Original Article

Is the Carotid Intima-Media Thickness Really a Good Surrogate Marker of Atherosclerosis?

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Aim: Intima-media thickness (IMT) is considered a surrogate measurement of atherosclerosis but this is still under debate.

Methods: To evaluate the relationship between carotid IMT and atherosclerosis, postmortem specimens of the distal segments of the left common carotid artery (CCA) from 133 Korean men aged from 20 to 78 years were used for histopathology and computer-assisted morphometry. Blood lipids and atherosclerosis-associated collagen and elastin were quantitatively analyzed.

Results: Correlation coefficients of IMT were smaller than those of intima thickness but IMT was well associated with age (r=0.55, p<0.00001), atherosclerosis score (or grade, AS, r=0.73, p<0.00001), plaque area (PA, r=0.72, p<0.00001), total cholesterol (TC, r=0.69, p<0.00001), low-density lipoprotein cholesterol (LDL-c, r=0.72, p<0.00001) and triglyceride (TG, r=0.38, p<0.001). Coronary artery stenosis (CAS) and coronary calcification were also well associated with age (p<0.00001), IMT (p<0.005) and PA (p<0.00001). When IMT was thicker than 1 mm, the possibility of carotid atherosclerosis accompanied with CAS and coronary calcification, TC, LDL-c and TG was much higher (CAS with coronary calcification, p<0.005; TC, p<0.00001; LDL-c, p<0.00005; TG, p<0.00001). Collagen tended to increase while elastin tended to decrease as AS increased (p<0.005); collagen increased and elastin decreased (p<0.00001) when comparing plaque to the plaque-free area in the same segment.

Conclusion: These results support that the carotid IMT in association with TC, LDL-c and TG can be used as a good surrogate marker of atherosclerosis and predictor of coronary heart disease. Plaque formation may influence significant quantitative changes in collagen and elastin.

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Key words; Carotid atherosclerosis, Collagen, Elastin, Morphometry, Serum lipids

Introduction

Since the mid-1990s, carotid intima-media thickness (IMT) by B-mode ultrasound has increasingly been used for cardiovascular disease. It has also been used as a surrogate endpoint to monitor the efficacy of therapy against atherosclerosis in clinical trials; however, regression or slowed progression of carotid IMT induced by cardiovascular drug therapies did not reflect a reduction in cardiovascular events¹ and current consensus guidelines are not available regarding the site and how carotid IMT sampling should be performed. Therefore, it is still under debate whether carotid IMT can be used as an independent risk factor, especially in younger subjects with intermediate risk. A previous study emphasized that the simultaneous measurement of carotid and femoral IMT should be performed to improve risk stratification in patients with coronary heart diseases². Furthermore, it may be practically difficult to differentiate intima and media accurately by ultrasound, although recently developed devices have higher resolution³⁻⁵.

On the other hand, it has been well established that collagen and elastin are the most abundant extra-
carotid Intima-Media Thickness and Atherosclerosis

Table 1. Age-associated atherosclerosis score (AS), intima thickness (INT), media thickness (MED), intima-media thickness (IMT) and plaque area (PA) (mean ± SD)

| Age group | Age (year) | AS  | INT (mm) | MED (mm) | IMT (mm) | PA (mm²) |
|-----------|------------|-----|----------|----------|----------|----------|
| 20-24 (n=7) | 23±2       | 1.9±2.0 | 0.15±0.13 | 0.63±0.11 | 0.77±0.19 | 0.28±0.59 |
| 25-34 (n=26) | 30±3      | 1.9±1.0 | 0.15±0.13 | 0.66±0.17 | 0.81±0.21 | 0.46±1.55 |
| 35-44 (n=32) | 40±3      | 2.8±1.0 | 0.26±0.14 | 0.73±0.16 | 0.98±0.18 | 1.71±4.67 |
| 45-54 (n=46) | 49±3      | 3.4±1.0 | 0.38±0.26 | 0.68±0.26 | 1.07±0.25 | 4.61±8.46 |
| 55-64 (n=12) | 60±2      | 4.1±1.5 | 0.52±0.37 | 0.78±0.13 | 1.30±0.42 | 9.16±9.40 |
| 65- (n=10) | 70±5      | 4.4±1.1 | 0.62±0.41 | 0.65±0.18 | 1.27±0.39 | 12.00±13.72 |
| Trend test |            |       |          |          |          |          |
| r=0.98 | r=0.98 | r=0.39 | r=0.92 | r=0.94 |          |          |
| p<0.0005 | p<0.0005 | NS    | p<0.001 | p<0.005 |          |          |

