Original Article

Quantitative assessment of carotid atherosclerotic plaque: Initial clinical results using ShearWave™ Elastography

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Abstract: Objective: In this study, we assessed the stiffness of homogeneous carotid plaques using quantitative measurements with shear wave elastography (SWE) and to evaluate its reproducibility. Methods: 199 participants (105 males and 94 females, median age 66±11 years old) underwent gray scale and SWE imaging. Gray scale images’ features and assessments were recorded. SWE imaging was used to acquire cine-loop data and quantify Young’s modulus. The inter-class correlation coefficient (ICC) was calculated to quantify reproducibility of measurements made by the two observers across the three acquisitions. Results: A total of 277 homogeneous carotid plaques (140 hyperechoic and 137 hypoechoic carotid plaques) were performed. Inter-operator reproducibility of stiffness values measured showed an excellent reliability with an ICC of 0.95 for mean stiffness value, 0.92 for minimum stiffness value, and 0.92 for maximum stiffness value. In hyperechoic carotid plaques, the peak middle part showed higher stiffness value as compared to the proximal or distal shoulder part of the plaques (all P<0.05). In hypoechoic plaques, the stiffness values peaked at the basal middle site (all P<0.05, with respect to the top, the proximal and distal shoulder), and bottomed at the peak middle site (all P<0.05). Statistically significant differences with respect to the mean, minimum and maximum stiffness values measured at the proximal, distal shoulder, and the peak middle part of the plaques were found between hyperechoic and hypoechoic plaques (all P<0.01). In both types of plaques, hypertension complicated by hyperlipidemia (P<0.05) and hyperlipidemia alone (P<0.05) were related to lower stiffness values. Hypertension alone was also related with lower stiffness values in hypoechoic plaques (all P<0.05). Conclusions: SWE imaging is a new noninvasive, reproducible and reliable imaging method for the assessment of carotid plaque, which can reflect the stiffness of the plaque and provide additional information for the quantitative assessment of carotid plaques.

Keywords: Shear wave imaging, elastography, ultrasound, carotid, plaque

Introduction

Stroke is one of the main causes of death and disability worldwide, with carotid artery disease responsible for 15%-20% of all strokes [1]. Current clinical protocols rely on measurement of the percentage of stenosis to determine suitability of patients for surgery. Previous clinical trials have shown that carotid endarterectomy is beneficial for symptomatic patients with stenosis over 70% [2]. However, the benefits of carotid endarterectomy for moderate stenosis or asymptomatic patients remain uncertain. As plaques in asymptomatic patients and/or smaller plaques may also rupture and cause stroke, there has been considerable interest in developing improved methods of identifying the morphologic characteristics of unstable or vulnerable plaque in order to improve clinical risk stratification for predicting stroke.

Conventional high-frequency carotid ultrasonography (US) as a fast, inexpensive, tolerable modality has been widely used to quantify carotid plaques. It can delineate the presence of plaque, quantify plaque size, determine its echogenicity, and detect morphologic characteristics of plaque vulnerability such as ulceration,
a large lipid or necrotic core and a thin fibrous cap [3-6]. However, the existing studies mainly focus on plaque classification based on echogenicity and stress and there were still deficiency in measuring the stiffness of the plaques. Real-time shear wave elastography (SWE) exploits acoustic radiation force to generate shear wave propagation in tissue [7]. It enables the quantification of the Young’s Modulus (YM) from the measurement of shear wave velocity. Previous studies have reported potential clinical value of SWE a wide range of tissues including the breasts [8-10], liver [11, 12], kidney [13], muscles [14, 15], thyroid [16-18], prostate [19], cervical lymph node [20] and salivary glands [21], though there are very few studies have considered vascular applications [22-25]. SWE imaging of carotid plaques is particularly challenging as plaques are often small, of heterogeneous tissue composition, and subject to pulsatile tissue motion because of their hemodynamic environment.

