Non-invasive assessment of endothelial function. Intra and inter-observer variability

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Summary

Background and objectives: Non-invasive evaluation of endothelial function with high resolution ultrasound has become a widely accepted tool in determination of high risk subjects for early atherosclerosis. Despite its simple appearance, ultrasonographic assessment of brachial artery changes, is technically challenging and has a significant learning curve. In the present study, we evaluate the intra and inter-observer variability in assessing peripheral endothelial function with high resolution ultrasound at a tertiary referral center. **Methods:** Assessment of endothelial function was performed by 2 physicians in 20 volunteers without evidence of coronary artery disease. Endothelial function is evaluated with a high frequency bidimensional ultrasound with a 10.0-MHz linear-array transducer used for the study. Each volunteer was examined by both observers using an identical protocol, measuring brachial artery diameter on three occasions. **Results:** Excellent correlation was observed for all brachial artery measures with a Spearman's correlation coefficient > 0.9 ($p < 0.0001$). Flow-mediated dilation (FMD) in the study population was of 10.45 ± 6.9%. Flow independent dilation (FID) was of 24.35 ± 7.63%. Intra-observer variability was of 2.1% for observer A and 1.1 % for observer B. **Conclusions:** Non-invasive assessment of endothelial function using brachial artery ultrasound is reproducible and can be performed with low intra and inter-observer variability.

Resumen

EVALUACIÓN NO INVASIVA DE LA FUNCIÓN ENDOTELIAL. ESTUDIO DE VARIABILIDAD INTRA E INTER-OBSERVADOR

Antecedentes y objetivos: La evaluación no invasiva de la función endotelial con ultrasonografía de alta definición se ha convertido en una herramienta ampliamente aceptada para la identificación de sujetos con alto riesgo de aterosclerosis temprana. A pesar de su simple apariencia, la medición de cambios en la arteria humeral requiere entrenamiento y presenta una curva de aprendizaje significativa. En este estudio, evaluamos la variabilidad intra e inter-observador para la medición de la función endotelial en un centro de referencia terciario. **Métodos:** La medición de la función endotelial fue realizada por 2 observadores en 20 voluntarios sin evidencia de enfermedad coronaria. Cada voluntario fue examinado por ambos observadores usando el mismo protocolo midiendo el diámetro de la arteria humeral en 3 ocasiones durante cada fase. Se usó un transductor lineal de 10 MHz. **Resultados:** Se obtuvieron excelentes correlaciones para todas las mediciones de la arteria humeral con un coeficiente de correlación de Spearman > 0.9 ($p < 0.0001$). La variabilidad intra-observador fue de 2.1% para el primero y 1.1% para el segundo observador. **Conclusiones:** La evaluación no invasiva de la función endotelial usando ultrasonido de la arteria humeral es reproducible y puede ser llevada a cabo con baja variabilidad intra e inter-observador.

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Key words: Inter-observer variability. Endothelial function. Flow-mediated dilation.
Palabras clave: Variabilidad inter-observador. Función endotelial. Dilatación mediada por flujo.

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Introduction

Non-invasive evaluation of endothelial function with high resolution ultrasound has become a widely accepted tool in determination of high risk subjects for early atherosclerosis.[1] Recently, it also has been associated with an increasing number of cardiovascular diseases like hypertension, heart failure or cardiac syndrome χ^2. Endothelial function is evaluated with a high frequency bidimensional ultrasound to obtain longitudinal images of the brachial artery in response to different stimuli *(Fig. 1)*.[3] The method is accurate and reproducible for measuring small changes in arterial diameter with low rates of inter-observer and intra-observer variability.[4] Despite its simple appearance, ultrasonographic assessment of brachial artery changes to external stimuli, is technically challenging and has a significant learning curve, so it is important to validate the method in every center where endothelial testing needs to be performed.

In the present study, we evaluate the intra-observer and inter-observer variability in assessing peripheral endothelial function with high resolution ultrasound at a tertiary referral center.

Methods

Assessment of endothelial function was performed in 20 volunteers without evidence of coronary artery disease; informed consent was obtained from all participating subjects. Each volunteer was examined by both observers using an identical protocol, measuring brachial artery diameter on three occasions. Bidimensional high frequency ultrasound with a 10.0-MHz linear-array transducer was used for the study.

