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Original Research Article

Measurement of endothelial function using photoplethysmography with continuous correction approach: a pilot study

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ABSTRACT

Background: Photoplethysmography flow-mediated dilatation (PPG-FMD) is an endothelial function measurement tool, and its use is increasing as it is easy to apply and relatively inexpensive. The analysis protocol of PPG-FMD has not yet been standardized. This study aimed to define the continuous correction method in PPG-FMD analysis and interpret the results obtained from a healthy population.

Methods: The study population consisted of 30 healthy persons. Endothelial function measurement was done with the PPG-FMD method. In the correction process of the PPG signal, a continuous (element-wise) correction approach was defined and applied. PPG-dilation indexes (PPG-DI) were calculated for each subject, and its correlation with demographic and anthropometric data were analyzed. Finally, the study group was divided into subgroups, and endothelial function results were compared.

Results: The median PPG-DI (IQR) value of the whole study group was 112% (60). The Pearson correlation coefficients (r) obtained in the analysis were as follows; r=-0.6278 for age, r=-0.4635 for BMI, r=-0.4628 for waist circumference, r=-0.4207 for systolic blood pressure, r=-0.2804 for diastolic blood pressure. In the subgroup analysis, groups with older age, high BMI, male sex, smoker, and high waist circumference had lower PPG-DI results.

Conclusions: PPG-FMD could be a good alternative in the endothelial function measurement, with its operator-independent and easily applicable nature. Although the measurement protocol is similar to USG-FMD, PPG-FMD contains much more technical details during the analysis process. We introduced a novel signal correction method, and we believe that this will be a basis for future studies on this subject.

Keywords: Endothelial function, Vascular health, Photoplethysmography, Flow-mediated dilation, Continuous correction

INTRODUCTION

The endothelium is the cell layer that forms the innermost layer of blood vessels surrounding the whole body. This cell layer, which is in constant contact with blood, has many vital functions besides forming a physical barrier. Endothelium is one of the essential building blocks of hemostasis with its functions, including vascular diameter and thrombosis regulation.[1]

Endothelial function may deteriorate with the contribution of aging, obesity, and many other factors, and this pathological condition is called endothelial dysfunction. Today, it is known that endothelial function is the first step of the atherosclerosis process.[2] Impairment in endothelial functions has been shown to be associated with cardiovascular risk.[3-5]

Measurement of endothelial function has been studied for many years. In the past, vasodilator agents such as acetylcholine were administered into the coronary artery or brachial artery, and the amount of dilatation in the vessel diameter was measured with the help of ultrasound imaging.[6] Then, in 1992, Celermajer et al succeeded measuring the endothelial function non-invasively.[7] This method is called flow-mediated dilatation (FMD), and it is still the gold standard for measuring endothelial function. In this method, the brachial artery is occluded for five minutes, and the amount of dilatation in the vessel diameter after occlusion is measured by ultrasound imaging. The vessel diameter in the post-occlusion stage is normalized to the baseline diameter, and the dilatation index is calculated in percentage. The nitric oxide (NO) released from the endothelium as a result of shear stress in the vessel wall is responsible for the dilatation that occurs after occlusion.[8,9]

Although the ultrasonography (USG)-FMD method continues to be the gold standard in non-invasive endothelial function measurement, it has some difficulties and disadvantages. First, the method requires a high-resolution USG device. The second disadvantage is the need for an experienced USG operator to apply the method correctly. Since the increase in the brachial artery diameter after occlusion will not exceed a few millimeters, minor errors caused by the operator or device can have significant consequences.

Due to the disadvantages of the USG-FMD method, new methods have been investigated in FMD measurements, and positive results have been obtained. One of the non-invasive endothelial function measurement methods is flow-mediated dilatation (PPG-FMD) with photoplethysmography. FMD has the potential to be used more widely due to its advantages, such as being relatively inexpensive, easily accessible, and applicable. In the PPG-FMD method, measurement protocol is almost the same as USG-FMD; however, a photoplethysmography sensor is used instead of an ultrasound device, and measurements are acquired from finger arterioles instead of brachial artery.[10] The PPG-FMD method has been tested in different patient groups, and its results have been compared with USG-FMD in some studies. Studies have shown that PPG-FMD has correlated results with USG-FMD and is effective in determining cardiovascular risk alone.[11-14]

One of the critical steps in the PPG-FMD analysis process is correction. Correction is the calibration of the systemic vasoregulatory effects of the pulse amplitudes in the active PPG signal using the control PPG signal. There is no standard correction method applied to the signal in the post-occlusive period in literature. Therefore, further studies are needed on signal correction for standardizing the PPG-FMD method.