The aim of our study was to measure stiffness values of carotid atherosclerotic plaques by using SWE and to discuss how stiffness measurements could be linked with plaque vulnerability to provide diagnostic information for patients’ management. To achieve this goal, we assessed the inter-operator reproducibility of SWE measurements, the difference in stiffness values between different types of carotid plaques, and the impact of underlying cardiovascular risk factors such as hypertension, hyperlipidemia, and hypertension complicated with hyperlipidemia on plaques stiffness values.

Materials and methods

Ethics statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Beijing Anzhen Hospital, Capital Medical University, China. Written informed consent was obtained from all subjects.

Participants

One hundred and ninety-nine participants with carotid atherosclerotic plaque who were referred to the Beijing Anzhen Hospital between May 2013 and November 2013 were included in the study. The participants consisted of 105 males and 94 females with a median age of 66±11 years. The inclusion was: (1) the thickness of plaque was more than 2 cm and length was more than 6cm; (2) the plaque was homogeneous (hypo- or hyperecho). The exclusion criteria was: (1) the thickness of plaque was less than 2 cm and length was less than 6cm; (2) the plaque was heterogeneous. Patients were defined as atherosclerosis if the plaque built up inside the arteries. Plaque was a sticky substance made up of fat, cholesterol, calcium, and other substances found in the blood. Over time, plaque narrowed the arteries or broken leading to cerebral thrombus. Plaques were classified as the thickness was more than 1.5 mm and local inside the arteries’ lumen. All of the participants underwent systematic investigations including routine laboratory tests. Hypertension was found on 50 patients, hyperlipidemia in 46, hypertension complicated with hyperlipidemia in 52, and none of these risk factors (normal group) in 51.

Conventional US and SWE

Ultrasound examinations were performed using Aixplorer® ultrafast ultrasound system (SuperSonic Imagine, Aix-en-Provence, France), which has the SWE™ imaging Mode. We used high-frequency linear array probe (frequencies: 2-10 MHz. In order to obtain a complete exposure of the carotid arteries, participants were placed in the supine position, with a pillow to ensure neck extension. The head was turned to 45° in the opposite direction of the ultrasound operator. Conventional gray scale imaging and color Doppler ultrasound were used to locate the plaque, measure its size and record its ultrasound characteristics. Hyperechogenicity and hypoechogenicity were assessed by comparing the echogenicity of the plaque with the echogenicity of the adventitia; plaques being more echogenic than the adventitia were classified as hyperechoic, whereas plaques being less echogenic than the adventitia were classified as hypoechoic. The subjective heterogeneity of the plaques was also assessed on the basis of the grey scale imaging.

The SWE mode was turned on at the precise location of maximum plaque thickness along the longitudinal axis. The stiffness information (Young’s modulus) was coded on a color scale ranging from 0 to 192 kPa. While in SWE Mode, the probe was held still for at least 3 seconds,
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Table 1. Types of carotid plaques and patients’ risk factors

<table>
<thead>
<tr>
<th>Types of plaques</th>
<th>Age</th>
<th>Sex</th>
<th>Hyperechoic plaques</th>
<th>Hypoechoic plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>No risk factor</td>
<td>64±9</td>
<td>Female 29</td>
<td>22</td>
<td>27</td>
</tr>
<tr>
<td>Hypertension alone</td>
<td>66±12</td>
<td>Male 25</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Hyperlipidemia alone</td>
<td>64±9</td>
<td>Female 16</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Hypertension + Hyperlipidemia</td>
<td>69±9</td>
<td>Male 24</td>
<td>28</td>
<td>34</td>
</tr>
<tr>
<td>TOTAL</td>
<td>66±11</td>
<td>Female 94</td>
<td>105</td>
<td>106</td>
</tr>
</tbody>
</table>

