SYSTEM MODELLING OF FLOW MEDIATED DILATION - EVALUATION OF TRANSFER FUNCTION PARAMETERS FOR INTERDAY AND INTRADAY REPEATABILITY

A Project Report

submitted by

J V RAGHUNATH

EE17B129

in partial fulfilment of the requirements
for the award of the degree of

DUAL DEGREE

DEPARTMENT OF ELECTRICAL ENGINEERING
INDIAN INSTITUTE OF TECHNOLOGY MADRAS.

JUNE 2022

THESIS CERTIFICATE

This is to certify that the thesis titled **SYSTEM MODELLING OF FLOW MEDIATED DILATION - EVALUATION OF TRANSFER FUNCTION PARAMETERS FOR INTERDAY AND INTRADAY REPEATABILITY**, submitted by **J V RAGHUNATH**, to the Indian Institute of Technology, Madras, for the award of the degree of **DUAL DEGREE IN ELECTRICAL ENGINEERING**, is a bona fide record of the research work done by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Chennai
Date: 17th June, 2022

Dr. Jayaraj Joseph
Research Guide
Assistant Professor
Dept. of Electrical Engineering
IIT Madras, 600 036

ACKNOWLEDGEMENTS

Foremost, I would like to thank my guide Dr. Jayaraj Joseph for his guidance over the entire project duration in terms of giving suggestions and discussions. I'm also grateful for my mentor Dr. Nabeel P M who gave ideas and corrected my understanding whenever I was stuck. I'm grateful to my lab mate Ms. Nimmi Sudarsan for the help with doubts, discussions and experiments

I thank all the authors whose papers I had referred, for their efforts which gave me different perspectives of understanding the concept.

ABSTRACT

KEYWORDS: Flow mediated-dilation(FMD) ; vascular age ; vasodilation ; shear stress ; FMD value ; transfer function ; Brachial artery

One of the leading causes of Cardio Vascular diseases(CVDs) is vascular aging. Endothelial dysfunction is a early biomarker of vascular aging. This is quantitatively described by the amount of Nitric Oxide (NO) produced upon shear stress on walls of brachial artery. Further, Influx of NO results in vasodilation which can be measured using FMD experiments. But the interpretation of FMD% value is difficult due to its inconsistency nature throughout days and within the day, i.e., different FMD% values on different days. To overcome this problem, this work proposes a standardised parameter which is more consistent than FMD% values. Consider brachial artery as the system, shear stress as its input and vasodilation as its output. Quantitatively vasodilation (FMD%) is normalised with shear stress, i.e., $\frac{FMD\%}{Shear\ stress}$ which iS transfer function of the system. The normalised FMD% parameters are found to be better than the FMD% value in terms of consistency and repeatability. This validates our assumption of FMD system model with its input and output. 4 non-hypertensive subjects in almost the same age group of 20's were taken to validate this approach for 5 consecutive days. The key aspect is that both FMD% and shear stress are estimated non-invasively using ultrasound probe.

TABLE OF CONTENTS

LIST OF FIGURES

ABBREVIATIONS

FMD	Flow Mediated Dilation
NO	Nitric Oxide
CVD	Cardiovascular disease
TIA	Transient Ischaemic Attack
COV	Coefficient Of Variation
MAD	Mean Average Deviation
SD	Standard Deviation
RC	Repeatability Coefficient
w.r.t	with respect to
VSM	Vascular Smooth Muscle

NOTATION

μ	Viscosity of blood
τ	Shear stress

CHAPTER 1

INTRODUCTION

1.1 Cardiovascular disease

There are many deaths caused due to cardiovascular disease. In 2019, 17.9 million deaths were estimated due to CVDs worldwide (32% of all deaths). The major reason for deaths in many countries is cardiovascular disease. It is estimated that one person dies in every **36 seconds** in US due to CVD. The disease variations in Asia(with 31% CVDs) is illustrated in the figure 1.1. It is mainly due to ignorance of individual's vascular health. Even young adults develop severe vascular diseases, if proper care is not taken. The CVDs are associated with a build-up of fatty deposits inside the arteries (atherosclerosis) and an increased risk of blood clots. It has also been linked to artery damage in organs like the brain, heart, kidneys, and eyes. There are many different types of cardiovascular disease,

- Coronary heart disease
- Stroke and TIA's
- Peripheral arterial disease
- Aortic disease

Coronary heart disease occurs when there is a reduced blood flow to muscle cells due to blockage which puts an increased strain in heart

A stroke occurs when a portion of the brain's blood supply is cut off, resulting in brain damage and possibly death whereas TIA is temporary brain's blood supply cut off

It is observed that predicting it earlier and taking necessary precautions and treatment is the key aspect to prevent it. Hence, we will be assessing one of the main early biomarkers of CVDs which is the extent of Endothelial dysfunction. We tried to quantize the biomarker with the health of artery which is the vascular age

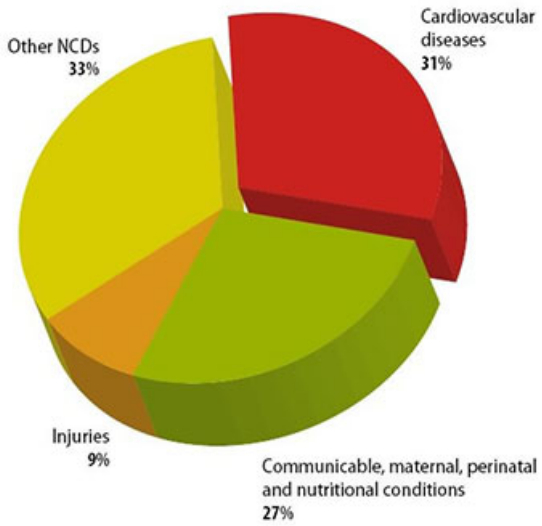

Figure 1.1: Variation of diseases in the population of Asia. (Photo courtesy of Global Atlas on Cardiovascular Disease Prevention and Control. Mendis S, Puska P, Norrving B editors. World Health Organization, Geneva 2011.)