Measurement of endothelial function

All measurements were performed in the morning after one night fasting state. The subjects were required to rest for 10 minutes on the testing bed in a temperature-controlled room. Every subject was positioned supine with the left arm in a comfortable position for imaging the brachial artery 3 centimeters above the antecubital fossa in the longitudinal plane. Baseline images of the brachial artery of the left arm were obtained. In order to provoke reactive hyperemia, ischemia was induced by the inflation of an occlusion cuff, positioned proximal to the site of imaging, during 5 minutes with a 200 mm Hg pressure. Images were recorded 60 seconds after deflation of the cuff. The flow independent response was evaluated using 2.5 mg of isosorbide dinitrate in a spray solution recording the images 5 minutes after the administration of the nitrate. All images were recorded at the beginning of the R wave. Flow-mediated dilation (FMD) was calculated as the percentage of dilation during reactive hyperemia in comparison with baseline values. Endothelial dysfunction was diagnosed if FMD was less than 9%.[3] For the assessment of interobserver and intraobserver variability, ultrasound investigation was performed by two independent recorders (A.S.G, A.K.). Observer A was a general physician trained by observer B for a period of 6 months previous to the study. Observer B was an experienced neurologist and sonographer.[5] Both observers had 6 month learning curve in the assessment of endothelial function.

Statistical analysis

All values are reported as mean ± SD. For analysis of the intra and inter-observer variability Spearman's correlation coefficient was employed. Spearman's rho is a measure of association between rank orders and was used only for numeric data. For nominal data Cohen's kappa was employed. All analysis were performed using SPSS software version 12.0.

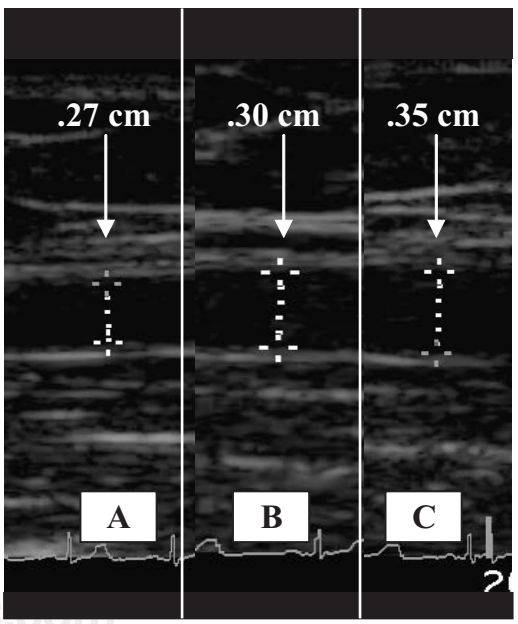

Fig. 1. Endothelial function test. A. Brachial artery diameter in baseline conditions B. Reactive hyperemia diameter C. Post-isorbid diameter. FMD = 11%. This is considered a normal test.

Results

Twenty patients were enrolled into the study (12 women and 8 men). The mean age of the participants was 43 ± 16 years. *Table I* provides baseline brachial artery diameters, responses to reactive hyperemia and isorbide challenge for the study cohort. Excelent correlation was observed for all brachial artery measures with a Spearman's correlation coefficient > 0.9 ($p < 0.0001$). FMD in the study population was of 10.45 ± 6.9%. Flow independent dilation (FID) was of 24.35 ± 7.63 % (Both results were means of the 3 measures recorded by each observer). Intra-observer variability was of 2.1% for observer A and 1.1% for observer B. *Fig. 2*, represents a Bland-Altman plot of FMD performed by both observers (Inter-observer variability). *Fig. 3*, is a Bland-Altman plot of FID also performed by both observers. 8 patients were found to have endothelial dysfunction according to the first observer against 9 according to the second observer for a Cohen's kappa of 0.89.

Table I. Correlation of measures of the brachial artery by observer A and observer B during different phases of the study.