Figure 1. Images in a 70-year-old man with hypertension and arteriosclerosis. A. B-mode ultrasound (US) shows a hyperechoic plaque on the posterior wall of the right common carotid artery bifurcation. B. SWE exhibits that the color of hyperechoic plaque is green and yellow. C. Schematic map shows SWE measuring position of the plaque.

with neither pressure from the operator nor breathing from the participant. Once the colored SWE map was stabilized, it was saved to the system. Quantification of the plaque stiffness was performed with the integrated quantification tool called Q-Box™, set at a diameter of 1 mm. Stiffness measurements were performed at the proximal and distal shoulders, the peak middle site and/or the basal middle site. Because of the shadowing of the hyperechoic plaques, stiffness measurements were performed at 3 different sites in hyperechoic carotid plaque, 4 different sites in hypoechoic carotid plaques. Mean, minimum and maximum stiffness values were recorded automatically by the system. Blue, green, and red areas on the elastogram corresponded to low Young’s modulus (soft), and high Young’s modulus (stiff), respectively, up to a maximum of 300 kPa.

In the present study, the reproducibility of the SWE technique for characterizing a carotid artery plaque was assessed independently by two physicians (one expert and one novice) in a double-blind fashion. Each physician performed 47 stiffness measurements on 14 carotid plaques on the same day, as described above. Data were acquired by two observers, each repeating the acquisition on three occasions.

Statistical analysis

SPSS 13.0 (SPSS Inc, Chicago, IL) was used for data analysis. Qualitative data were expressed as percentage and were compared using Chi-square test. Normally distributed continuous data are presented as means ± standard deviation (SD) and were compared using t tests. Non-normally distributed continuous data are presented as the median and interquartile range, and were compared using the rank-sum tests. Inter-operator agreement was assessed using the intra-class correlation coefficients (ICC). Differences were considered statistically significant when P<0.05.

Results

Types of carotid plaques

Types of carotid plaques and patients’ characteristics are given in Table 1. Hyperechoic carotid plaques (n=140) and hypoechoic carotid plaques (n=137) was detected in 106 and 93 participants, respectively. The SWE appearance of hyperechoic plaques showed colors related to increased stiffness (green, yellow or
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Figure 2. Images in a normal 71-year-old woman. A. B-mode US shows a hypoechoic plaque on the posterior wall of the right common carotid artery bifurcation. B. SWE exhibits that the color of hypoechoic plaque is blue. C. Schematic map shows SWE measuring position of the plaque.

Table 2. Comparison of stiffness values (kPa) measured at 3 different sites in hyperechoic plaque

<table>
<thead>
<tr>
<th>Stiffness value</th>
<th>Mean (kPa)</th>
<th>Min (kPa)</th>
<th>Max (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal shoulder</td>
<td>51.8±16.3</td>
<td>42.2±16.9</td>
<td>60.8±18.6</td>
</tr>
<tr>
<td>Distal shoulder</td>
<td>50.8±19.3</td>
<td>41.6±17.8</td>
<td>60.2±21.1</td>
</tr>
<tr>
<td>Peak middle site</td>
<td>56.6±17.0</td>
<td>47.8±15.9</td>
<td>59.4 (50.6, 75.2)</td>
</tr>
</tbody>
</table>

p value

<table>
<thead>
<tr>
<th>Proximal shoulder</th>
<th>Distal shoulder</th>
<th>Peak middle site</th>
<th>Distal shoulder</th>
<th>Peak middle site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.613</td>
<td>0.024*</td>
<td>0.006*</td>
<td>0.002▲</td>
</tr>
<tr>
<td></td>
<td>0.784</td>
<td>0.137</td>
<td>0.065</td>
<td></td>
</tr>
</tbody>
</table>

Note. *: One-sample Kolmogorov-Smirnov Test is not normal and compute the function and special variables of SQRT. ▲There was statistically significant difference between proximal shoulder and peak middle site in mean and minimum stiffness values of plaques. ▲There was statistically significant difference between distal shoulder and peak middle site in mean and minimum stiffness values of plaques.

red, Figure 1). Appearance of these hypoechoic plaques in SWE Mode showed colors related to low stiffness values (mainly dark blue or blue, Figure 2). The length and thickness of hyperechoic plaques and hypoechoic plaque was 0.51 (0.4, 0.78) cm and 0.15 (0.13, 0.19) cm, and (1.28±0.62) cm and 0.23 (0.20, 0.28) cm respectively.

