Normalization of Flow-mediated Dilation to Brachial Artery Material Property: A Feasibility Study*

Nimmi Sudarsan, Rahul Manoj, Raj Kiran V, Nabeel P M, *Member IEEE,* Dinu S Chandran, and Jayaraj Joseph

***Abstract*— Endothelial reactivity (ER) is widely measured using flow-mediated dilation (FMD) of brachial artery. Conventional measurement of FMD is influenced by factors such as input shear stress, arterial transmural pressure, diameter and thereby arterial material properties (Ɛ). Thus, for a reliable interpretation of FMD, it has to be normalized with respect to the above confounding factors. Normalization of FMD with shear stress at the time of measurement has been reported to reduce measurement variability. However, its widespread usage among the research community is limited. In this work, we examine the feasibility of normalizing the brachial FMD index (FMD%) to Ɛ : extrema (Ɛp), baseline (Ɛb) and extrema change (ΔƐ) post-ischemia using its inter-day variability against FMD. In-vivo measurements were performed on 10 participants for 2 consecutive days and simultaneous pressure-diameter cycles were collected to estimate the material properties during reactive hyperemia (RH). The box-whisker plot reveals differences in the mean and deviation of FMD to $FMD_{|Ɛb}$. A significant value for repeatability (ICC ≥ 0.6) was obtained for normalized FMD ($FMD_{|Ɛb}$) for specific stiffness index (β), pressure-strain elastic modulus (Ep), and local pulse wave velocity (PWV) as compared to FMD. Hence, normalization of FMD% to arterial Ɛ can potentially improve the measurement reliability of ER assessment.**

***Clinical Relevance*— This pilot study demonstrates the feasibility of brachial artery stiffness assessment during FMD and its potential use for normalizing the standard FMD measurement.**

I. Introduction

Vascular endothelial cells line the circulatory system, forming an anticoagulant barrier between the vessel wall and the blood [1]. Several studies have supported the significance of vascular inflammatory response in the pathogenesis of cardiovascular illnesses by inducing endothelial cell activation/dysfunction. The influence of the endothelium on vascular tone is characterized by the creation and release of various vasoactive chemicals that relax or constrict the vessel. These chemicals are classified into two types: endothelium-derived relaxing factors (EDRFs) and endothelium-derived constricting factors (EDCFs) [2]. Endothelial dysfunction is attributed to a reduction in EDRF bioavailability, notably NO, and an increase in EDCFs [3]. This imbalance impairs endothelial-dependent vasodilation, which is a functional feature of endothelial dysfunction.

The most common noninvasive evaluation of endothelial function is the measurement of brachial artery flow-mediated vasodilation (FMD). The measurement involves ~120 s of baseline diameter measurement, ~300 s of ischemia and ~180 s of reactive hyperemia. The relative change in diameter pre and post-ischemia gives the FMD index (FMD%). However, FMD % is influenced by variations in blood pressure, shear stress and arterial stiffness etc. [4]. Guidelines for the ultrasonic evaluation of brachial artery FMD [5] were published in 2002 in an effort to standardize FMD measurement across researchers. Since then, considerable research has been done to provide a more reliable and precise assessment of endothelium function. Given that physical pressure-related manipulations of SR tend to influence measurement of FMD, recent research has focused on whether FMD data should be adjusted for the shear rate stimulus [6]. However, studies have reported that the FMD to shear rate normalization method does not comply to the assumptions required for reliable conclusions [7]. Thus, there is an impending requirement of methods to improve the repeatability of FMD for reliable assessment of endothelial reactivity [8].

Addressing the need for standardization of FMD to improve it as a repeatable and reliable marker for endothelial reactivity (ER) assessment, we have developed a standardization technique using the brachial artery material properties (MP) estimated during the reactive hyperemia (RH) procedure. Simultaneous pressure and diameter cycles were recorded from the brachial artery to estimate the MP in terms of functional stiffness indices: specific stiffness index (β), pressure-strain elastic modulus (Ep), arterial compliance (AC), and local pulse wave velocity (PWV) [9][10]. Further, attempts were done to standardize FMD to: 1) Estimated stiffness during baseline measurement (Ɛb), 2) Extrema stiffness during RH (Ɛp), and 3) Difference in extrema stiffness during reactive hyperemia and baseline (ΔƐ). The normalized FMD ($FMD_{|Ɛ}$) was then compared with FMD for repeatability analysis.

The measurement protocol, data acquisition and processing is explained in Section II. A detailed discussion on the results and observations are discussed in Section III, followed by

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Nimmi Sudarsan, Rahul Manoj and Raj Kiran V are with the Department of Electrical Engineering, Indian Institute of Technology Madras, Chennai – 600036, India (e-mail: nimmi_sudarsan@smail.iitm.ac.in)

Nabeel P M is with Healthcare Technology Innovation Centre, Indian Institute of Technology Madras, Chennai – 600113, India.