In the present study, we aimed to develop a new correction method for the PPG-FMD measurement signal. We also aimed to test the method in healthy subjects and compare the results with the literature.

METHODS

Study type, population and duration

The study was a cross-sectional epidemiological study involving a single healthy group. Participants were selected on a voluntary basis from the relatives of the patients who applied to Alanya Alaaddin Keykubat University Training and Research Hospital between March 2020 and May 2020. Permission was obtained from the local ethics committee for the study. The purpose, content, and importance of the study were explained to all participants, and their consents were obtained.

Study population and characteristics

The present study was a cross-sectional epidemiological study involving a single healthy group. Those older than eighteen and volunteers were included in the study. The study's exclusion criteria were determined as having any chronic illness that would affect peripheral blood circulation, such as cardiovascular disease, diabetes mellitus, thoracic outlet syndrome, or peripheral arterial disease. A total of 30 people, 15 men and 15 women participated in the study. The demographic characteristics of the participants are shown in Table 1.

The age, gender, smoking status, and medication use of participants were recorded with a simple questionnaire. The height, weight, waist circumference, and blood pressure values of the individuals were measured by a physician and recorded.

Endothelial function measurements

Flow-mediated dilatation measurements were made with PPG-FMD setup and standard FMD protocol. Two PPG signals were acquired simultaneously. Left hand PPG was the active channel where occlusion was applied, and the right hand PPG was the control channel used for correction of the left PPG to eliminate systemic vasoactive effects. PPG data was recorded digitally and then was transferred to the MATLAB environment for further analysis. PPG-FMD measurements were made with Biopack MP36 data acquisition module (California, USA) and two Biopack SS4LA photoplethysmography sensors (California, USA). MATLAB (Massachusetts, USA) was used for PPG-FMD analysis.

Continuous correction method

In the correction process of the active PPG signal, a continuous (element-wise) correction approach was used. In this approach, the first step was creating a correction matrix using the right-hand PPG signal. Correction matrix was created with this formula.

$$C(n) = 1 - \frac{A(n) - m}{A(n)}$$

Where C (n) =correction matrix includes n number of elements, n=number of the pulse wave in the post-occlusive period, A (n) =the amplitude series of control channel PPG signal with n number of pulse wave, and m=mean pulse wave amplitude value of control PPG signal baseline part.

In the second step, a moving average with a subset size of 30 is applied to the correction matrix to prevent distortion via very large or small amplitude values caused by motion artifacts. The corrected active channel PPG signal was then obtained by multiplying each post-occlusive amplitude values by the corresponding correction matrix element. In Figure 1, a part of the PPG signal, the raw and corrected pulse wave amplitude signals of an individual, was shown.

After obtaining corrected amplitude values, normalization was applied, and the final PPG-FMD result graphic was created. Normalization of the amplitudes values was done using the following formula.

$$N(n)(\%) = \frac{A(n) - A_{Bsl}}{A_{Bsl}} \times 100$$

Where N (n) is the normalized pulse amplitude series of active channel PPG, A_{Bsl} is the mean pulse wave amplitude in the pre-occlusion (baseline) period, and n is the pulse wave number.

After obtaining the normalized amplitude series, curve fitting was applied to the signal to acquire a smooth and continuous signal (Figure 2).

In this step, we again used a moving average with a subset size of five. Since our signal is less error sensitive at this stage, we used a smaller subset size to avoid losing data to be used in further analysis.

