Functional carotid ultrasound markers of subclinical atherosclerosis in men with cardiovascular risk factors

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Abstract

Aim: The aim of this study was to compare the most commonly used ultrasound parameters of elasticity and stiffness of the arterial wall in detection of carotid subclinical atherosclerosis (SA) in vascular healthy men with one of the most prominent cardiovascular risk factors (CVRF).

Methods: Total of 120 vascular healthy men between 33 and 59 years of age ($\bar{x}$(SD) = 49.7(6.93)) were allocated into hypertension, diabetes, smokers and control group of respondents without CVRF. Ultrasound examination of carotid arteries was used to measure intima-media thickness and maximal and minimal lumen diameter. Along with the blood pressure of all the respondents, the following markers of elasticity/stiffness of arterial wall were calculated: distensibility coefficient (DC), compliance coefficient (CC), Young's elasticity modulus (YEM) and β stiffness index (β).

Results: DC, CC and β indicated significantly lower elasticity and higher stiffness of arterial wall in hypertension and diabetic groups of respondents without CVRF (multiple comparison, $p<0.001$). There were significant changes in functional characteristics of carotid arteries present between respondents-smokers and the control group detected by DC and β (multiple comparison, $p<0.05$). There were 94 respondents (78%) with subclinical carotid atherosclerosis criteria. ROC analysis indicated that DC, β and CC (AUC 0.811, 0.810, 0.799) are good markers for SA.

Conclusions: In conclusion, it is possible to use an ultrasound in detection of changes of elasticity/stiffness in arterial wall caused by the major CVRF in vascular healthy men. DC and β seem to be the best indicators of the presence/absence of subclinical carotid atherosclerosis.

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Introduction

Subclinical atherosclerosis (SA) is a latent precursor of cardiovascular disease, but the complete prevalence remains unfamiliar (1). There is a high level of clinical interest in detecting vascular changes by non-invasive methods, as it could contribute to identifying individuals at high risk of cardiovascular incident, who might also be in need of more aggressive approach to risk factors (2). SA indicates the cumulative effect of all known and unknown risk factors together, thus providing better data than the data evaluation of only known risk factors (3). Different types of ultrasound tests of subclinical atherosclerosis bear different prognostic features. There was an independent correlation recognized between the changes of the elastic properties of the carotid arteries and cardiovascular outcome (4) as well as ischemic stroke (5). The aim of this study was to compare the most commonly used ultrasound parameters of elasticity and stiffness of the arterial wall in the detection of carotid SA in vascular healthy men with one of the most prominent cardiovascular risk factors (CVRF).

Patients and methods

The research was conducted from October 2011 to June 2014 and it included 120 men from eastern Croatia (Osijek and 40 km radius) between 33 and 59 years of age ($\bar{x}$(SD)=49.7 (6.93)), who had never had a vascular disease. The respondents were patients referred to diagnostic procedures or specialist examinations at the Clinical Hospital Center Osijek; they were diabetic patients hospitalized at the Department of Endocrinology at the Clinical Hospital Center Osijek and volunteers (who were mostly part of the control group). Three risk factor criteria including hypertension, diabetes type II and smoking were indicators in forming three groups; the risk factors being present over the last five years. The control group consisted of respondents without CVRF.

To eliminate the possibility of arterial hypertension (according to the American Heart Association), each respondent's blood pressure was set at <140/<90 (6). To exclude the diabetes, fasting blood glucose (FBG) level was set at <7.0 mmol/L and the value of glycated hemoglobin (HbA1c) was set at ≤6.5% (according to the American Diabetes Association) (7). Respondents smokers were men who smoked for longer than five years and more than 10 cigarettes a day on average, while non-smokers were identified as men who have not actively been smoking for the last five years, and in time prior to that no more than 10 cigarettes per day in a period of one year (8). Excluding factors were the presence of another CVRF, i.e. anamnestic or medical history data of arterial disease, both present or past (i.e. myocardial infarction, angina pectoris, ischemic cardiomyopathy, cerebral infarction, transient cerebral ischemic attack, peripheral arterial disease, abdominal aortic aneurysm and cardiac arrhythmia) or previous therapeutic procedure of arterial disease (i.e. percutaneous transluminal angioplasty, endarterectomy, intra-arterial stent implantation, arterial bypass).

