New fully automated software for assessment of brachial artery flow-mediated dilation with advantages of continuous measurement

Brakiyal arterde akıma bağlı vazodilatasyon değerlendirilmesi için sürekli ölçüm yapabilen yeni bir yazılım yöntemi

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ABSTRACT

Objective: Flow-mediated dilation (FMD) is used to evaluate endothelial functions. Computer-assisted analysis utilizing edge detection permits continuous measurements along the vessel wall. We have developed a new fully automated software program to allow accurate and reproducible measurement.

Methods: FMD has been measured and analyzed in 18 coronary artery disease (CAD) patients and 17 controls both by manually and by the software developed (computer supported) methods. The agreement between methods was assessed by Bland-Altman analysis.

Results: The mean age, body mass index and cardiovascular risk factors were higher in CAD group. Automated FMD% measurement for the control subjects was 18.3±8.5 and 6.8±6.5 for the CAD group (p=0.0001). The intraobserver and interobserver correlation for automated measurement was high (r=0.974, r=0.981, r=0.937, r=0.918, respectively). Manual FMD% at 60th second was correlated with automated FMD % (r=0.471, p=0.004).

Conclusions: The new fully automated software© can be used to precise measurement of FMD with low intra- and interobserver variability than manual assessment. *(Anadolu Kardiyol Derg 2012; 12: 553-9)*

Key words: Flow mediated dilation, endothelial function, reliability and validity

ÖZET

Amaç: Akıma bağlı vazodilatasyon (ABV) endotel fonksiyonlarını değerlendirmek için kullanılır. Bilgisayar destekli yazılımlar damar duvarı boyunca ve damarların kenar çizgilerini kullanarak sürekli ölçüm yapabilmektedir. Biz bu amaçla endotel fonksiyonlarını değerlendirmek için kullanılan ABV ölçümlerinin doğru, tekrarlanabilir ve devamlı olarak yapılabilmesi için yeni bir yazılım yöntemi geliştirip manuel ölçüm yöntemleriyle karşılaştırdık.

Yöntemler: Çalışmaya 18 koroner arter hastası (KAH) ve 17 sağlıklı kontrol olgusu alındı. Tüm olguların ABV ölçümleri hem manuel olarak, hem de bilgisayar destekli yeni yazılımla ölçülerek değerlendirildi.

Bulgular: Ortalama yaş, vücut-kitle indeksi, kardiyovasküler risk faktörleri koroner arter hastalığı grubunda daha yüksek bulundu. Otomatik olarak kontrol grubunda ölçülen ABV % değeri 18.3±8.5, KAH grubunda 6.8±6.5 bulundu (p=0.0001). Otomatik ölçüm için gözlem içi ve gözlemciler arası korelasyon yüksekti (r=0.974, r=0.981, r=0.937, r=0.918, sırasıyla). Altmış saniyede ölçülen % ABV değeri ile otomatik ölçülen ABV değeri korele bulundu (r=0.471, p=0.004). Metotlar Bland-Altman analizi yardımıyla değerlendirildi.

Sonuçlar: Yeni tam otomatik ölçüm yazılımı gözlemci ve gözlemciler arası düşük değişkenlik oranlarıyla ABV ölçümü için kolaylıkla kullanılabilir. *(Anadolu Kardiyol Derg 2012; 12: 553-9)*

Anahtar kelimeler: Akıma bağlı vazodilatasyon, endotel fonksiyonu, güvenilirlik ve geçerlilik

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Introduction

Currently, with increasing technological advances, computerized systems are used in every field. With advanced imaging systems, images are obtained from which more quality and reliable evaluations could be done. Evaluations on these images are performed by human eye at times and image processing systems at other times. Studies in the field of image processing reduce human control in visual evaluation and instead, increase computer control. This reduces errors of human origin and increase speed. Studies such as those on processing on retinal view and those on processing on ultrasound view are being performed in order for image processing systems to be used in medical field (1, 2). Images from medical imaging systems are subjected to image processing techniques and information from this process is used by specialist users and this information is used for decision-making.