Dinu S Chandran is with the Department of Physiology, All India Institute of Medical Sciences, New Delhi 110029, India.

Jayaraj Joseph is with the Department of Electrical Engineering, Indian Institute of Technology Madras, Chennai – 600 036, India.

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limitations and future scope. The study conclusions are outlined in Section IV.

II. Materials and Methods

A. FMD standardization protocol

The normalization of FMD% to estimated stiffness matrices was done using following equations,

$$\mathrm{FMD}_{|\mathcal{E}\mathrm{p}}\,\% = \frac{FMD}{\varepsilon_{extrema}} \quad (1)$$

$$\mathrm{FMD}_{|\mathcal{E}\mathrm{b}}\,\% = \frac{FMD}{\varepsilon_{baseline}} \quad (2)$$

Where $\varepsilon_{extrema}$ and $\varepsilon_{baseline}$ are the extrema stiffness matrix post deflation and baseline stiffness pre-inflation. FMD was also normalized to the difference in stiffness ($\Delta\mathcal{E}$) using equation (3),

$$\mathrm{FMD}_{|\Delta\mathcal{E}}\,\% = \frac{FMD}{\Delta\mathcal{E}} \quad (3)$$

$$\Delta\mathcal{E} = \varepsilon_{extrema} - \varepsilon_{baseline} \quad (4)$$

The beat-to-beat estimation of stiffness matrices requires the continuous, simultaneous acquisition of pressure and diameter throughout an FMD intervention. For this, an in-house cuff-based continuous pressure measurement system was developed and pressure measurements along side ultrasound imaging based diameter measurements were recorded.

B. Data acquisition and Processing

The pressure signals from the brachial artery were obtained using a cuff based continuous pressure monitoring device which consist of 1) A brachial bladder cuff to occlude at a sub diastolic set pressure. 2) An air pump (max. 500 mmHg) to inflate the cuff. 3) Three solenoid valves for fast/slow deflation and bypassing. 4) A pressure sensor (Honeywell 24PCCFA6D) to measure the pressure in the cuff and 5) A 32-bit ARM micro controller unit (LPC4370FET256, NXP Semiconductors) that regulates inflation and deflation. The digitized pressure signals from the microcontroller along with additional information (Patient information, anthropometric measurements, and timestamps of start of baseline, intervention and recovery) were received via USB and a LabVIEW (National Instruments, USA) based program was used for data processing.

The diameter signals were acquired using an ultrasound transducer (7-15 MHz) using B mode scanning (Sonix Touch+, BK Medicals, US) and analyzed using a continuous edge-detection and wall-tracking software (FMD Studio, Quipu, Netherlands). The instantaneous diameter, flow and shear data were saved for further analysis. Once the pressure signals were calibrated to the systolic and diastolic blood pressure (SBP-DBP), they were leakage compensated and the corresponding diameter cycles were taken to calculate the stiffness matrices: β, Ep, AC and PWV.

Pressure and diameter cycles obtained during the experiment were analyzed using a LabVIEW based platform and 10 corresponding cycles during baseline, intervention and recovery (~0 to 90 s after cuff deflation) were taken to estimate $\mathcal{E}$. Further, the extrema and averaged baseline values were used to normalize FMD to $\mathrm{FMD}|_{\mathcal{E}}$ using equations (1), (2) and (3). FMD and $\mathrm{FMD}|_{\mathcal{E}}$ indices for two consecutive days were estimated and compared for repeatability.

TABLE I. Participant Demography and Parameter description

Parameter	Value Mean ± SD
Number of participants	10
Male/Female	6/4
Age (years)	25 ± 4
Systolic blood pressure (mmHg)	122.79 ± 6.19
Diastolic blood pressure (mmHg)	72.60 ± 6.77
Systolic-extrema diameter (mm)	3.68 ± 0.73
End-diastolic diameter (mm)	3.23 ± 0.43
FMD%	15.82 ± 9.04

C. Study Population and Procedure

An in-vivo study was performed on 10 participants (6 males: 4 females; 25±4 years) over 2 consecutive days (20 measurements). The study was carried out in a controlled laboratory environment (23 ± 2 ^{0}C) at the Healthcare Technology Innovation Centre, Indian Institute of Technology Madras, India. Prior to the trial, participant information such as lifestyle and medical history were collected. People who were taking drugs, had cardiometabolic problems, smoked, or had a history of tobacco use were excluded from the study. While reviewing the study procedure, the suggested FMD recommendations were taken into account [4]. The study adhered to the guidelines of the Helsinki Declaration (revised in 2013). The study's objectives and methodologies were communicated to participants and an informed consent collected. None of the test participants were compensated.