Inter-operator reproducibility

Inter-operator reproducibility of stiffness values measured at the same site on the same day showed excellent reliability with an ICC of 0.95 for mean stiffness value, 0.92 for minimum stiffness value and 0.92 for maximum stiffness value, respectively.

Stiffness differences within and between plaques

We found that the mean and the minimum stiffness values were significantly higher at the peak middle site (Table 2) (all P<0.05) as compared to stiffness values measured at the proximal shoulder and distal shoulder of hyperechoic plaques. Comparison of stiffness values among 4 different sites of hypoechoic plaques were given in Table 3. We found that the mean and the minimum stiffness values peaked at the basal middle site and bottomed at the peak middle site (all P<0.05). Compared to the other 3 sites of hypoechoic plaques, the peak middle site had a lower maximum stiffness values (P<0.05). In addition, we also compared the stiffness values between hyperechoic and hypoechoic carotid plaques, we found mean, minimum, maximum stiffness values measured at the proximal shoulder, the distal shoulder and at the peak middle site were significantly different between hyperechoic and hypoechoic plaques (all P<0.01). The hyperechoic plaques usually had higher stiff values than hypoechoic plaques.

Influence of cardiovascular risk factors on plaque stiffness

Hyperechoic plaques: No differences with respect to the stiffness values measured at any
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Table 3. Comparison of stiffness values (kPa) measured at the 4 different sites in hypoechoic plaques

<table>
<thead>
<tr>
<th>Site of Measurement</th>
<th>MEAN (kPa)</th>
<th>MIN (kPa)</th>
<th>MAX (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal shoulder</td>
<td>15.7±8.2</td>
<td>7.5 (3.0, 14.9)*</td>
<td>22.3±10.7</td>
</tr>
<tr>
<td>Distal shoulder</td>
<td>17.4±8.7</td>
<td>11.1±7.9</td>
<td>24.8±11.8</td>
</tr>
<tr>
<td>Basal middle site</td>
<td>19.9±9.2</td>
<td>14.5±8.5</td>
<td>25.8±12.0</td>
</tr>
<tr>
<td>Peak middle site</td>
<td>11.3±7.5</td>
<td>5.7 (2.0, 12.4)*</td>
<td>15.5±9.2</td>
</tr>
</tbody>
</table>

Table 4. The p values of stiffness values comparison in hyperechoic plaques among different etiologies

<table>
<thead>
<tr>
<th>Site of Measurement</th>
<th>MEAN (kPa)</th>
<th>MIN (kPa)</th>
<th>MAX (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal shoulder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal shoulder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak middle site</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *: One-sample Kolmogorov-Smirnov Test was not normal and compute the function and special variables of Lg10. ▪: Equal variances were not assumed in One-way ANOVA and compared with Tamhane’s T2. ▪: There was statistically significant difference between proximal shoulder and basal middle site in mean and minimum stiffness values of plaques. ▲: There was statistically significant difference between distal shoulder and basal middle site in mean and maximum stiffness values of plaques. ●: There was statistically significant difference between distal shoulder and peak middle site in mean and minimum stiffness values of plaques. ○: There was statistically significant difference between peak middle site and basal middle site in mean, minimum and maximum stiffness values of plaques.