Endothelial dysfunction precedes overt vascular disease by years and may itself be a potentially modifiable cardiovascular disease risk factor (3). Although no gold standard for the measurement of endothelial function exists, the measurement of flow-mediated dilation (FMD) in the brachial artery, assessed with Doppler ultrasonography, is the most studied method and shows the most promise for clinical application. It is a well-tolerated, noninvasive, and low-risk procedure. Brachial arterial FMD reflects endothelium-dependent vasodilator function, which is a surrogate marker of atherosclerosis (3, 4). FMD describes the increase in diameter of an artery, response to increased blood flow. The conventional approach to FMD calculation expresses the diameter at selected time point after cuff deflation relative to the baseline diameter. FMD measurement has relied on manual assessment of vessel diameter using visual inspection of single frame. Although the measurement of brachial artery FMD is a noninvasive index of endothelial function, technique- and patient-related limitations and the lack of a "gold-standard" test for endothelial function presently preclude its widespread use as a clinical screening tool (3, 5, 6). Technically, measurement of brachial artery diameter can be difficult. Vessel sizes are generally in the 3 to 5 mm range, with the absolute caliber often changing by only 0.2 to 0.4 mm, even in healthy subjects. When it is appreciated that the resolving power of many electronic caliper systems is approximately 0.1 mm, the potential for significant interpretative error, especially between observers, is understandable. Although most reported interobserver variability rates are low (<5%), they are usually expressed as a percentage of the baseline diameter; when expressed as the percent increase above baseline diameter, variability is more profound. Placement of calipers using visual inspection - as a crucial point of diameter measurement-is operator dependent and susceptible to error (7). Although at present, this technique remains very operator sensitive, novel edge-detection software packages and expected improvements in imaging hardware show promise for superior detection with less attendant interobserver variability (7, 8), another potential problem relates to image timing. Manual assessment of FMD gives little information about the time course of change in arterial diameter after cuff deflation. The variation in the literature regarding the time points for post deflation measurement of diameter changes is between 60-180 seconds. Even multiple time points used still it may fail to identify the true peak diameter (9). The conventional protocol assumes maximum dilation occurs around the selected time point, which are 60, 90 and 120 seconds after the cuff deflation. Bressler et al. (10) demonstrated highly variable peak flow after cuff deflation; fewer than half the subjects tested had achieved peak hyperemic flow at 60 seconds. The average time to peak flow in that study was 81 seconds, with a range of 40 to 140 seconds. Because of the wide baseline variability in the normal response to increased flow, present techniques may fail to detect the true peak FMD. Frequent (eg, every 20 seconds) monitoring of flow in a longer period (eg, 3 minutes) after cuff deflation, with measurements taken at the time of greatest flow, is one potential solution. This limitation may also be overcome with systems that can allow for continuous diameter assessment and thus facilitate the detection of peak vessel dilation. Continuous edge-detection and wall tracking system, which calculates arterial diameter, result in more realistic calculation compared to the traditional manual method. Thus, in order to promote endothelial function testing in clinical practice we need new, easy, operator-and time-independent methods. Computer-assisted analysis utilizing edge detection permits continuous measurements along the vessel wall. We have developed new fully automated software program to allow accurate and reproducible measurement. Computer-assisted analysis permits multiple measurements along the vessel wall and would be expected to increase the precision of the measurements.

The present manuscript describes the protocol for a typical FMD study, the computerized system for image acquisition and analysis, and the results of clinical repeatability testing in normal volunteers and coronary artery disease (CAD) patients.