Materials and Methods

Preparation of Materials

The study protocol was approved by the ethics committees of the institutes. Korean men who had died from external causes (including suicide, homicide, falls and traffic accidents) during 2005-2009 were enrolled. The whole length of either CCA was carefully removed within 24 h after death (Table 1). Each postmortem specimen was perfused with 10% buffered formalin in a bath overnight to prevent warping the vessel contour and divided into 3 equal segments, i.e., proximal, middle and distal (including the carotid bulb). The histological sections were stained with hematoxylin and eosin for general microscopic examination and with Verhoeff-Van Gieson for computed morphometry, according to a previous study. Left main coronary artery was macroscopically examined for stenosis (coronary artery stenosis, CAS) and calcification. Reduction of the diameter of the coronary artery by less than 50% was regarded as moderate and more than 50% as severe.

Classification of Atherosclerosis and Morphometry

Tissue sections were histopathologically graded for scoring (atherosclerosis score, AS) according to the guidelines of the American Heart Association as follows: 1 (adaptive intimal thickening with isolated macrophage or foam cells), 2 (fatty streak with multiple foam cells but still no evidence of tissue damage), 3 (preatheroma with multiple foam cell layers and deposits of extracellular lipids; evidence of tissue damage), 4 (atheroma with confluent and disruptive core of extracellular lipid), 5 (fibroatheroma with multiple lipid cores, calcification and fibrous cap), and 6 (complicated plaque; extensive calcification and disruption of the lumen surface) (Fig. 1B-1D).

INT, MED and PA were measured as previously described. In brief, the circumferences of the lumen (endothelium), internal elastic membrane, and external elastic membrane were measured, respectively, and INT, MED and IMT were calculated by the formula for the circumference: INT=(B-A)/2π, MED=(C-
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containing elastin was stored at \(-20^\circ\text{C}\) until assay. Total collagen and elastin were assayed by dye precipitation with commercial kits (Sircol Collagen and Fas-tin Elastin Assay; Biocolor Ltd, Carrickfergus, UK).

Blood samples were taken from only 82 subjects (since the remainder was not suitable for assay because of excessive blood loss). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglyceride (TG) were assayed using enzyme precipitation (Wako Diagnostics, Osaka, Japan) and mass spectrometry.

Statistics

Overall, 30.8% of the distal, 11.3% of the middle and 14.3% of the proximal segment of the left CCA, and 30.1% of the distal, 12.8% of the middle and 13.5% of the proximal segment of the right CCA had AS 4-6 (clinical) atherosclerosis; 41.4% of the distal, 18.0% of the middle and 21.1% of the proximal segment of the left CCA, and 35.3% of the distal, 18.8% of the middle and 17.3% of the proximal segment of the right CCA had various sizes of plaque(s) with or without calcification. These findings confirm that the distal segment is a common site of CCA ath-