Table 5. Comparison of stiffness values (kPa) measured at the 4 different sites in hyperechoic plaques

<table>
<thead>
<tr>
<th>Site of Measurement</th>
<th>MEAN (kPa)</th>
<th>MIN (kPa)</th>
<th>MAX (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal shoulder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal shoulder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak middle site</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. *: One-sample Kolmogorov-Smirnov Test was not normal and compute the function and special variables of Lg10. ▪: Equal variances were not assumed in One-way ANOVA and compared with Tamhane’s T2. ▪: There was statistically significant difference between normal ones and patients with hyperlipidemia in mean stiffness values at distal shoulder of plaques. ▲: There was statistically significant difference between patients with hypertension and hyperlipidemia in maximum stiffness values at proximal shoulder and minimum stiffness values at peak middle site of plaques. ○: There was statistically significant difference between patients with hypertension and hyperlipidemia in maximum stiffness values at proximal shoulder and minimum stiffness values at peak middle site of plaques.

Site of measurement were observed between normal patients and patients with hypertension alone (Table 4). Significantly lower stiffness values were observed at the distal shoulder of hyperechoic plaques in patients with concomitant hyperlipidemia alone as compared to normal patients (mean stiffness) or hypertensive patients (minimum stiffness). When compared to normal patients, patients with hypertension complicated with hyperlipidemia had a statistically significantly lower mean and minimum stiffness values at the proximal shoulder and the peak middle site of hyperechoic plaques. There was no statistically significant difference between patients with hypertension and hyperlipidemia in both and patients with hypertension or hyperlipidemia alone.

Hyperechoic plaques: No differences were observed between stiffness values measured in normal patients and patients with hypertension alone, at any site of measurement (Table 5). No differences between any groups of patients were observed for stiffness values measured at the peak middle site of hyperechoic plaques. Significantly lower values were observed at the proximal and the distal shoul-
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Table 5. The p values of stiffness values comparison in hypoechoic plaques among the different etiologies.

<table>
<thead>
<tr>
<th></th>
<th>Proximal shoulder</th>
<th>Distal shoulder</th>
<th>Basal middle site</th>
<th>Peak middle site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEAN</td>
<td>MIN</td>
<td>MAX</td>
<td>MEAN</td>
</tr>
<tr>
<td>Normal</td>
<td>0.414</td>
<td>0.063</td>
<td>0.999</td>
<td>0.606</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.164</td>
<td>0.560</td>
<td>0.663</td>
<td>0.272</td>
</tr>
<tr>
<td>Hypertension+ hyperlipidemia</td>
<td>0.031</td>
<td>*0.200</td>
<td>0.090</td>
<td>0.068</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0.027</td>
<td>*0.014</td>
<td>0.310</td>
<td>0.105</td>
</tr>
<tr>
<td>Hypertension+ hyperlipidemia</td>
<td>0.004</td>
<td>*0.003</td>
<td>0.016</td>
<td>0.022</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>0.341</td>
<td>0.435</td>
<td>0.805</td>
<td>0.386</td>
</tr>
</tbody>
</table>

Note. *: Equal variances were not assumed in One-way ANOVA and compared with Tamhane’s T2. ▲There was statistically significant difference between normal ones and patients with hyperlipidemia in mean stiffness values at basal middle site of plaques. ▪There was statistically significant difference between normal ones and patients with hypertension with hyperlipidemia in mean stiffness values at proximal shoulder of plaques. ●There was statistically significant difference between patients with hypertension and hyperlipidemia in mean and minimum stiffness values at proximal shoulder of plaques. ♦There was statistically significant difference between patients with hypertension and hypertension with hyperlipidemia in mean, minimum and maximum stiffness values at proximal shoulder and mean and minimum stiffness values at distal shoulder of plaques.

