reactive oxygen species, cytokine and CRP as inflammatory ones(12-16). In present study, we emphasized that atherosclerosis was associated with a low grade inflammation as indicated by high hs CRP level in atherosclerosis patients. Our result was comparable with a previous longitudinal study in 37 HD patients with mean hs CRP of 7.01 ± 16.06 mg/L(17). Current evidence from experimental and clinical studies showed that CRP may contribute to the pathogenesis of atherosclerosis through a variety of mechanisms(6). First, CRP has a potential pathological role in foam cell formation by binding to damaged cell and activates the complement system(6,18). Second, CRP binds to enzymatically degraded lipoproteins and localizes with these lipid particles in early human atherosclerosis lesions(6,19). Finally, CRP is a potent stimulator of tissue factor production by monocytes which may link to inflammation and coagulation(6,20).

Carotid intima-media thickness (CIMT) measured by high-resolution B-mode ultrasonography and CAVI are noninvasive tools that have been used to detect atherosclerosis in its subclinical phase. While CIMT is a marker of structural change of atherosclerosis, CAVI is used to detect arterial stiffness that reflects its functional consequences. We found that CIMT was significantly higher in the presence of atherosclerosis, and it increased progressively from non-atherosclerotic subgroup to established atherosclerosis subgroup. Several studies in hemodialysis patients have shown that CIMT and plaque occurrence in the carotid arteries are useful indicators of coronary atherosclerosis(21-23). Robert Ekart et al(24) found that patients with CIMT more than 1 mm had significant higher risk of death from cardiovascular disease, in comparison with patients with CIMT less than 1.0 mm. In our hemodialysis population, subclinical atherosclerosis subgroup had CIMT of ≥ 1 mm, which may indicate a higher risk of cardiovascular event. In the present study, we used CVI as a non-invasive method to detect arterial stiffness or arteriosclerosis since it has been reported to be more reproducible and less influenced by blood pressure. We found that CAVI positively

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**Fig. 1** The correlation of CAVI and maximum carotid intima-media thickness (r = 0.44, p < 0.001)

**Fig. 2** Relationship between CAVI and carotid grading obtained from B-mode ultrasonography. (grade 0 = no atherosclerosis, grade 1 = fibrous streak, grade 2 = soft plaque, grade 3 = calcified plaque)
correlated with CMIT and the severity of atherosclerosis or plaque grading assessed by ultrasound of carotid arteries. It appears that CAVI may be a useful tool to assess the cardiovascular risk. However, additional validation studies based on clinical outcome are needed. There are some pitfalls and concerns in the CAVI measurement. Since CAVI measure PWV from aorta to ankle, it therefore reflects stiffness of the aorta, femoral artery and tibial artery as a whole. The aorta is an elastic vessel, while the femoral artery and tibial artery are muscular vessels under the control nerves. Accordingly, vasospasm of these muscular vessels could affect the CAVI result. To avoid such effect, patients should be kept resting on a bed for a while before the test to minimize vascular stress. In patients with a severe arteriosclerotic femoral artery with ankle-brachial index (ABI) of less than 0.9, CAVI was apparently low. Thus, CAVI in such case should be regarded as a false value.

There are some limitations in the present study. First, the study population was not general HD population (diabetes patients are only 16%), this might be from selection bias. Since the CAVI measurement needs at least one arm for apply the pressure cuff and ECG monitor, the patients who had already had vascular surgery of both arms or arrhythmia (that found more frequently in diabetic patients) are excluded in the study. Second, abnormality of CAVI levels used in the present study was from manufacturer’s references, which derived from the Japanese population. It may not apply to Thai population. The reference for diagnosis of abnormal CAVI may need data from age and sex matched normal control in Thai population. Finally, clinical outcomes were not evaluated in the present study. Additional prospective study studies on clinical outcomes are required to verify the benefit and usefulness of CAVI.

In conclusion, the prevalence of atherosclerosis and arterial stiffness are high in HD population. High hs CRP levels are common and associated with atherosclerosis. CAVI may be a useful clinical marker for evaluating arterial stiffness and positively correlated with arterial intima-media thickness.

Potential conflicts of interest
None.