The participants were advised to rest for 10-15 minutes in a supine position before the assessment, while the operator connected the brachial cuff for continuous blood pressure readings. Oscillometric BP readings were taken on the brachial artery before and after using an automated BP monitor (SunTech®247TM, SunTech Medical, Halma, UK). Continuous blood pressure measurement was taken along with cine loops of B-Mode diameter throughout the intervention (~600 s). Data synchronization was ensured by enabling time stamps. For assessment of repeatability, FMD measurements of the participants, were repeated the next day at the same time.

D. Statistical Analysis

Continuous variables are presented as mean ± standard deviations. The repeatability of measurements were analysed by means square estimates of between/within measurements for Day 1 and 2 using the ANOVA 2 factor test (without replication). The correlation between measurements was calculated using Intraclass correlation coefficient (ICC) which is the ratio between the variance of interest group and total variance in measurements. A value of ICC greater than 0.4 is considered as moderately repeatable and greater than

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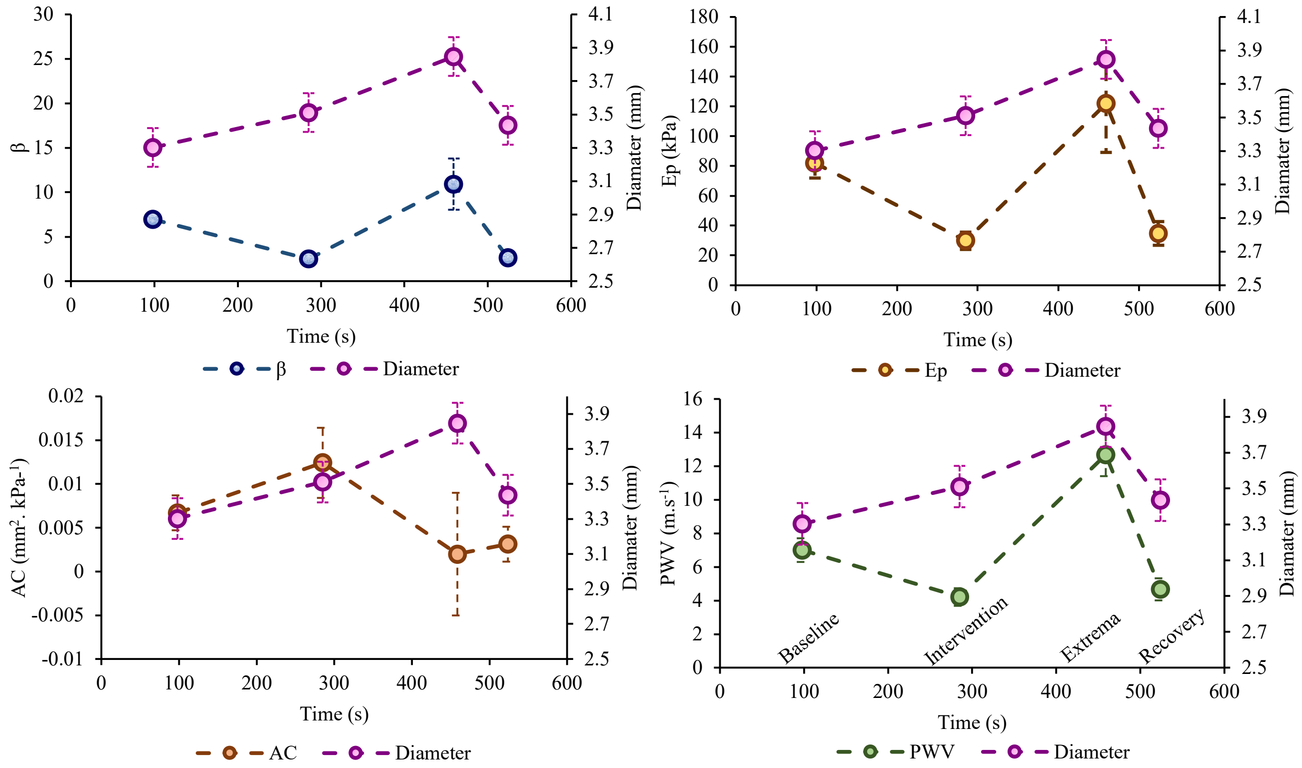

Fig 1. Diameter, β, Ep, AC and PWV during, baseline, intervention, extrema and recovery points.

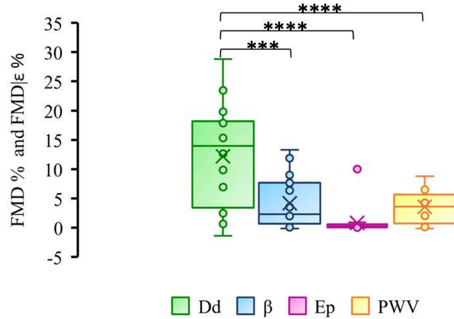

Fig 2. (b) Box_plots showing the mean and standard deviation of FMD versus FMD|ε

0.7 as highly repeatable. The Box plots were used to examine the patterns and degree of variance between two independent measurements.

