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Noninvasive Detection of Endothelial Function in Normal Subjects, Asymptomatic Patients at Risk of Atherosclerosis and Patients with Coronary Artery Disease

Background/Objective: The endothelial dysfunction is associated with atherosclerosis. The dilatory reaction of atherosclerotic vessels in response to occlusion is reduced. This reduction could be of value in atherosclerosis determination. This study aimed at comparing brachial artery response to occlusion and administration of nitroglycerine in three groups: coronary artery disease patients, individuals with coronary disease risk factors but no coronary disease, and normal subjects.

Patients and Methods: The participants included 23 healthy individuals, 22 subjects with cardiovascular risk factors (diabetes mellitus, smoking, hypertension or hypercholesterolemia), and 57 angiographically proven coronary patients. The brachial artery diameter was measured by color Doppler ultrasound at rest, 5 minutes after inflation of the cuff, and 5 minutes after sublingual administration of nitroglycerine pearl.

Results: The vessel's diameter increased the least in the coronary artery disease and coronary risk factor groups in comparison to normal subjects (p=0.003 and 0.048, respectively). Vessel dilatation in response to nitroglycerine did not differ in healthy individuals from the coronary patients or the risk factor group (p=0.96 and 0.77, respectively).

Conclusion: Doppler ultrasound may be used as a noninvasive method to identify subjects with endothelial dysfunction at high risk of coronary artery disease who need intervention or more invasive procedures.

Keywords: endothelial dysfunction, color Doppler, ultrasonography, vasodilatation, atherosclerosis

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Introduction

Atherosclerosis is a disseminated process, beginning during childhood by deposition of lipid in the intima of systemic arteries.[1] There are many atherosclerosis risk factors starting early in life that may cause endothelial damage long before the disease is clinically apparent.[1] Endothelial injury and changes are the earliest signs of the disease before they become clinically apparent. Endothelial injury induces thrombosis, leukocyte adhesion and proliferation of smooth muscle cells in the arterial wall. [2] These changes interfere with the normal endothelial functions of: 1) maintaining the vascular tone, 2) balancing blood fluidity and thrombosis, and 3) controlling the vascular inflammatory process.

In fact, endothelial function could serves as an indicator for cardiovascular health which can be used for patient care and evaluation of new therapeutic strategies.

Endothelium controls vascular tone by influencing the vascular smooth muscle. In especial situations such as shear stress applied by occlusion and release of

blood flow in peripheral vessels (i.e. after releasing inflation of the cuff around the arm), nitric oxide releases from the endothelium of the vessel wall, causing vasodilatation. [2] This phenomenon is called "flow-mediated vasodilatation" (FMD). [2-5]

Individuals prone to atherosclerosis, suffer endothelial dysfunction. In these cases, traditional and novel coronary artery disease (CAD) risk factors initiate a chronic inflammatory process (that results in vasoconstriction and increase pro-thrombotic products), and the endothelial cells adopt a pro-thrombotic phenotype. These inflammatory factors lead to atherosclerotic plaque formation; and finally, endothelial dysfunction participates in a "positive feedback loop" with atherosclerosis.

Endothelium-dependent vasodilation is one of the features altered through atherosclerosis and can be assessed by several methods, including quantitative coronary arteriography, brachial artery catheterization with venous occlusive plethysmography, vascular tonometry and measurements of vascular stiffness, and brachial artery ultrasound with FMD.

According to previous studies, patients with advanced atherosclerosis, show a reduced endothelial response to increased blood flow. [1-5] Moreover, sublingual nitroglycerine acts directly on the mural smooth muscle causing a vasodilatation independent of the endothelium.[1,2,5-7] Therefore, endothelial function could be assessed by evaluation of endothelial response to the techniques provoking shear stress and the release of nitric oxide, resulting in vasodilatation (FMD). This change in vessel diameter can be quantified by several methods. High frequency ultrasound imaging of peripheral arteries provides a detailed picture of the arterial wall anatomy.[1, 2, 4, 5, 7-9] After inducing vasodilatation, we could assess endothelial response by using a high frequency linear array transducer in patients and the control groups and evaluate the association of responses in different groups with different variables and risk factors, and the subtle changes in vessel diameter at high precision.