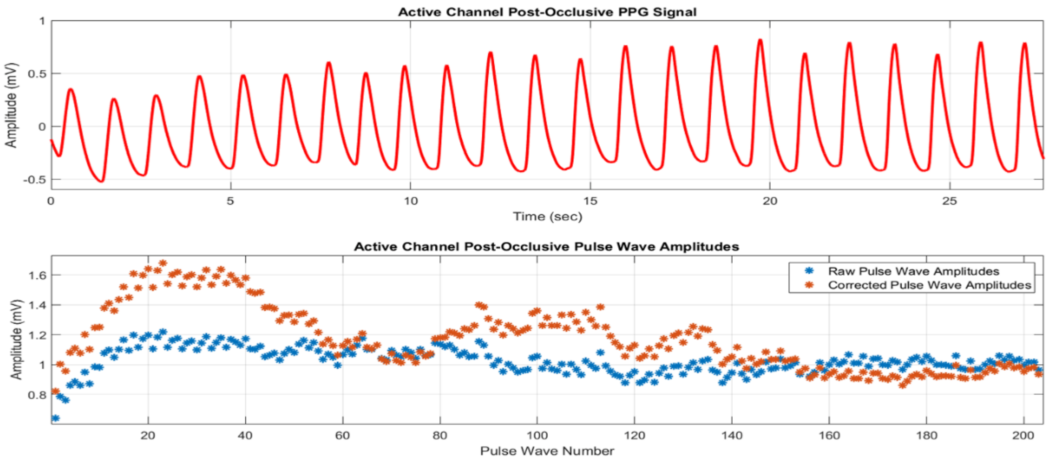

Figure 1: (a) A part of the left-hand post-occlusive PPG signal of an individual. Note that pulse wave amplitudes increase gradually, (b) pulse wave amplitudes scatter plot of the post-occlusive signal. Blue dots represent the raw amplitudes series, while orange dots represent the corrected amplitudes scatter. Note that the raw and corrected signal differ substantially.

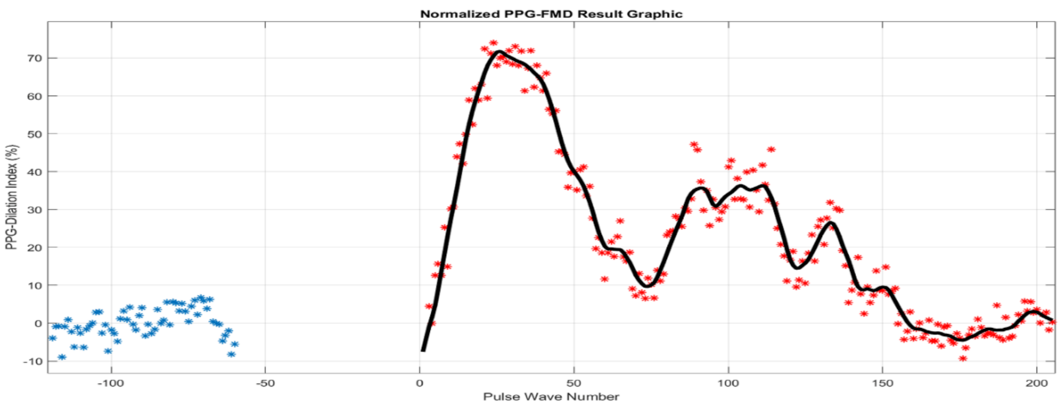

Figure 2: PPG-FMD result graphic of a participant. X-axis represents pulse wave number starting from the moment the occlusion ends. Y-axis is the dilation index normalized to the mean amplitude of the baseline signal. Blue dots are baseline signal amplitudes, while red dots are post-occlusive period amplitudes. The continuous line is the post-occlusive result plot fitted curve with moving average.

Finally, we took the maximum value in each person's final result graphic and defined it as PPG-dilation index (PPG-DI) belonging to that person. The PPG-DI value is an indicator of the maximum increase in pulse wave amplitude in the post-occlusion period. Physiologically, it corresponds to the maximum dilatation response mediated by the post-occlusive blood flow and shear stress on the vessel wall.

Statistical analysis

GraphPad Prism software version 7 (GraphPad software, USA) was used to store and analyze the collected data. Descriptive statistical tools such as normality analysis, frequency distribution, median and interquartile range (IQR) were used in the data analysis. Continuous data were presented as median (IQR). Chi-square test was used when comparing categorical data between groups, and student's t-test, Mann-Whitney U or one-way analysis of variance (ANOVA) was used for comparison of continuous variables.

After calculating PPG-DI values for the whole study population, subgroup analyzes and comparisons were made. In subgroup analysis, the population was grouped according to age, BMI, gender, smoking status, and waist circumference, and then PPG-DI results were compared.

RESULTS

Demographic data and other measures

A total of 30 participants were included in the study. 15 (50%) of the participants were male, and 15 (50%) were female. Nine (30%) of the participants were current smokers. Participants' median (inter-quartile range) age was 48 years (31.3), height 169 cm (8.7), weight 72.5 kg (17), body mass index 24.3 kg/m^2 (6.7), waist circumference 97 cm (9.3), SpO_2 value 98.5% (2), systolic band pressure 123 mmHg (20) and diastolic blood pressure was 80 mmHg (10).