	Observer A	Observer B
Baseline diameter	.34 ± .06	.34 ± .06
Reactive diameter	.38 ± .07	.38 ± .07
Isorbide diameter	.42 ± .06	.42 ± .07
% FMD	10.95 ± 6.95	9.96 ± 6.87
% FID	24.35 ± 8.11	24.36 ± 7.35

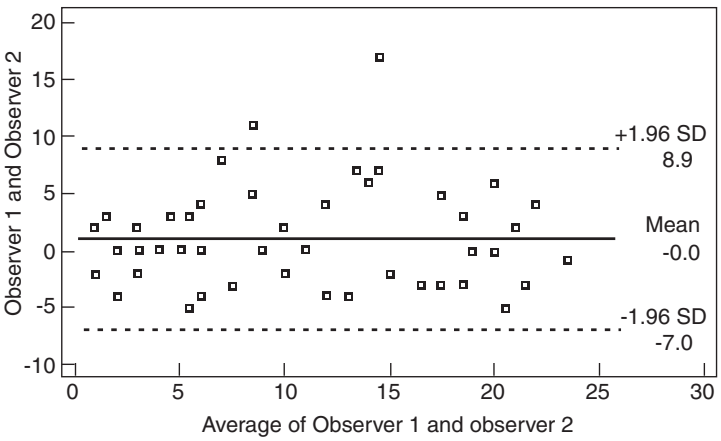

Fig. 2. Bland-Altman plot representing the mean of FMD measurements performed by both observers plotted against the difference of these measurements. Slope = 0.1934 (p = 0.811) Intercept = 0.777 (p = 0.44).

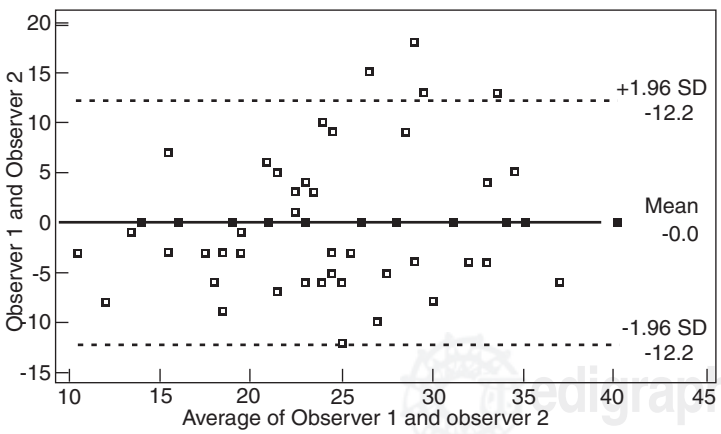

Fig. 3. Bland-Altman plot representing the mean of FID measurements performed by both observers plotted against the difference of these measurements.

Discussion

There are many different ways of measuring endothelial function; it can be assessed either in the coronary or the peripheral circulation.[6,7] Currently, the gold standard for testing endothelial function is by intracoronary infusion of acetylcholine, but it is limited by the risk of the invasive procedure.[8] To non-invasively assess endothelial function other tests have been designed. High-resolution brachial artery ultrasonography is used to study vasodilator response induced by physiologic reactive hyperemia. Other blood vessels can be used for the study (e.g. radial artery), but brachial artery is the most widely accepted.[9] In our study we found that FMD in the brachial artery can be measured with high reproducibility and low intra and inter-observer variability. In spite of that, small differences at the moment of measuring brachial artery diameter can produce important changes in the FMD percentage, so is important to obtain at least 3 different images during each phase of the study. We also observed greater intra and inter-observer variability during the nitrate phase in comparison to basal and hyperemia states, probably due to differences in pharmacokinetics of isorbide according to each subject of study. It is important to emphasize, that according to various studies, the effects of methodology on the outcome of FMD could be of importance.[10] In our study we use upper arm occlusion to induce hyperemia; various studies have demonstrated that upper arm occlusion produces higher FMD values in comparison with lower arm occlusion.[11] Length of occlusion is important as well, a longer duration of the ischemic trigger has shown

to increase dilation response, using less than 4 minutes for occlusion can significantly affect the FMD response.[12]

Study limitations

This study population was not aleatorized and was composed by a heterogeneous group of subjects that might have concomitant illnesses (e.g. hypertension, hyperuricemia). Probably explaining the high prevalence of endothelial disfunction. However, the main objective of the study was only to assess intra and inter-observer variability and not to evaluate incidence and prevalence of endothelial disfunction.

Conclusions

Non-invasive assessment of endothelial function using brachial artery ultrasound is reproducible and can be performed with low intra and inter-observer variability.

Conflicts of interest. None.

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