The carotid arteries ultrasound scan of all respondents has been performed in B-mode by the same examiner (KB), by linear probe 7.5 MHz on Aloka Pro Sound 5000, Tokyo, Japan. Carotid atherosclerotic plaque was defined by *Mannheim*carotid intima-media thickness and plaque*consensus (*9, 10) and it was observed in the segment including 30 mm distal common carotid arteries (CCA), carotid bulb and proximal 20 mm internal carotid arteries on both sides of the neck. Intima-media thickness was measured by the standard protocol on the far wall of the distal segment of both CCA, 10 mm proximal of the starting point of bifurcation (9), with multiangle insonation (anterolateral, lateral and posterolateral) (11), in diastolic blood flow phase with maximum magnification of the image. The mean of carotid intima-media thickness (cIMT) is set for the right and left side for each respondent. Ultrasound criteria for defining SA were the presence of atherosclerotic plaque on carotid arteries and/or cIMT values ≥75th percentile of the control group (11, 12). The measurement of the minimal diastolic diameter of CCA between two lines of adventitia-media was performed 10mm proximal of bifurcation (13), three times on each side, and the lowest value was taken as the referential one. The maximum systolic and minimum diastolic lumen diameter of common carotid arteries in intima-media area of close and far arterial wall was measured in maximum systolic expansion of the artery and minimal lumen width, during the relaxation of the artery at the end of diastole (14). Measuring was performed four or five times on each artery, with the maximum image magnification, along with the examination of previously recorded and stored images over three to five cardiac cycles (15). The results of the maximum and minimum diameter were an average of two maximal systolic and an average of two minimal diastolic lumen diameters for each respondent.

Just before the first and during the carotid arteries diameter measurement, the blood pressure was also measured on the upper arm side that corresponded to the test of the current carotid artery. It was taken by automatic electronic sphygmomanometer Omron M6 Comfort, Kyoto, Japan, which was validated according to the international protocol of the European Society of Hypertension (16). The conversion factor of the measured blood pressure from mmHg to kPa is 0.13.

Ultrasound elasticity markers of the carotid wall - distensibility coefficient (DC) and compliance coefficient (CC) were calculated according to the following:

$$DC = \frac{\left(\frac{2\Delta D}{D_D}\right)}{\Delta P}[kPa^{-1}] \text{ (15, 17),}$$

$$CC = \frac{\pi D_D \Delta D}{2\Delta P}[m^2 kPa^{-1}] \text{ (17, 18).}$$

Stiffness markers of arterial wall – Young's elasticity modulus (YEM) and beta stiffness index (β) were calculated according to the following:

$YEM = \frac{E_P D_D}{2CIMT}[kPa]$, where $E_P = \frac{\Delta P D_D}{\Delta D}[kPa]$ (18, 19) and

$E_P = \frac{\Delta P D_D}{\Delta D}[kPa]$ where $STRAIN = \frac{\Delta D}{D_D}[\%]$ (18,20).

In the above expressions, D_D is CCA lumen diameter at the end of diastole, ΔD is pulsatile diameter change, i.e. the difference in systolic and diastolic diameter of the CCA lumen, ΔP is pulse pressure, i.e. the difference in systolic and diastolic pressure readings, E_P is Peterson's (elastic) modulus, ln is natural logarithm, and strain is the change in lumen diameter of the CCA during cardiac cycle (expressed in percentage).

Total cholesterol was quantitatively measured by enzymatic spectrophotometric method, while hsCRP was measured by turbidimetry (Beckman Coulter AU 680 analyzer). Blood glucose was measured quantitatively by UV enzymatic test (hexokinase method) on Beckman Coulter AU 680 analyzer. HbA1c was quantitatively measured by turbidimetric inhibition immunoassay (TINIA) principle on Dimension clinical chemistry system RxL, Siemens.

Statistical analysis

Wilcoxon's signed-rank test, when applied to 17 respondents, did not indicate statistically significant difference in the first and second (performed within three months) ultrasound parameters of the following: cIMT, diameter, ΔD. Furthermore, statistically significant difference was not found in the number of subjects with or without carotid atherosclerotic plaque.

Figure 1. *Distensibility coefficient of the respondents with the cardiovascular risk factor.*

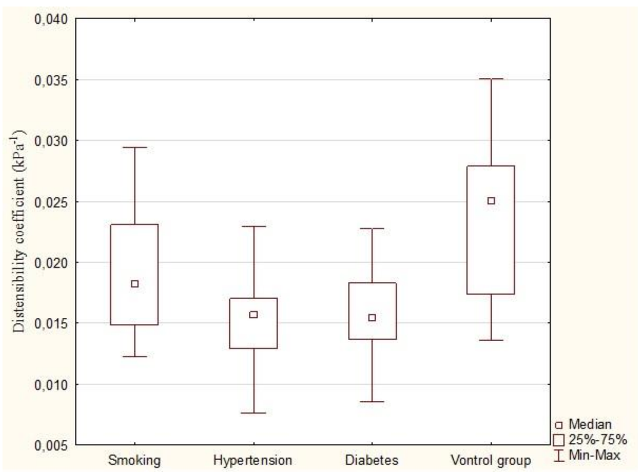

Figure 2. *ROC curve of functional ultrasound tests of the carotid arteries with subclinical atherosclerosis prediction.*