The median PPG-DI value of the whole study group was calculated as 112% (60). The minimum and maximum values were found to be 29% and 251%, respectively.

Table 1: Characteristics of the study group (n=30).

Characteristics	Median	IQR
Age	48	31.3
BMI (kg/m^2)	24.3	6.7
Weist circumference (cm)	96.5	9.3
Systolic blood pressure (mmHg)	123	20
Diastolic blood pressure (mmHg)	80	10
SpO_2 (%)	98.5	2

Correlation analysis results

The correlation between PPG-DI values and age, weight, BMI, waist circumference, systolic, and diastolic blood pressure values of the study population was investigated. The Pearson correlation coefficients (r) obtained in the analysis were as follows; r=-0.6278 for age, r=-0.4635 for BMI, r=-0.4628 for waist circumference, r=-0.4207 for systolic blood pressure, r=-0.2804 for diastolic blood pressure (Figure 3).

Subgroup endothelial function comparisons

The study group was divided into subgroups according to age, BMI, gender, smoking status, and waist circumference, and the endothelial function values of the groups were compared (Figure 4). Subgrouping was done using two-decade segments for age and median values for BMI and waist circumference.

Participants were divided into three groups according to their age: 20-39, 40-59, and 60 and above. Each age group consisted of 10 participants. The median PPG-DI values were calculated as 148% (68) for the 20-39 group, 97% (59) for the 40-59 group, and 85% (58) for the 60+ age group. In the one-way variance analysis, the difference between endothelial function results belonging to age groups was found to be statistically significant, F (2, 27) =8.018, p=0.0018. In paired group comparisons, 20-39 years versus 40-59 years and 20-39 years versus 60+ age differences were statistically significant (p=0.0345 and 0.0015, respectively). The difference from 40-59 versus 60+ age groups was not statistically significant (p=0.4199). In the next analysis, the study population was divided into two groups as BMI below and above 24.3 kg/m^2 (n=15 for each group). While the median PPG-DI value of the BMI <24.3 group was 127% (73), the value of the BMI ≥24.3 group was calculated as 100% (61). The difference between the endothelial function results of the two groups was statistically significant (p=0.0365).

To understand the effect of gender on endothelial function, the study population was divided into two groups as female (n=13) and male (n=17). Endothelial function measurement results were calculated as 126% (68) for women and 93% (61) for men. The difference in endothelial function results between the two groups was not statistically significant (p=0.0839).

To test the effect of smoking, the study group was divided into two groups as non-smokers (n=21) and smokers (n=9). The PPG-DI value of the non-smoking group was 118% (77), while the value of the smoking group was 100% (60). The difference between the two groups was not statistically significant (p=0.7954).

In the final analysis, the study group was divided into two groups as waist circumference <97 cm (n=15) and ≥97 cm (n=15). PPG-DI results were 136% (71) for the <97 cm group and 100% (57) for the ≥97 cm group. The difference between the two groups was statistically significant (p=0.0298).