Analysis of Collagen, Elastin and Serum Lipids

AS-associated \((n = 7\) each) and plaque-associated \((n = 42)\) collagen and elastin were quantified. In brief, soluble collagen was extracted according to the following modification. Tissue was minced, treated in 0.5 M acetic acid containing pepsin (1 mg/mL) at 4°C for 30 min and homogenized. Homogenate was incubated for 24 h at 4°C on an orbital shaker. After centrifuga-
tion, a tissue pellet was suspended in 0.5 M acetic acid containing pepsin for 24 h at 4°C. The final supernatant containing collagen was precipitated by adding NaCl to a final concentration of 5% at 4°C. Collagen pellet was collected by centrifugation and subsequently dissolved in 0.5 M acetic acid. Water-soluble elastin was extracted as follows. Homogenate was treated with 0.25 M oxalic acid and incubated at 96°C for 60 min. After centrifugation, the tissue pellet was suspended in 0.25 M oxalic acid 2 times. Supernatant containing elastin was stored at \(-20^\circ\text{C}\) until assay. Total collagen and elastin were assayed by dye precipitation with commercial kits (Sircol Collagen and Fastin Elastin Assay; Biocolor Ltd, Carrickfergus, UK).

Blood samples were taken from only 82 subjects (since the remainder was not suitable for assay because of excessive blood loss). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglyceride (TG) were assayed using enzyme precipitation (Wako Diagnostics, Osaka, Japan) and mass spectrometry.

Fig. 1. Schematic illustration of a cross section (1A) and microphotographs of common carotid artery with atherosclerosis (1B-1D, Verhoeff-van Gieson staining) and medial elastic fibers (1E-1F, Verhoeff-van Gieson staining). Atherosclerosis score (AS) 2 section shows adaptive intimal thickness with multiple foam cells (arrow, 1B); AS 4 section shows extracellular lipid accumulation within the intima (1C); and AS 6 section shows predominant calcification (1D). On the other hand, elastic fibers still show waviness in subclinical (AS 3) atherosclerosis (1E) but degradation and loss of waviness appear as atherosclerosis progresses (1F, AS 6). Abbreviations: A, endothelium; B, internal elastic membrane; C, external elastic membrane; P, plaque; cal, calcification; fc, fibrous cap; l, lumen; lc, lipid core. Scale bar=0.5 mm (1B-1C), 1mm (1D) and 0.1 mm (1E-1F).
Data are expressed as the mean ± SD and compared using the paired t-test. Statistical differences among the age, INT, MED, IMT, AS, PA and serum lipids were analyzed by ANOVA. If a difference was found, correlation coefficients were computed and lin-
carotid regression analyses were performed. The prevalence of plaque, CAS and coronary calcification was analyzed by Chi-square test. AS-associated changes of collagen and elastin were analyzed by a trend test. $P < 0.05$ was considered significant.

**Results**

**General Findings**

By macroscopy, fatty streaks were found focally but dispersed along the vessel wall in association with raised lesions with aging. No intact histology was found. They showed various degrees of atherosclerotic changes but did not include surface defects, hema-
toma or thrombosis. Moderate to severe CAS was accompanied in 129 subjects (42.4%; 19 moderate and 53 severe); coronary artery calcification was found in 159 subjects (44.4%; 19 moderate and 56 severe) (Fig. 1E-1F). In severe CAS, the mean ages of subjects without and with calcification were $47 \pm 7$ and $56 \pm 11$, respectively ($p < 0.0001$). These findings suggest that older subjects are liable to have CAS and coronary artery calcification (CAS with calcification, $\chi^2 = 40$, $p < 0.0001$); however, surface defects, plaque rupture and hemorrhage were not found.

**Atherosclerosis Score (AS), Wall Thickness, Plaque Area (PA) and coronary Artery Stenosis (CAS)**

By light microscopy, 55 out of 133 (41.4%) subjects had CCA plaques. Secondary changes were found in the media: the elastic fibers were frayed and thinned with loss of waviness in accordance with increasing age and AS (Fig. 1E-1F).

As shown in Table 1, age-associated AS, INT, IMT and PA tended to increase but MED did not. INT and IMT increased with age ($\text{INT}, r = 0.56, p < 0.00001$; $\text{IMT}, r = 0.55, p < 0.00001$); AS and PA increased with age, INT and IMT (Fig. 2). By light microscopy, 55 out of 133 (41.4%) subjects (42.4%; 19 moderate and 53 severe); coronary artery calcification was found in 159 subjects (44.4%; 19 moderate and 56 severe) (Fig. 1E-1F). In severe CAS, the mean ages of subjects without and with calcification were $47 \pm 7$ and $56 \pm 11$, respectively ($p < 0.0001$). These findings suggest that older subjects are liable to have CAS and coronary artery calcification (CAS with calcification, $\chi^2 = 40$, $p < 0.0001$); however, surface defects, plaque rupture and hemorrhage were not found.