We tried to determine the changes of the FMD in patients with CAD and the subject with cardiovascular risk factors as compared with the normal controls.

Patients and Methods

One hundred and thirteen patients were randomly chosen from the patients who underwent coronary arteriography. Eleven patients were excluded because they lacked information or did not match our inclusion criteria. Thus, a total of 102 patients took part in the study. The participants were grouped in three group: 23 healthy individuals (with no risk factor, and normal coronary arteriogram), 22 persons with risk factors for cardiovascular disease (but normal coronary arteriogram), and 57 patients with coronary artery disease (CAD) proven by coronary arteriography. The risk factors included diabetes mellitus, smoking, hypertension and hypercholesterolemia. The patients had to fast for 8 to 12 hours prior to the assessments.

The participants were informed about the project and under the supervision of the researcher cardiologist all the vasoactive medications influencing the vascular tonicity were withdrawn for 4 half-lives. The participants were advised not to do any exercise, or take coffee, or smoke 6 to 8 hours prior to the assessment. The ambient temperature was kept between 24 and 26 degrees centigrade.

The subject was asked to lie down comfortably for 10 minutes before the first scan. The diameter of the artery was measured using a 7.5 MHz linear array transducer and a Hitachi EUB525 Doppler ultrasound system. An ATI graphic card captured the images. The probe angle (θ) was 60 degrees, with the gate of 1.5mm. The diameter of the artery was measured on the gray scale scan, from one echogenic layer of endothelium to the other side. An area, 4-10 cm above the antecubital fossa was chosen, and the flow velocity and the diameter of brachial artery at the end of diastole (according to the color changes in systolic and diastolic phases on color Doppler sonography) were measured. Then, the cuff was applied distal to the transducer and inflated up to a pressure above the systolic pressure, sustained for 4-5 minutes, and again the velocity and diameter of the artery were measured 45 seconds before and 60 seconds after deflation of the cuff. Fifteen minutes after deflation of the cuff, the same measurements were repeated. A nitroglycerine pearl (0.4mg) was given to the subject afterwards and the measurements were repeated 5 minutes after. All the measurements were repeated 3 times and an average was obtained. The obtained information was analyzed by SPSS, using ANOVA test.

Table 1. Results of baseline measurements in the three groups

	Control group (n=23)	Risk Factors group (n=22)	CAD group (n=57)	P (ANOVA)
Age (Mean±SD)	51.37±16.88 (25-72)	55.71±17.23 (22-70)	55.89±12.07 (34-78)	0.418
Baseline diameter (mm) (Mean±SD)	4.2±0.59 (3.5-5.5)	4.6±0.79 (3.6-6.2)	4.3±0.59 (3.6-6)	0.07
Baseline Flow (L/Min)	0.16±0.07	0.14±0.08	0.14±0.08	0.54

SD=standard deviation, CAD=Coronary Artery Disease

Differences considered statistically significant when P-values were lower than 0.05.

Results

There was no significant difference between the ages of three groups (p=0.4) (Table 1). The baseline diameter of the artery at the beginning of the assessment was not significantly different in three groups (p=0.07) (Table1). The rate of arterial dilation (the change in diameter from the baseline diameter divided by the baseline diameter, D-D0/D0) after cuff inflation was significantly different in the control group and the cardiovascular risk factor group (p=0.048). Also, the diameter change in patients with CAD was different from the healthy group (p=0.003) (Table 2).

The velocity of blood flow at rest was not different in three groups (p=0.54). The velocity after cuff inflation was higher in healthy persons in comparison with the CAD group (p= 0.045), but not the risk factor group (Table 2).

After administering nitroglycerine to the subjects, dilatation of the artery was observed in all 3 groups with no significant difference between the healthy subjects and the subjects with risk factor (p=0.77) and the CAD patients did not differ from the controls (p=0.96) (Table 2).