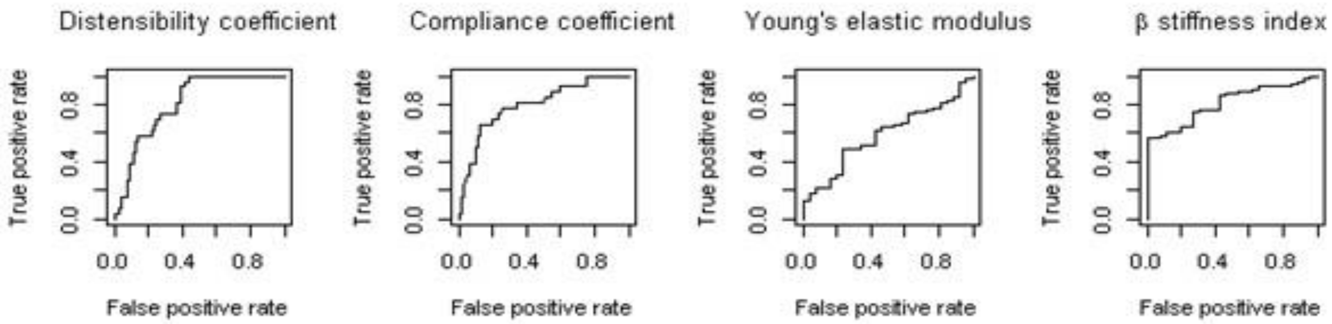

Table 1. *Average value (±SD) of hemodynamic and metabolic variables of respondents with respect to cardiovascular risk factor.*

MEN (N=120)	CARDIOVASCULAR RISK FACTOR			Control group (N=30)	p
	Hypertension (N=28)	Diabetes (N=30)	Smoking (N=32)		
	x̄(SD)				
Age, years	49,7(8,16)	50,9(5,69)	49,1(6,94)	49,1(6,53)	0,741‡
Duration of the risk factor, years	8,2(3,72)	10,8(4,32)	26,1(6,79)	-	<0,001§
Systolic blood pressure, mmHg	145,1(16,46)	128,8(9,38)	127(10,32)	126(12,18)	<0,001§
Diastolic blood pressure, mmHg	90,6(10,84)	81,7(5,37)	81,6(7,15)	80,3(8,17)	<0,001§
Total cholesterol, mmol/L	5,73(0,874)	5,81(2,415)	5,95(1,024)	5,69(0,97)	0,904‡
HsCRP*, mg/L	2,012(1,371)	3,018(2,579)	3,52(4,964)	1,096(0,701)	<0,001‖
Fasting plasma glucose, mmol/L	5,25(0,692)	9,99(2,658)	5,24(0,745)	4,96(0,419)	<0,001§
HbA1c†, %	5,62(0,375)	9,21(2,02)	5,68(0,437)	5,52(0,301)	<0,001§

**high-sensitivity C-reactive protein; †glycosylated hemoglobin A1c; ‡classical ANOVA; §Welch's ANOVA; ‖Kruskal-Wallis ANOVA*

In order to verify the difference in the expected numerical values of arterial walls, depending on their groups, T-test, i.e. ANOVA test was used. In some cases, in order to verify the difference in data distribution, it was necessary to use Kruskal-Wallis test, Mann-Whitney U test for independent samples, or Wilcoxon's signed-rank test for paired samples. The effectiveness of the numerical variable in subclinical atherosclerosis risk assessment is discussed because of its sensitivity and specificity, but also because of the analysis of the ROC curve results. Significance level was set at 0.05 in all of the tests. Statistical analysis was carried out in the data analysis program R (https://cran.r-project.org, package ROCR and pROC) and Statistica (StatSoft, version 11, http//www.statsoft.com/company).

Results

Table 1 presents basic hemodynamic and metabolic variables of respondents with respect to CVRF. Statistically significant difference in age and levels of total cholesterol has not been found between the control group and risk factor groups (p=0.741, i.e. p=0.904). The hypertension group indicated the highest values of blood

Table 2. *Ultrasound carotid arteries' parameters of elasticity and stiffness with respect to the risk factor.*

MEN (N=120) Functional ultrasonic parameter	CARDIOVASCULAR RISK FACTOR			Control group (N=30)	p[¶]
	Hypertension (N=28)	Diabetes (N=30)	Smoking (N=32)		
	x̄(SD)				
DC[*], kPa^{-1}	0,0157 (0,0129-0,017)	0,0154 (0,0136-0,0183)	0,0182 (0,0148-0,0231)	0,0251 (0,0173-0,0279)	<0,001
CC[†], $10^{-7}m^2kPa^{-1}$	4,81 (4,15-5,56)	4,26 (3,65-5,24)	5,21 (4,7-6,49)	6,46 (5,42-7,27)	<0,001
YEM[‡], kPa	623,2 (553-717,3)	529,9 (428,4-616,6)	533,2 (394,7-615,5)	444,2 (368,6-547,4)	<0,001
β[§]	4,86 (4,78-5,05)	4,87 (4,72-4,99)	4,71 (4,46-4,92)	4,38 (4,27-4,75)	<0,001