**Table 2.** Atherosclerosis score (AS, or grade)-associated changes of age, intima thickness (INT), media thickness (MED), intima-media thickness (IMT) and plaque area (PA) (mean ± SD)

| AS         | Age (year) | INT (mm) | MED (mm) | IMT (mm) | PA (mm²) |
|------------|------------|----------|----------|----------|----------|
| 1 ($n = 19$) | 30 ± 6     | 0.07 ± 0.03 | 0.63 ± 0.14 | 0.71 ± 0.15 |          |
| 2 ($n = 25$) | 40 ± 11    | 0.14 ± 0.05 | 0.75 ± 0.11 | 0.89 ± 0.12 |          |
| 3 ($n = 48$) | 45 ± 11    | 0.26 ± 0.09 | 0.72 ± 0.18 | 0.99 ± 0.20 | 0.25 ± 0.55 |
| 4 ($n = 21$) | 47 ± 11    | 0.47 ± 0.16 | 0.66 ± 0.12 | 1.13 ± 0.22 | 4.63 ± 2.83 |
| 5 ($n = 15$) | 57 ± 11    | 0.50 ± 0.05 | 0.62 ± 0.14 | 1.32 ± 0.26 | 17.75 ± 6.28 |
| 6 ($n = 5$)  | 61 ± 11    | 1.08 ± 0.38 | 0.66 ± 0.21 | 1.75 ± 0.37 | 26.85 ± 12.61 |
| Trend test  | $r = 0.98$ | $r = 0.97$ | $r = -0.31$ | $r = 0.96$ | $r = 0.90$ |
|             | $p < 0.0005$ | $p < 0.005$ | NS        | $p < 0.005$ | $p < 0.01$ |

When comparing subjects with and without CAS, age, INT, IMT, prevalence of coronary calcification, AS and PA were significantly different (Fig. 4). All parameters were significantly higher in the subjects with severe CAS. These data indicate that CAS may...
be severe when aged subjects have thicker carotid IMT with larger plaques.

Collagen and Elastin
Collagen in AS 1 and AS 6 was $0.19 \pm 0.06$ and $0.63 \pm 0.14 \, \mu g/mg$ tissue ($p<0.001$), and elastin in AS 1 and AS 6 was $142.46 \pm 9.98$ and $89.49 \pm 9.31 \, \mu g/mg$ tissue ($p<0.005$), respectively. As shown in Fig. 5A, collagen tended to increase and elastin to decrease as atherosclerosis progressed (collagen, $r=$
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The elastic artery is not absolutely round and the vessel wall structure cannot be clearly differentiated, whereas high resolution ultrasound is non-invasive and provides highly reproducible measurements of the carotid artery. Therefore, carotid IMT has been used widely as a surrogate of atherosclerosis because it has consistently been shown to be associated with cardiovascular risks and the prevalence of coronary heart disease\(^{15,16}\). However, Finn et al.\(^ {17}\) recently described that carotid IMT is unable to distinguish lesions with a necrotic core from those with a fibrous cap and collagen-rich content.

### Discussion

Carotid IMT measured by B-mode ultrasound may be over- or under-estimated in accordance with the insonation angle because the outline of the large elastic artery is not absolutely round and the vessel wall structure cannot be clearly differentiated, whereas high resolution ultrasound is non-invasive and provides highly reproducible measurements of the carotid artery. Therefore, carotid IMT has been used widely as a surrogate of atherosclerosis because it has consistently been shown to be associated with cardiovascular risks and the prevalence of coronary heart disease\(^ {15,16}\). However, Finn et al.\(^ {17}\) recently described that carotid IMT is unable to distinguish lesions with a necrotic core from those with a fibrous cap and collagen-rich content.