Methods

Study protocol

This experiment is conducted as a joint study between Department of Cardiology of Medical Faculty and Department of Computer Engineering of Çanakkale Onsekiz Mart University. FMD has been measured in 18 coronary artery disease (CAD) patients and 17 healthy controls. All of the studies were analyzed by two operator blinded to the subject's clinical status both manually (the conventional method) and by the software developed (computer supported) methods. Each patient was examined by two methods at two time points in order to calculate the intra-assay correlation. Two measurements by the same operator were used to calculate intra-assay correlation coefficient and measurements for the same patient by different

operators were used for inter-assay correlation co-efficient. Number of the patients was evaluated as 12 considering that FMD difference between the control and study groups would be at 25%. Eighteen patients were included in the study group. The patients in the study group were chosen from the patients with CAD with high risk factors with expected endothelial dysfunction. The subjects in the control group were chosen from the healthy volunteers. The study was approved by the local Ethics Committee. Written informed consent was obtained from each patient. Investigations were in accordance with the Declaration of Helsinki.

After a 12-hour fasting, no smoking for 12 hours, and no alcohol intake for 3 days, individuals were examined in a dark, temperature-controlled, quiet room after 20 minutes of rest. Brachial artery FMD was assessed using a 15-MHz linear array transducer (Agilent 5500, Andover, MA). The artery was imaged above the antecubital fossa in the longitudinal plane. FMD was measured as the dilatory response to reactive hyperemia induced by inflation of a blood pressure cuff on the forearm to suprasystolic levels for 3 minutes. One minute, 90 seconds and 120 seconds after cuff deflation, the brachial artery diameter was remeasured. Measurements were taken at end diastole (corresponding to an R wave). The arterial diameter was measured using a digital caliper on the image at a comparable site at baseline and after cuff release. The four measurements were averaged, and the percent increases from baseline diameter were calculated for FMD responses. FMD was expressed as percentage change =100× [brachial artery diameter at peak hyperemia minus the diameter at rest]/brachial artery diameter at rest. Three measurements were averaged at baseline and after cuff deflation, and the averages were used in the analysis.

The software protocol

The image may be directly loaded on the software and doesn't require to be divided into stages such as basal hyperemic phase. It is new advantage that it enables choosing the area of interest and that it doesn't need to be synchronized with electrocardiography. A flexible logic is used that excludes the artifacts during the measurements. The software can yield the function of arterial diameter with time as a graphic. The data may be taken as an excel file. The percent of FMD is provided through the ratio of averaged baseline and maximum diameter.

Measuring diameter of brachial artery

Video image from brachial artery taken by the physician through a medical imaging system is transferred to the computer with file extension*.*vob. On this video image lasting averagely ten minutes, changes of arterial diameter after inflating the cuff of a sphygmomanometer and then deflating it on the region of brachial artery on the forearm is seen. Video image by the operator takes recording continuously from the long axis of brachial artery prior to (basal), during and following the procedure. The segment of blood vessel part of the tissue is captured by thresholding and then borders for each part namely top and bottom respectively are determined. This constitutes the whole main preprocessing part of overall system. Edge detection and measurement FMD is studied in Mark et al. (7) and Woodmann et al. (9). In our implementation after the base step, the measurement is online which means that it does not require passing the data for the second time or more. This improves the speed. Furthermore, the algorithm do sampling on frames and pixels based on given interval vales instead taking into account the whole frame and each pixel in the frame. This reduces the time needed to respond in an online manner as well. Diameter of the brachial artery is measured at more than 20 points for each frame on the software newly developed. The new software developed measures the diameter of brachial artery, calculates the changes in the diameter, records the retrieved information in the computer and reports the results. The software was developed in the setting of MATLAB 2009 (11). The video image with the file extension *.*vob taken from the medical imaging system was converted to file with extension *.*avi. By this way, large sized video files (about 100 MB) was reduced and it was enabled to work faster.