Discussion

To our knowledge, it had been demonstrated that the stiffness of carotid plaques was quantitatively assessed in vivo. Unlike other organs, the stiffness of the carotid plaque is changing along the cardiac cycle due to the deformation of the vessels walls. When the vessels have plaques, they could be regarded as thicker vessels wall. The feasibility of measuring the arterial stiffness using SWE has been demonstrated both in an in vitro arterial model and in an in vivo common carotid artery model of one volunteer [23]. Garrard et al. [22] reported the preoperative SWE using in carotid plaque and postoperative histological assessment were compared. Ramnarine KV et al [25] assessed inter- and intra-observer reproducibility of SWE measurements in vessel phantoms simulating soft and hard carotid plaque under steady and pulsatile flow conditions. They found that that SWE can quantify Young’s modulus of carotid plaque phantoms with good reproducibility. In the present study, we found that the reproducibility of stiffness measurements of carotid plaques in vivo was excellent with an ICC of no less than 0.92. Our findings were consistent with the previous report.

It is well known that hyperechoic plaques are mainly composed of calcium, which may promote a ‘stiffer’, more stable plaque. Whereas hypoechoic plaques rich in lipids, results in a “softer” and more unstable plaques. In the present study, we found that hyperechoic plaques had significantly higher stiffness values than in hypoechoic plaques. A previous study has demonstrated that shoulder region of the plaque had higher densities of mast cells and lipids responsible for plaque softness than the core region of the plaque [26]. Our results confirmed that the basal middle site of hypoechoic plaques had higher stiffness values than the proximal and the distal shoulders. Another interesting finding of our study was that the peak middle site of the plaques was the softest region as compared to other measurements sites. Previous histological and hemodynamic studies demonstrated that the plaque location that is the most prone to instability and rupture is the proximal shoulder [27, 28]. In this study, we found the statistically significant differences with respect to the stiffness values at the proximal site of hyperechoic and hypoechoic plaques in patients with hyperlipidemia and/or hypertension. These findings confirmed the above opinion.

It has long been recognized that conventional US imaging can discriminate between homogeneous and heterogeneous plaques in gray scale ultrasound images. In this study, we found differences with respect to the stiffness values among different measurement sites,
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regardless of the homogeneous hyperechoic plaques or homogeneous hypoechoic plaques. The findings suggest that SWE imaging had a higher sensitivity in the assessment of homogenous plaque’s stiffness as compared to the conventional gray scale ultrasound imaging. The “homogeneous” plaques in gray scale ultrasound images were heterogeneous in stiffness value of SWE images, particularly in the homogeneous hyperechoic plaques, due to softer regions on two shoulders in stiffness value of SWE.

Lipid-laden macrophage or foam cell is usually considered to be a hallmark of hyperlipidemia [29]. Atherosclerosis is a systemic vascular disease that can remain asymptomatic for several decades, but is associated with hypertension and hyperlipidemia. Hypertension complicated with hyperlipidemia will increase the risk of atherosclerosis. Our results show that the stiffness values of carotid plaques in normal patients were higher than in patients with hyperlipidemia alone or patients with concomitant hypertension and hyperlipidemia. Said differently, atherosclerotic plaques in patients with hypertension and hyperlipidemia or hyperlipidemia alone were softer than plaques in normal patients. The increased growth response of vascular smooth muscle is one of the characteristics of atherosclerosis in hypertension patients’ arteries [29]. The present study showed that hypertensive patients had a higher stiffness values compared to patients with hypertension and hyperlipidemia; however there were no significant differences can be found when compared to normal patients. Besides, we also found no statistically significant differences with respect to the stiffness values between patients with hyperlipidemia alone and patients with hypertension and hyperlipidemia or hypertensive patients. Further studies with a larger number of sample size or measurement positions are still needed to confirm our findings.

Conclusions

The evaluation of stiffness differences within a carotid artery plaque can be considered as additional information to the evaluation of the plaque stability, which already took into account several factors such as plaque size, plaque morphology, thickness of the fibrous cap, and forces on each part of the plaque. SWE is a noninvasive, reproducible and reliable imaging technique which could be as an addition of early detection of vulnerable plaques in the carotid artery.

Acknowledgements

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Disclosure of conflict of interest

None.

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