References


ปัจจัยพยากรณ์โรคหลอดเลือดแดงแข็งในผู้ป่วยฟอกเลือด

มาโนช รัตนสมปัตติกุล, กุลนุช ชาญชัยรุจิรา, ลีนา องอาจยุทธ, ทวี ชาญชัยรุจิรา

ภูมิหลัง: นอกจากปัจจัยเสี่ยงของการเกิดภาวะหลอดเลือดแดงแข็งที่พบในผู้ป่วยทั่วไป ผู้ป่วยฟอกเลือดพบว่ายังมีปัจจัยเสี่ยงเสริมอื่นๆเพิ่มขึ้น เช่น มีภาวะอักเสบเพิ่มขึ้นที่พบมากขึ้นและระดับ high-sensitive C-reactive protein (hsCRP)ในเลือดสูงขึ้น ระดับ homocysteine ในเลือดสูงขึ้น รวมถึงมีผลชีวภาพมั่นคงต่ำทำให้ความยืดหยุ่นของหลอดเลือดลดลง

วัตถุประสงค์: เพื่ศึกษาปัจจัยการเกิดภาวะหลอดเลือดแดงแข็ง และประเมินความยืดหยุ่นของหลอดเลือดแดงและปัจจัยเสี่ยงต่างๆในผู้ป่วยฟอกเลือด

วัสดุและวิธีการ: ผู้ป่วยฟอกเลือด 105 รายอายุเฉลี่ย 53 ± 15.5 ปี ระยะเวลาฟอกเลือดเฉลี่ย 82 ± 59.5 เดือน ทุกรายจะได้รับการตรวจ common carotid artery intima-media thicknesses (CIMT), atherosclerotic plaque โดย B-mode ultrasonography การวินิจฉัยภาวะหลอดเลือดแดงแข็งโดยจากประวัติในเวชระเบียน และจากการตรวจทางกายโดยละเอียด ผู้ป่วยทุกรายจะได้รับการตรวจ cardiovascular ankle index (CAVI) เพื่อใช้ในการประเมินการลดลงของความยืดหยุ่นของมันหลอดเลือดแดง และได้รับการตรวจเลือดเพื่อหาปัจจัยเสี่ยงต่างๆรวมถึงการตรวจ hsCRP และระดับ homocysteine

ผลการศึกษา: ผู้ป่วยฟอกเลือดพบภาวะหลอดเลือดแดงแข็งถึงร้อยละ 79 (วินิจฉัยจากประวัติโรคหลอดเลือดหัวใจและการตรวจทางกายโดยละเอียด atherosclerotic plaque) เมื่อเปรียบเทียบกับกลุ่มที่ไม่มีภาวะหลอดเลือดแดงแข็งพบว่ากลุ่มที่มีภาวะหลอดเลือดแดงแข็งมีค่าเฉลี่ยของ CIMT และ serum hsCRP สูงกว่ากลุ่ม (1.9 ± 0.8 มม. vs. 0.8 ± 0.6 มม., 0.001; 6.5 ± 8.3 มก./ล. vs. 3.3 ± 5.5 มก./ล., p = 0.03, ตามลำดับ) ในขณะที่ผลการตรวจภาวะหลอดเลือดแดงแข็งมีความสัมพันธ์ชัดเจนกับการตรวจวัด CAVI (69% vs. 54.5%, p = 0.28) ในทั้งสองกลุ่มไม่ต่างกัน อย่างไรก็ตามพบว่า CAVI มีความสัมพันธ์ชัดเจนกับภาวะหลอดเลือดแดงแข็งอย่างเดียว (69% vs. 54.5%, p = 0.28) ดังนั้นการตรวจภาวะหลอดเลือดแดงแข็งอยู่ในกลุ่มที่มี carotid plaque (soft plaque และ calcified plaque)

สรุป: ตัวชี้วัดภาวะหลอดเลือดแดงแข็งและการลดลงของความยืดหยุ่นของมันหลอดเลือดแดงพบว่ามีส่วนเกี่ยวข้องกับภาวะหลอดเลือดแดงแข็งอย่างชัดเจน ซึ่งมีความสัมพันธ์กับภาวะหลอดเลือดแดงแข็งตามลำดับในการตรวจภาวะหลอดเลือดแดงแข็งอยู่ในกลุ่มที่มี carotid plaque (soft plaque และ calcified plaque)

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