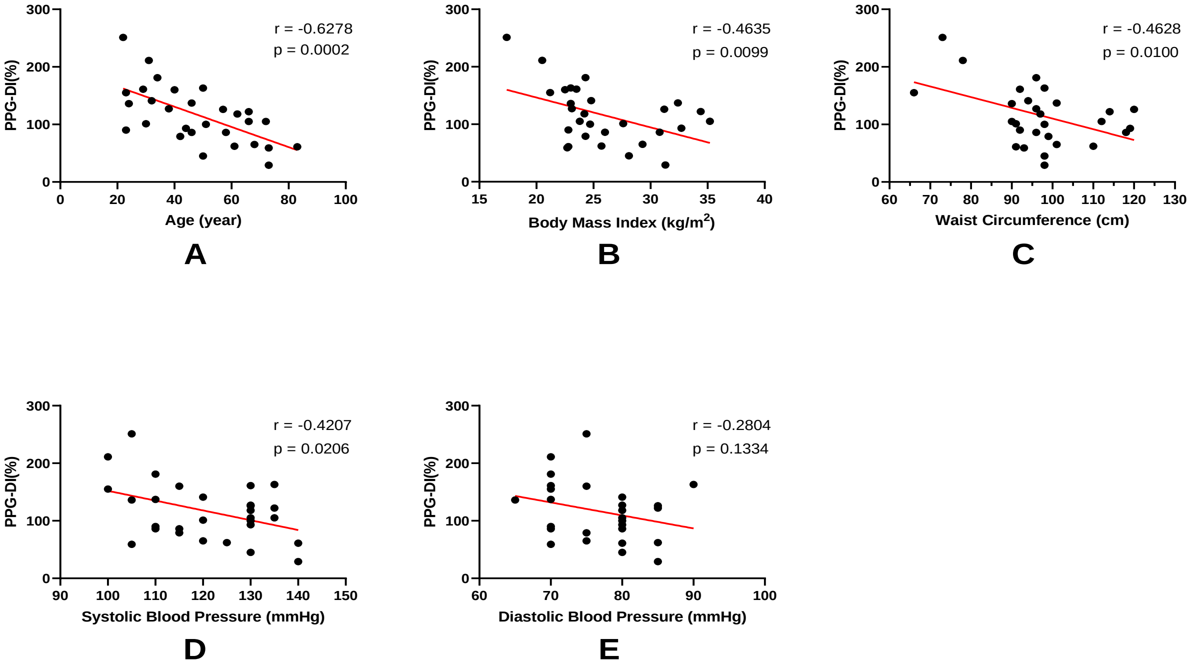

Figure 3: Correlation analyzes between endothelial function and other continuous variables. Pearson correlation coefficient (r) and correlation statistical significance values (p) are presented in each graph, (a) dilatation index (DI) correlation with age, (b) DI correlation with body mass index, (c) DI correlation with waist circumference, (d) DI correlation with systolic blood pressure values, and (e) DI correlation with diastolic blood pressure values.

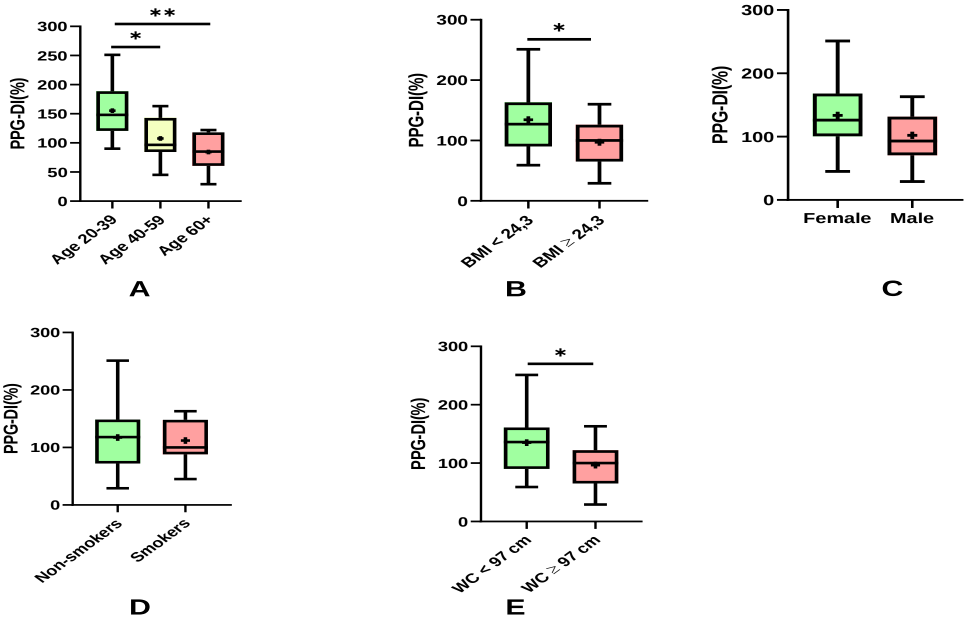

Figure 4: Endothelial function comparisons of study population subgroups. The lines inside the boxes indicate the median value, the lower and upper borders of the boxes indicate the first and third quartile values, the lower and upper lines border the minimum and maximum values, and the + sign inside the boxes indicates the average value. The *sign indicates the statistical significance of the difference between groups. BMI: body mass index, WC: waist circumference, (a) age subgroup analysis, age 20-39 versus age 40-59 versus age 60+, total ANOVA p=0.0018, *p=0.0345, **p=0.0015, (b) BMI subgroup analysis, BMI <24.3 versus BMI ≥24.3, *p=0.0365, (c) gender subgroup analysis, female versus male, p>0.05, (d) smoking subgroup analysis, non-smokers versus smokers, p>0.05, and (e) waist circumference subgroup analysis, WC <97 cm versus WC ≥97 cm, *p=0.0298.