Preparing the video image

The raw video image of the blood vessel taken from the medical imaging system was enhanced by image processing techniques to obtain the best result (12). On the video image, information areas such as caption and scale are present in addition to the zone of blood vessel. At first, cropping is made at predefined sizes in order to make clear the area on which the blood vessel appears. On the image of blood vessel, gray scale conversion and conversion into the binary (white and black) format with threshold value of 1.9 was done using Otsu's method (13). Thresholding is easily done because of significant differences between gray levels of vessel area and muscular area, but rarely if needed, otsu is used with different parameters to get better segmentation. The threshold value may be changed to more accurate results for different cases. The new image on which the vessel area appears white and muscular areas out of the vessel appear black is transferred as a parameter to the next preprocessing step. Entire operation proceeds independent from the operator. Name, risk factors, protocol number and arterial tension value may be entered to the software prior to the procedure.

Pre,processing the video image

The image is not at the same quality on 5000 frames constituting the video file. Image's artifacts occur due to movement during inflation and deflation of the cuff and taking the images and the artery cannot be distinguished on same frames. In order to minimize errors from these factors, the video file is pre-processed to eliminate the faulty frames. At this step, each frame on the video is processed by the software and arterial borders are established. In order to draw the arterial borders, points are taken with certain intervals on the upper border of the black

Figure 1. The distance between the points establishing the borders of brachial artery, edge detection

area symbolizing the vessel area on the white and black image by making row-column screening on the frame and the points corresponding to them on the lower border are established. Two lines drawn by unifying the lower and upper points express the arterial borders. These lines taken for each frame have slope. Ideal borderlines are taken for the video by averaging the slopes of appropriate range. Points on the other frames are excluded because they are considered as faulty. The artifacts and estimated measurements are expressed as "outlier" and "inlier", respectively. The measurements considered as artifact are established in the case of having change in pixel length more than two-folds in standard deviation relative to the previous frame. This also expresses the 95% confidence interval.

Calculating the arterial diameter

The distance between the points establishing the borders of brachial artery, obtained from the previous step, is averaged. Thus, an arterial diameter value is calculated for each frame. As seen in Figure 1, these points and the diameter value are presented on the user interface and recorded in the computer. After calculating diameter value for each frame on the video, these values are averaged. Standard deviation is obtained according to this mean diameter value. On the user interface, such information as number of the frames being processed and mean diameter, and changes in the diameter of blood vessel are presented to the user. The user is asked to choose two areas on the diameter/time graphic on which changes of brachial artery diameter is shown along the video image. These areas chosen by the user correspond to the frames at certain time intervals. Percentage of change is calculated by comparing the diameter values at these

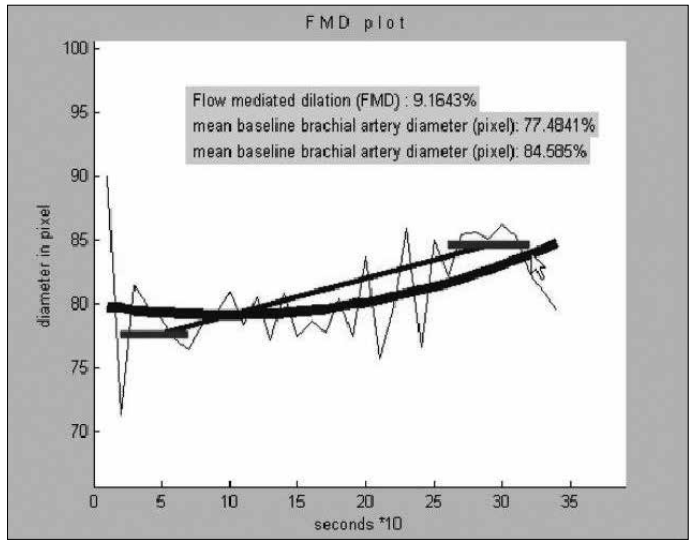

Figure 2. The user was asked to choose two points on the diameter/time graphic. These points were chosen by the user correspond to the frames at certain time intervals. Percentage of change was calculated by comparing the diameter values at these time intervals and reported to the user and stored as output

time intervals and reported to the user and stored as output (Fig. 2). The file in which such information as new video image on which arterial margins appear, positions of input and output files, percentage of change and average width for each case and name of the patient is recorded is kept in the computer.