III. Results and Discussion

TABLE I details the participants' baseline demographics, and FMD%.

A. *FMD normalization to baseline stiffness*

The mean value of diameter during baseline was 3.28 ± 0.67 mm and that of β, Ep AC, and PWV were 6.95 ± 0.72, 81.84 ± 9.88 kPa , 0.0067 ± 0.002 mm^2 kPa^{-1} and 6.99 ± 0.71 ms^{-1} respectively. The baseline values were in line with those reported in literature [11]. Though the stiffness response due to RH follows the diameter response, the percentage change in stiffness from baseline to intervention (reduced by 64% for β , Ep and 40% for PWV) for and recovery (increased by 57% for β, 48% for Ep and 81% for PWV) is different from that of diameter (Fig. 1), owing to the direct influence of pressure. The AC response (increased by 85% during intervention and reduced by 70% during recovery) is associated with reduced peripheral artery diameter impending to its inverse relationship [12].

TABLE II. ICC values comparing FMD and FMD|ε

Normalization method	β	Ep	AC	PWV
FMD$_{\|\varepsilon b}$	0.65	0.71	0.24	0.57
FMD$_{\|\varepsilon p}$	0.35	0.32	0.36	0.34
FMD$_{\|\Delta\varepsilon}$	0.29	0.24	0.39	0.26

The ICC values for FMD normalized to baseline β, Ep and PWV are significant (ICC > 0.5) as compared to the moderate repeatability of FMD% (ICC < 0.5) (Table II). The higher ICC values also demand further analysis to explore possible trends of material response to shear on larger cohorts. The box-plot reveals the difference in mean values of FMD and FMD|ε (Fig. 2).

B. *FMD normalization to extrema stiffness*

The post ischemia value of extrema β, Ep and PWV were 10.91 ± 2.88, 121.85 ± 32.81 kPa and 12.68 ± 1.29 ms^{-1}. The mean pressure observed during baseline was 95.52 ± 6.47

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mmHg, which was decreased by 3.34% leading to an elevation in the average value of β, Ep and PWV and a decrease in AC ($0.003 \pm 0.0.002$ $mm^2.kPa^{-1}$) during RH (Fig. 1). This is similar to previous studies, reporting that distending blood pressure increases pulse-wave velocity and incremental elastic modulus while decreasing distensibility and compliance [13]. This also throughs light on further investigation of independent predictability of arterial stiffness on ER. Normalization of FMD to $FMD_{|\varepsilon p}$ has only a low repeatability with AC (ICC = 0.36) demanding larger studies in this line.

C. FMD normalization to extrema change in stiffness

The group average value of difference in stiffness pre and post ischemia were 3.96 ± 3.81 for β, 40.01 ± 32.59 kPa for Ep, -0.0034 ± 0.004 $mm^2.kPa^{-1}$ for AC and. 6.11 ± 1.28 ms^{-1} for PWV. The ICC values gave a poor repeatability as compared to FMD confirming the inapplicability of this method to normalize FMD. This can be statistically due to the different trends of stiffness matrices during baseline and post ischemia.

D. Limitations and Future Scope

The study limitations, particularly the use of a small sample space for the inter-day repeatability investigation, should be highlighted when interpreting the reported observations. However, data was acquired through controlled experiments and can be increased for future study and to corroborate the given assumptions. Thus, the reported relationships are trustworthy and, to a large extent, correlate with allied in-vivo studies. More attempts are being made to translate this work into a clinical environment by recruiting large human cohorts and developing automated systems that simultaneously measure pressure [14]and diameter [15] cycles and analytical models that connect arterial stiffness response to direct estimates of stiffness for normalisation of FMD. Multimodal acquisition of functional stiffness indices directly from target artery [10], [16] during RH can improve the accuracy and repeatability to a large extend.

IV. Conclusion

This work investigates the feasibility of normalization of standard flow-mediated dilation index with extrema, baseline and difference values of stiffness markers measured in response to an input shear stress. 10 healthy participants were recruited for the study and inter-day measurements were taken for two consecutive days. A significant intraclass correlation value was obtained when FMD was normalized to baseline values of stiffness owing to its applicability in reducing subject specific variations in measuring endothelial response to shear. The varying AC trend in response to shear demands extending the study to a larger population. Further, development of devices for direct estimation of stiffness from brachial artery is required for improved reliability. Efforts by our group are in progress to realize methods employing such comprehensive analytical models for reliable assessment of ER.

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