Discussion

Endothelium is a large organ (1.8kg, 700m²) that secretes numerous factors regulating vascular tone, cell growth, platelet and leukocyte interaction and thrombogenicity including nitric oxide.[4] Endothelium could sense and respond to many internal and external stimuli through complex cell membrane receptors, signal transduction mechanisms, synthesis and release of several regulatory factors .[3] Endothelial dysfunction is believed to play a key role in the development of atherosclerosis, hypertension and heart failure.

Endothelial function could be assessed by evaluation of endothelium dependent flow mediated vasodilatation (FMD) induced by techniques provoking shear stress and the release of nitric oxide, resulting in vasodilatation. This change in vessel diameter can be quantified by several methods and used as an index of vasomotor function. High frequency ultrasound imaging of peripheral arteries has been used to assess FMD. [1, 2, 4, 5, 7-9] External ultrasound imaging provides a detailed picture of arterial wall anatomy. [2] Using high frequency linear array transducer, small change in vessel diameter could be detected. Limits for axial resolution of 7MHz ultrasound in near field is 0.1-0.2mm, and ultrasound calipers are accurate to 0.1 mm.[2] With 7MHz linear transducer, there is a low coefficient of variation for measurements of vessel diameter and a high correlation between control measurements.[2]

Using Doppler ultrasound, we demonstrated that the FMD was reduced in patients with CAD and in subjects with cardiovascular risk factors. Atherosclerosis is a diffuse process not limited to coronary arteries, and further assessments of endothelial function in brachial artery may predict the risk of CAD in asymptomatic individuals with CAD risk factors. Other studies have found similar results. [1,2,5,6]

Table 2. Results of FMD and TNG-induced flow in three groups

	Control group	Risk Factor group	CAD group	P1	P2
FMD (%)	0.85±0.97	0.16±0.07	0.16±0.07	0.003	0.048
TNG Induced Dilatation (%)	0.11±0.098	0.10±0.07	0.11±0.1	0.96	0.77
Increased Flow Ratio	0.67±0.3	0.23±0.75	0.37±0.42	0.048	0.22

P1= For CAD and control group; P2= For Risk Factor and control group; FMD= Flow Mediated Dilatation; TNG= Nitroglycerin; CAD=coronary artery disease; Increased Flow = F1-F0/F0

According to this study, there was no difference in endothelial response to nitroglycerine (TNG) between the three groups, which was also indicated by two other studies.[5,6] The finding is reasonable since TNG acts directly on the mural smooth muscle independently of the endothelium. However, one study has reported a difference between the healthy subjects and those with risk factors in their brachial artery response to TNG, but not in the femoral artery of the hypercholestrolemic children and adult smokers.[2]

We also showed that the increase in blood flow ratio in response to cuff release was less in the patients with CAD in comparison to the healthy subjects; but not in the individuals with risk factors. Similar results were found in one study [2], yet another study showed a difference in the flow between the healthy subjects and those with risk factors.[6]

Endothelium function measurement may predict the likelihood that patients with risk factors might develop cardiovascular disease, but cannot define which individual actually has atherosclerosis.[1] A long-term prospective study is required to determine which risk factors and abnormal endothelial function would lead to clinical atherosclerosis later in life .[1]

Measuring endothelial function has become a useful way in atherosclerosis research. [3] Endothelial function is proportionately related to the risk of cardiovascular disease. The interventions which lower the risk of cardiovascular disease, also improve endothelial function .[10-13]

Endothelial function measurements need great experience and expertise before they can be used as a screening tool. Some software has been developed to measure the arterial diameter in real time studies, which can be used to obtain more accurate results .[14]

Endothelial dysfunction can identify the patients at risk of CAD at a pre-clinical stage, set new risk factors for CAD (screening), and screen the patients who need more intensive workup.[15]

Conclusion

Applying Doppler ultrasound, this study demonstrated that vessel dilation in response to hyperemia is reduced in patients with cardiovascular risk factors and patients with CAD. The increase in blood flow secondary to cuff inflation-deflation was also reduced in CAD. These results may have clinical implication in identifying asymptomatic subjects at risk of CAD and sparing them the more invasive procedures. Also it may help setting new risk factors for CAD.

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