Statistical analysis

SPSS 15.0 software (Chicago, Il, USA) was used for statistical analysis. We hypothesized that the new fully automated

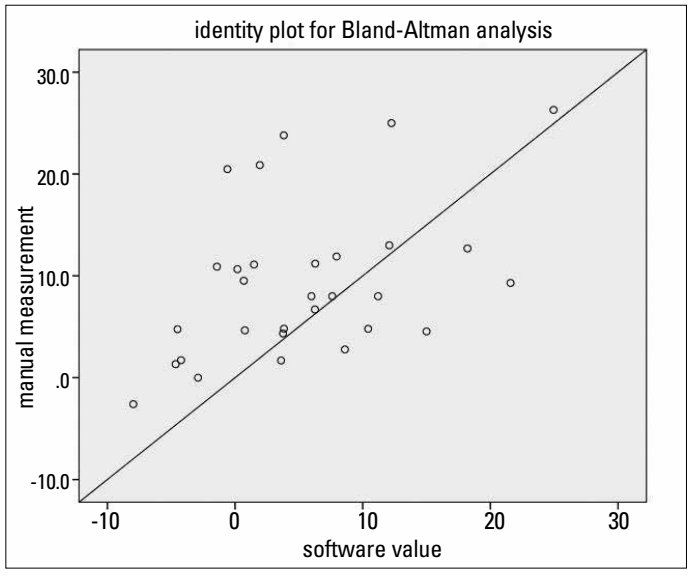

Figure 3. Identity plot for Bland-Altman analysis

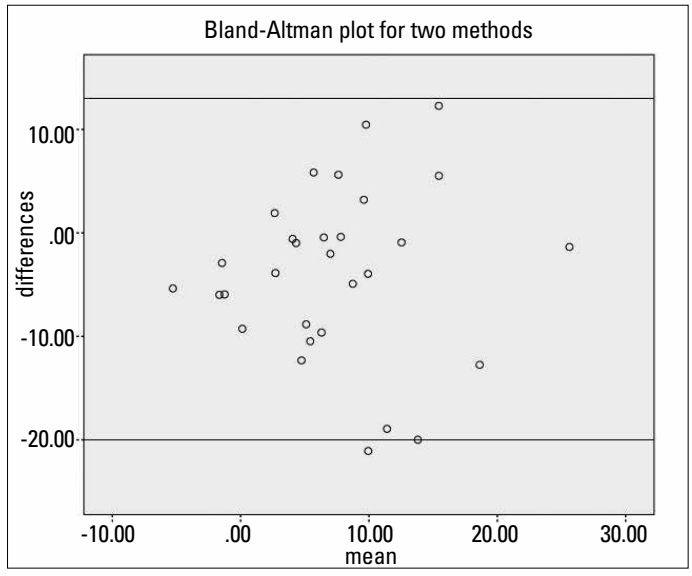

Figure 4. Bland-Altman plot for two methods

software for brachial artery flow mediated dilation can measure FMD precisely with low intraobserver and interobserver variability. Normality was assessed with the Shapiro-Wilk test. Categorical data were analyzed by the Chi-square test. The Spearman rank correlation method was used as a nonparametric measure of association for correlations. A p value 0.05 was considered statistically significant. Assuming 80% power and of 0.05, eight subjects with matched controls would be required, in a parallel designed study, to detect an absolute 2.5% change in FMD. A Bland-Altman analysis shows that the software can be used instead of manual measurement. As can be seen in Figure 3, points for measurement for plot of identity of Bland-Altman are lined up closely to the line y=x, giving agreement for the methods. The Bland-Altman in Figure 4 shows the mean of the different measurement on x-axis and their deviations on y-axis with values of -3.9364 and 8.21986 for mean and standard deviation respectively. The all points are seen within the $\mu \pm 2^* \sigma$ this concludes that the software is used for FMD.