[*]distensibility coefficient; [†]compliance coefficient; [‡]Young's elasticity modulus; [§]beta stiffness index; [||]interquartile range; [¶]Kruskal-Wallis test

pressure, while FBG and HbA1c were significantly higher in diabetic respondents (Table 1). Smokers indicated statistically significant higher values of hsCRP when compared to other risk factor groups and control group ($p<0.001$). Ultrasound markers of carotid arteries' elasticity – DC and CC – indicated differences between the control group and groups of respondents with hypertension and diabetes (multiple comparison, $p<0.001$), while indicating the difference of smokers in DC, but not in CC (multiple comparison, $p=0.033$, i.e. $p=0.055$) (Table 2, Figure1).

The analysis of β stiffness index indicated significantly higher arterial stiffness in respondents belonging to the hypertension and diabetic groups (multiple comparison, $p<0.001$), as well as smokers (multiple comparison, $p=0.028$) in comparison with the control group. YEM values, as the second arterial stiffness indicator, proved to be the highest in the hypertension group, i.e. they were significantly higher than those in the control group ($p<0.001$) (Table 2). Pearson's correlation coefficient indicated a significant positive correlation of age and β (0.507; $p<0.001$), and negative correlation of age and DC (-0.496; $p<0.001$), independent of CVRF presence/absence. ROC analysis of functional vascular ultrasound parameters with carotid SA prediction was done with respect to the SA ultrasound criteria – cIMT values ≥75^{th} percentile in the control group and/or presence of the atherosclerotic plaque in carotid arteries (Figure2, Table 3). There were 94 out of 120 respondents (78%) with one or two subclinical carotid atherosclerosis criteria and 26 (22%) without any of the mentioned criteria.

Of all the functional ultrasound tests in predicting SA, the following seem to be the best indicators: DC (AUC 0.811), ß (AUC 0.810) and CC (AUC 0.799). The borderline value of DC to discriminate between the respondents with and without SA was set at ≥0.0231 kPa^{-1} (sensitivity 0.872, specificity 0.538), and for ß≤4.47 (sensitivity 0.872, specificity 0.538). ROC analysis of the ultrasound tests of carotid arteries has been lower in participants with CVRF (N=90, group+ =78, group- =12). According to AUC data, DC, CC, YEM and ß were lower (0.806, 0.784, 0.513 and 0.798) than in ROC analysis, which also included the respondents from the control group.

Discussion

Given that numerous research has already reported the correlation of age and arterial elasticity/stiffness indicators (18, 21-23), which has been corroborated in our research as well, the absence of significant age difference has been a key prerequisite of this research. As expected, the highest difference in blood pressure values was found in the hypertension group, while FBG and HbA1c were found in respondents with diabetes. HsCRP was significantly higher in smokers than in non-smokers ($p<0.001$), similar to previous research (24, 25).

In this research, DC, CC and ß index indicated statistically significant lower elasticity and higher stiffness of the carotid arterial wall in respondents with diabetes, hypertension and smokers when compared to healthy participants without CVFR. These findings corroborate the results found in previous research (21, 26-28). Similar to our results, Sharret et al. found higher arterial wall stiffness in respondents with diabetes, but they have also found the correlation between smoking and higher elasticity of the arteries (29). By contrast, our research found ß and DC to be the ultrasound parameters that indicate statistically lower elasticity and higher stiffness of the arterial wall in smokers in comparison with the control group ($p<0.05$). The previous literature did not account for the comparison of values of different functional ultrasound parameters in detecting subclinical carotid atherosclerosis. ROC analysis indicated that DC, ß, and CC seem to be valid predictors of the subclinical carotid atherosclerosis in vascular healthy men, independently of the presence/absence of the CVRF. YEM, when compared to other functional ultrasound tests, did not indicate significant differences in determining the presence/absence of the SA of the observed groups of men.

Automatic measurement of the oscillation of carotid arteries' lumen width was not performed in this research due to technical malfunction of necessary appliances. According to Prado et al., some recent research used standardized manual approach, which could be as precise as automatic measuring (18). Cuadrado Godia et al. have reported good reproducibility of CCA diameters measured with B/M-mode sonography in their results (30).

Conclusions

To conclude, the results of this research indicate the possibility of ultrasound detection of early arteriosclerotic changes in elasticity and stiffness of carotid arteries in vascular asymptomatic hypertensive and diabetic respondents, as well as smokers. DC and ß seem to be the most prominent ultrasound markers, which could differentiate between male respondents with or without SA better than CC and YEM.

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Competing interests
None to declare.

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