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**Fig. 4.** Differences between subjects with coronary artery stenosis (CAS), none \((n=74)\), moderate \((n=19)\) and severe CAS \((n=40)\) in case. All parameters were significantly higher in the subjects with severe CAS. *, **analyzed by ANOVA; *by Chi-square test; *between none and moderate CAS; **between moderate and severe CAS.
significant associations were found between the change in IMT and the change in total cholesterol but not that in TG. Interestingly as shown in Table 3 and Fig. 4, AS, PA, the prevalence of CAS and coronary calcification was well associated with IMT. When IMT was thicker than 1 mm, AS, PA, and the prevalence of severe CAS, LDL-c and TG were much higher than in subjects with IMT thinner than 1 mm. IMT was well related with AS when IMT was thinner than 1 mm and was well related with AS and PA when IMT was thicker than 1 mm. In addition, INT, IMT, AS, PA and the prevalence of coronary calcification were significantly higher when CAS was severe (compared to subjects without CAS or moderate CAS). These data suggest that if the IMT was thicker than 1 mm, the possibility of carotid atherosclerosis accompanied with CAS and calcification becomes significantly higher and IMT may be closely associated with TC, LDL-c and TG; if IMT is thinner than 1 mm, CAS may not be severe and coronary calcification is rare. Our findings are core and, moreover, in most cases measurements of PA or plaque volume are generally considered better predictors of an inflammatory process consistent with atherosclerotic disease rather than IMT. In addition, Inaba et al. concluded that the ultrasound assessment of carotid plaque (plaque thickness), compared with that of carotid IMT, had higher diagnostic accuracy for the prediction of future coronary artery disease events from their meta-analysis of population-based studies. We agree with the results of these previous studies in part, but meta-analysis studies have certain limitations because of significant differences in the methodological quality, publication bias or different protocols of carotid ultrasound for assessment of IMT or plaque. However, as shown in Table 1-2 and Fig. 2, IMT is well associated with age, AS and PA, although correlation coefficients of IMT are relatively smaller than those of INT. IMT was also well correlated with TC, LDL-c and TG (Fig. 3), as previously described. These findings are consistent with previous studies, however, the debate continues.

Fig. 5. Atherosclerosis score (AS)-related changes of collagen and elastin (A) and comparison of collagen and elastin between the plaque area [plaque (+)] and plaque-free [plaque (-)] area in the same segment (mean ± SD) (B). Collagen tends to increase (r = 0.96, p < 0.005) and elastin to decrease (r = −0.95, p < 0.005) as AS increases. In addition, there are significant differences in collagen and elastin between plaque (+) and plaque (−).
consistent with those of previous studies using ultrasound. These findings also mean that calcification indicates more severe stenosis than a necrotic core, as in a previous study. Histological sections can give exact, accurate and reproducible measurements with few errors but it is not applicable in practice. Carotid IMT mainly represents medial hypertrophy or hypertensive thickening of smooth muscles in the media, whereas intimal thickening is the first manifestation of atherosclerosis; therefore, measurement of IMT is practical and gives meaningful information for the prediction of coronary heart disease risk. For these reasons, the IMT may be used as a good surrogate marker of atherosclerosis and as a predictor of coronary heart disease.

It is widely accepted that the first recognized lesion of atherosclerosis is a fatty streak containing intracellular and extracellular lipids and foam cells of macrophage and/or smooth muscle cell (SMC) origin. This is accompanied by lipid pools located in the deeper intima in area rich in proteoglycan, a component of the ECM, with a generalized loss of SMCs and speckled calcification; however, there are recognized differences in the distribution of atherosclerosis relative to abundant ECM protein including collagen and elastin. In our study, collagen and elastin showed quantitative changes from AS. Collagen in the plaque and elastin. In our study, collagen and elastin showed relative to abundant ECM protein including collagen and elastin in atherosclerosis is still controversial. Specific collagens may be involved in the modulation of cellular responses via specific receptors and signaling pathways and in the migration of the cells to the site of lesion growth; collagens may also be involved in the calcification of lesions, and glycation of collagen may promote atherogenesis. In addition, elastin is a critical regulatory molecule that controls the phenotypic modulation, proliferation and migration of SMCs; however, elastin expression and SMC proliferation are coupled inversely. Taken together with our and previous studies, this implies that both quantitative and qualitative changes in collagen and elastin may occur as atherosclerosis progresses.