Results

The mean age, body mass index and cardiovascular risk factors were higher in CAD group (Table 1 and 2). Automated FMD % measurement for the control subjects was 18.3±8.5 and 6.8±6.5 for the CAD group (p=0.0001) (Table 3) (Fig. 3). Manual FMD % measurement for the control subjects was %13.4±6.2 and 8.8±7.1 for the CAD group at the 60th second (p=0.011). The intraobserver correlation for automated measurement was high (r=0.974, r=0.981) in Figure 5. Interobserver correlation for automated measurement was r=0.937 and r=0.918. Manual FMD % at 60th second was correlated with automated FMD % (r=0.471, p=0.004).

Discussion

We describe studies on a computerized edge-detection and wall-tracking software program to allow more accurate and reproducible measurement. Manual measurement yields limited results due to low number of samples from the sections and due to the fact that measurement can't be done from each section and the measurements are taken at certain time points and thus are criticized by some authors (14-16). The software we developed eliminates these limitations. Thousands of measurements taken during entire process are much more accurate compared to manual measurements. Furthermore, giving a graph of the continuous measurement instead of a measurement at a certain time point enables determining maximum dilatation time pre-

Table 1. Demographic and anthropometric variables of patients and controls

Variables		Patients group	Control group	*p
Age years	Mean	35.6	54.9	0.290
	Standard Deviation	11.9	11.9	
	Percentile 25	28.0	51.0	
	Percentile 75	35.0	63.0	
Height, cm	Mean	170	168	0.544
	Standard Deviation	7	7	
	Percentile 25	165	165	
	Percentile 75	172	172	
Weight, kg	Mean	69	75	0.310
	Standard Deviation	12	10	
	Percentile 25	65	71	
	Percentile 75	75	80	
Body mass index, m/kg^2	Mean	0.24	0.27	0.707
	Standard Deviation	0.04	0.03	
	Percentile 25	0.21	0.24	
	Percentile 75	0.27	0.28	

Data are presented as mean, SD and 25th-75th percentile
Bland-Altman test

Table 2. Baseline characteristics of the groups

Variables		Patients group			Control group			*p
		n	%, within group	%, of total	n	%, within group	%, of total	
Sex, male:	1	10	58.8	40.0	15	83.3	60.0	0.290
Female:	2	7	41.2	70.0	3	16.7	30.0	
Diabetes mellitus	0	14	82.4	48.3	15	83.3	51.7	0.612
	1	3	17.6	50.0	3	16.7	50.0	
Hypertension	0	14	82.4	46.7	16	88.9	53.3	0.160
	1	3	17.6	60.0	2	11.1	40.0	
Smoking	0	13	76.5	50.0	13	72.2	50.0	0.804
	1	4	23.5	44.4	5	27.8	55.6	
Hyperlipidemia	0	14	82.4	60.9	9	50.0	39.1	0.919
	1	3	17.6	25.0	9	50.0	75.0	
Myocardial infarction	0	17	100.0	50.0	17	94.4	50.0	0.439
	1	0	0.0	0.0	1	5.6	100.0	
Percutaneous coronary	0	17	100.0	50.0	17	94.4	50.0	0.439
intervention	1	0	0.0	0.0	1	5.6	100.0	

Data are presented as number/percentage
*Chi-square test

Table 3. Flow mediated dilation values were measured by automated software

Flow mediated dilation (FMD), %		Patients group	Control group
Observer EE	Mean	18.74	6.97
	Standard Deviation	8.95	7.07
	Percentile 25	10.11	3.64
	Percentile 75	24.98	10.04
Observer BK	Mean	19.18	6.53
	Standard Deviation	8.48	8.85
	Percentile 25	12.10	4.36
	Percentile 75	22.84	8.43
Manual FMD measurement at 60th second	Mean	13.41	8.78
	Standard Deviation	6.19	7.07
	Percentile 25	11.40	5.20
	Percentile 75	15.10	11.50
Manual FMD measurement at 90th second	Mean	10.8	9.6
	Standard Deviation	5.6	7.8
	Percentile 25	4.6	4.5
	Percentile 75	14.2	12.3
Manual FMD measurement at 120th second	Mean	11.4	9.2
	Standard Deviation	5.6	7.3
	Percentile 25	8.0	4.5
	Percentile 75	13.0	11.2

Data are presented as mean, SD and 25th-75th percentile
*Bland-Altman test, EE: Ertuğrul Ercan, BK: Bahadır Kırılmaz

cisely. For this reason, it was aimed to develop the software of calculating FMD.