DISCUSSION

USG-FMD is a non-invasive method for endothelial function evaluation. Although used in research, the difficulty of its application and the high cost of ultrasonic devices prevent it from being widely used in clinical settings. The challenges associated with traditional USG-FMD tests point to more practical and affordable methods such as PPG-FMD. The measurement of endothelial function with PPG-FMD has been investigated for about 15 years, and its use in clinical settings is increasing.

Sourij et al evaluated the effect of pioglitazone treatment on endothelial function using the PPG-FMD method in 42 patients with newly diagnosed diabetes mellitus.[15] After a treatment period, the improvement in endothelial function was found significantly higher in the treatment group than in the placebo group. In another study, endothelial functions of 31 healthy subjects and 52 participants with cardiovascular risk were evaluated simultaneously with PPG-FMD and USG-FMD methods.[16] It was seen that the endothelial function values calculated by both methods were significantly lower in the cardiovascular risk group, and the results of the two methods were correlated. In a study by Kuznetsova et al PPG-FMD measurement was applied to 311 volunteers from the FLEMENGHO cohort study.[17] At the end of this study, it was observed that PPG-FMD values were higher in women than men, in non-smokers compared to smokers, in normal-weight subjects compared to obese. In another recent study, PPG-FMD measurement was performed in 317 patients before coronary angiography, and it was investigated whether the PPG-FMD increases the success of age-adjusted Framingham risk score in predicting coronary artery disease (CAD).[18] In this study, it was concluded that endothelial function measured from the finger is an independent predictor for coronary artery disease and can be used in the detection of patients with high risk for CAD.

In the present study, we developed a new methodology on PPG signal correction and tested the method in healthy subjects. We used a continuous correction approach, while other groups use only a small portion of the post-occlusive signal in the correction process. This approach corrects the whole post-occlusive signal, not just the maximum amplitude part, and thus, it enables further analysis on the PPG-FMD result chart. This further analysis includes but is not limited to maximum slope in PPG-FMD result curve, area under the curve in time until amplitude returns to baseline level, frequency analysis of the curve. These analyses can provide information about the person's vascular health and cardiovascular risk, but they were not investigated as they were outside the current study scope.

In the correlation analysis, it is seen that PPG-DI values are negatively correlated with all variables. The negative correlation of these variables, which are the classic risk factors for atherosclerosis and cardiovascular diseases, with endothelial function is consistent with both physiopathological mechanisms and literature knowledge. On the other hand, to understand the relationship between these variables and microvascular endothelial function, more subjects should be investigated in multi-center trials, including different groups of patients.

When the subgroup analyzes is examined in our study, it is seen that the endothelial function decreases with age and that the difference between the 20-39 versus 40-59 and 20-39 versus 60+ age groups is statistically significant. Furthermore, even the difference between the 40-59 and 60+ groups was not statistically significant; the older group had a lower FMD-DI index.[19,20] Besides, the differences between BMI and waist circumference subgroups were found to be statistically significant. These results are consistent with both the atherosclerosis process's physiopathological infrastructure and the literature knowledge.[17,21]

We see that the endothelial function measurement of the female population was higher than the male population; however, the difference was not statistically significant (126% versus 93%). This result is consistent with our current literature knowledge.[17] It is known that the increase in the risk of cardiovascular disease and the decline in endothelial functions occur in women at an older age than men.[22] It is also known that the menopause process is effective on endothelial functions as in all vascular pathologies.[23] Therefore, studies that will examine menopause and hormone levels in larger women groups are needed to better understand the effect of gender on PPG-FMD values.

Limitations

The major limitation of the study was the small sample size. Another limitation is that the study was conducted in a single center. The proposed method and results may represent the general population when validated in larger populations and different patient groups in a multicenter study.

CONCLUSION

Our results in a healthy population are compatible with the literature knowledge and endothelial function physiopathology. We have introduced a signal correction approach that will allow further analysis of the PPG-FMD result signal. We believe that our study will be a basis for future studies on this subject.

USG-FMD method is the gold standard in endothelial function measurement; however, we believe it will leave its place to easily applicable methods. PPG-FMD could be a good alternative, with its operator-independent and easily applicable nature.