It has been shown that the distal (including carotid bulb) segment is the most vulnerable site of CCA atherosclerosis as in this study. The prevalence rates of clinical atherosclerosis (AS 4-6) and plaque formation were approximately 2-3-fold higher in the distal segment than in the other segments. It has been well recognized that the CCA bifurcation, proximal internal carotid artery as well as carotid siphon (tortuous segment of internal carotid artery) are the most frequent sites of carotid atherosclerosis; however, the reason is still uncertain. Evidence shows that wall shear stress (WSS) and local hemodynamic load are important for endothelial cell function and gene expression. Atherosclerosis might be closely related to fluid dynamics, since atherosclerotic plaques preferentially originate in areas of disturbed flow, such as regions of curvature, bifurcation and branching of vessels. Gnasso et al. have found that IMT is inversely related to WSS values in the CCA. When atherosclerosis progresses, WSS of the distal segment may be significantly lowered, and low WSS can cause arterial wall damage from an increased fluid residence time and transportation of atherogenic particles, the inference of endothelial metabolism or increased adhesion of platelets and macrophages to the arterial wall. These events may result in local inflammation and increased local production of mitogenic substances, and finally cause the formation of plaque and thickening of the intima-media complex.

In this study, all subjects showed variable degrees of atherosclerotic changes, including intimal thickening, atheroma with or without calcification or fibrosis and medial degeneration, with aging. Age was well related with AS and PA, end-point parameters of atherosclerotic progression (Fig. 2). It has been well established that atherosclerosis is primarily a generalized disease of vascular aging. It does not manifest clinical signs until middle age, in which it is characterized by a focal lipid-rich deposit of atheroma with some lesions becoming thick, fibrotic and calcified. Atheroma may involve the formation of plaques, which results in thickening and hardening of the arterial wall. Abundant experimental and clinical studies have
found that aging is associated with chronic low-grade inflammation\(^4\), which predisposes the vasculature to the development of atherosclerosis. The inflammatory process can promote oxidative stress; therefore, a vigorous metabolic cycle can influence both age-related vascular oxidative stress and inflammation\(^4\).

There are limitations of this study. Measurements of carotid wall thickness and plaque area using tissue sections (microslides) may be superior in accuracy and reproducibility to B-mode ultrasound or magnetic resonance; however, the definitive weakness of the histological method is difficulties in the collection of fresh specimens and limitation of specimen numbers. Accordingly, the total number of subjects was relatively small, particularly those aged under 24 and over 65 in this study. In addition, traceable recent data on the blood chemistry of the subjects were too few to be considered statistically; therefore, cardiovascular risks including smoking, alcohol intake, obesity, diabetes and others could not be considered. Furthermore, chemical analysis of the blood was performed in only 61.7% of all subjects (82 out of 133), since some subjects had massive blood loss and circulatory collapse. Finally, internal carotid arteries were not investigated and the coronary arteries were examined only macroscopically. Some coronary arteries, particularly from older subjects with severe stenosis and calcification, were not suitable for histology because they had undergone angioplasty and stent replacement. These limitations may not compatibly explain the results of this study.

In conclusion, carotid IMT may be a good surrogate of carotid atherosclerosis and coronary vascular events. Blood lipids, particularly TC, LDL-c and TG, may contribute to the thickening of IMT, and quantitative changes of ECM proteins (collagen and elastin) may mutually affect the progression of atherosclerosis and plaque formation.

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