In the present study, image processing was performed on the arterial image taken through medical imaging system. By this way, arterial width calculated previously manually was enabled to be calculated automatically in the computer setting and additionally, enabled such information as percentage of change to be obtained. Output data of the software was recorded in the computer setting for presentation of the videos and graphics to the user and in order to retrieve the information that would be required later. In the present study, processing the data from the medical imaging system was not real-time. Real-time processing and analysis of the results may be enabled by integrating into the medical imaging system.

Innovations and advantages of the software

Any movable file may be used directly to be processed in the software. The image from ultrasound device may be studied without dividing into such sequences as basal or hyperemic phases. By the means of the software we recently developed, such dividing is not required. In contrast to other software's, choosing the region of interest is not required on the image from the ultrasound device (7, 17, 18). On entire image area, the region of interest is established by the software. The image may be processed easily as single file. Electrocardiography is not required to be evaluated in synchrony for the image to be processed. Thus simultaneous electrocardiographic recording is not required for the patients. On examination of each frame, measurement is made on more than 20 points on each frame

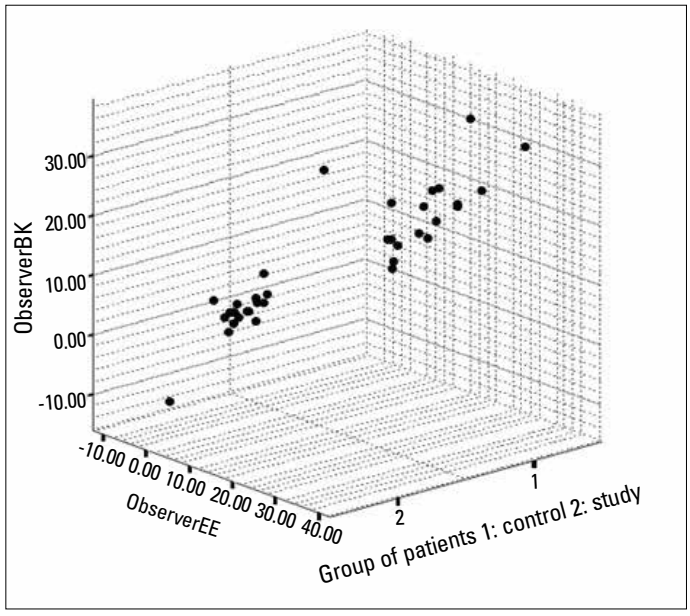

Figure 5. FMD values in the control and study group provided by the new software
FMD - flow-mediated dilatation

along the long arterial axis and these measurements are averaged and standard deviation is calculated. All data obtained may be taken as an excel file. Thus, the correction for the average of consecutive images taken once again with electrocardiography is made by the software itself. The images yield accurate results not only on the cases with excellent image quality but also on those with poor image quality. Time/arterial diameter graphic is obtained at the end of the procedure. The software allows the operator to make selection for the measurements of basal arterial diameter and maximal diameter from the time graphic. Measurement for this purpose doesn't have to be spot. The sections made average once again all image sections at the time interval chosen by the operator. Arterial FMD is calculated by dividing the maximal diameter to the basal diameter and presented numerically.

Conclusion

Our method overcomes the variability of FMD measurement seen with conventional manual analysis. This is obvious in healthy subjects and in patients with CAD. We are planning new studies about applicability of this software. The software is easy to use in the clinical setting in patients and should allow the more widespread evaluation of endothelial dysfunction, in particular in subjects suspected to be at risk of CAD.

Conflict of interest: None declared.

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