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REFERENCES

1. Godo S, Shimokawa H. Endothelial Functions. Arterioscler Thromb Vasc Biol. 2017;37(9):108-14.
2. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol. 2004;15(8):1983-92.
3. Yeboah J, Folsom AR, Burke GL, Johnson C, Polak JF, Post W, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: The multi-ethnic study of atherosclerosis. Circulation. 2009;120(6):502-9.
4. Abdel Hamid M, Bakhoum SWG, Sharaf Y, Sabry D, El-Gengehe AT, Abdel-Latif A. Circulating Endothelial Cells and Endothelial Function Predict Major Adverse Cardiac Events and Early Adverse Left Ventricular Remodeling in Patients with ST-Segment Elevation Myocardial Infarction. J Interv Cardiol. 2016;29(1):89-98.
5. Ayer A, Mills C, Donovan C, Christenson RH, Ganz P, Dubin RF. Associations of microvascular dysfunction with cardiovascular outcomes: The cardiac, endothelial function and arterial stiffness in ESRD (CERES) cohort. Hemodial Int. 2019;23(1):58-68.
6. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, et al. Paradoxical Vasoconstriction Induced by Acetylcholine in Atherosclerotic Coronary Arteries. N Engl J Med. 1986;315(17):1046-51.
7. Celermajer DS, Sorensen KE, Gooch VM, Miller L, Sullivan ID, Lloyd JK, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340(8828):1111-5.
8. Green DJ, Dawson EA, Groenewoud HMM, Jones H, Thijssen DHJ. Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. Hypertension. 2014;63(2):376-82.
9. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, et al. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. Circulation. 1995;91(5):1314-9.
10. Allen J. Photoplethysmography and its application in clinical physiological measurement. Physiol Meas. 2007;28(3).
11. Zahedi E, Jaafar R, Mohd Ali MA, Mohamed AL, Maskon O. Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. Physiol Meas. 2008;29(5):625-37.
12. Kuznetsova T, Van Vlierberghe E, Knez J, Szczesny G, Thijs L, Jozeau D, et al. Association of digital vascular function with cardiovascular risk factors: A population study. BMJ Open. 2014;4(3).
13. Mashayekhi G, Zahedi E, Movahedian Attar H, Sharifi F. Flow mediated dilation with photoplethysmography as a substitute for ultrasonic imaging. Physiol Meas. 2015;36(7):1551-1571.
14. Si D, Ni L, Wang Y, Liu J, Yang J, Yang P. A new method for the assessment of endothelial function with peripheral arterial volume. BMC Cardiovasc Disord. 2018;18(1).
15. Sourij H, Zweiker R, Wascher TC. Effects of pioglitazone on endothelial function, insulin sensitivity, and glucose control in subjects with coronary artery disease and new-onset type 2 diabetes. Diabetes Care. 2006;29(5):1039-45.
16. Zahedi E, Jaafar R, Mohd Ali MA, Mohamed AL, Maskon O. Finger photoplethysmogram pulse amplitude changes induced by flow-mediated dilation. Physiol Meas. 2008;29(5):625-37.
17. Kuznetsova T, Van Vlierberghe E, Knez J, Szczesny G, Thijs L, Jozeau D, et al. Association of digital vascular function with cardiovascular risk factors: A population study. BMJ Open. 2014;4(3).
18. Du B, Si D, Zhao D, Zhao Y, Wagatsuma K, He Y, et al. A new peripheral endothelial function measurement improves prediction of symptomatic coronary artery disease. J Int Med Res. 2020;48(6).
19. Skaug EA, Aspenes ST, Oldervoll L, Mørkedal B, Vatten L, Wisløff U, et al. Age and gender differences of endothelial function in 4739 healthy adults: The HUNT3 fitness study. Eur J Prev Cardiol. 2013;20(4):531-40.
20. Yavuz BB, Yavuz B, Sener DD, Cankurtaran M, Halil M, Ulger Z, et al. Advanced age is associated with endothelial dysfunction in healthy elderly subjects. Gerontology. 2008;54(3):153-6.
21. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. 2016;118(4):535-46.
22. Maas AHEM, Appelman YEA. Gender differences in coronary heart disease. Netherlands Hear J. 2010;18.12:598-603.
23. Celermajer DS, Sorensen KE, Spiegelhalter DJ, Georgakopoulos D, Robinson J, Deanfield JE. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J Am Coll Cardiol. 1994;24(2):471-6.

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