1.2 Vascular aging

Vascular aging, the leading cause of cardiovascular disease, is linked to structural and functional changes in the vasculature, as well as deficiencies in the synthesis and release of endothelium-derived vasoactive substances. We estimate the vascular age by capturing these changes earlier. Comparison is given in figure 1.2

Effects of vascular aging

Aging effects on artery are listed down below,

- It looses its elastic behaviour and also the elastic gradient which is essential for smooth blood flow
- Endothelium dysfunction
- Slow thickening of Intima-media layer ($\sim 0.17mm/10years$)
- Increased arterial wall outer diameter

1.3 Endothelium Function

- Endothelium is single layer of squamous endothelial cells that lines the Intima layer and the first layer incontact with the Lumen

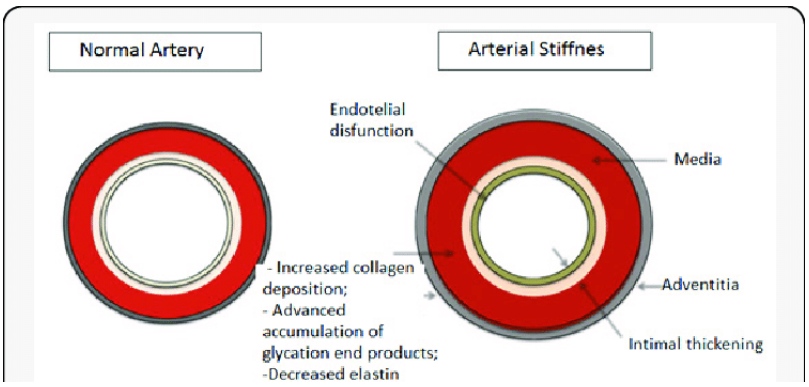

Figure 1.2: Comparison of artery before(left) and after(right) aging. source : [Finkler *et al.* (2019)]

- Upon activation of endothelial cells, they release various chemicals for vasodilation, vasoconstriction (Endothelin), etc
- The major vaso-dilator is found to be Nitric Oxide(NO), which is activated by shear stress on the Endothelial cells
- Upon shear stress, Ca^{2+} influx happens which reacts with L-arginine in presence of catalysts and eNOS(endothelial NO synthase) to form NO
- Now, the produced NO enters smooth muscle cells turning GC to cGMP which inturn relaxes the smooth muscle cells of artery leading to vasodilation
- There are two phases of NO release from endothelium :
  1. Brief transient Calcium - Calmodulin - dependent NO burst
  2. Sustained phase of low "NO" production lasting as long as shear is imposed, which occurs at resting Ca^{2+} concentration

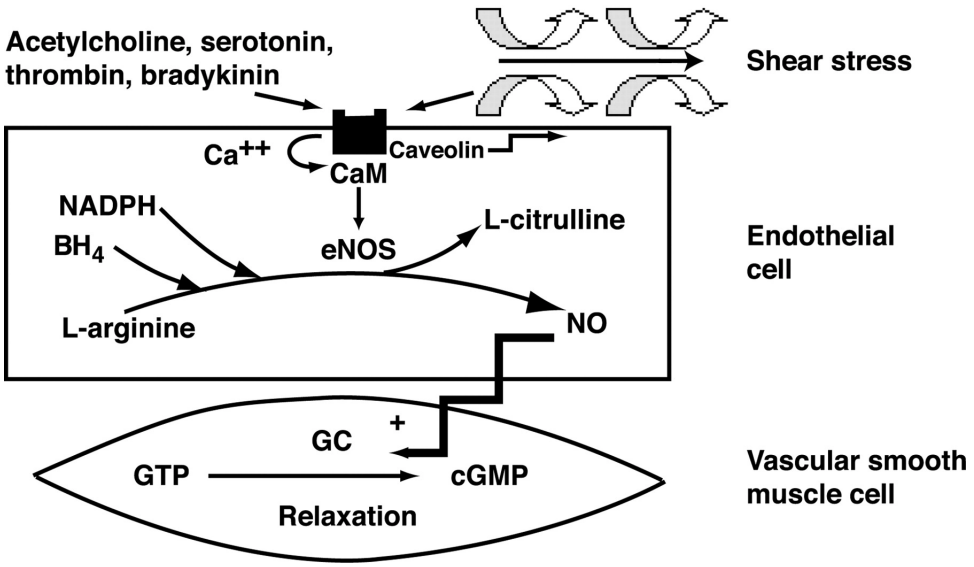

Figure 1.3: NO reaction chain in vasodilation

1.4 Endothelium Function analysis

Endothelium dysfunction is one of the early biomarkers of vascular aging and we quantitatively describe it by the amount of NO produced upon shear stress in brachial artery. This NO produced comes under brief transient calcium dependent NO burst. We relate the amount of NO produced by its effect which is vasodilation. FMD testing was created about 20 years ago as a noninvasive method of examining vasodilator performance in vivo in the brachial artery

There are 3 stages in a FMD experiment:

- Baseline period - Rest
- Intervention period - Cuff inflation
- Post deflation period - Quick cuff deflation

$$\text{FMD\%} = \left(\frac{\text{Maximum end diastolic diameter in post deflation - Average end diastolic baseline diameter}}{\text{Average end diastolic baseline diameter}}\right)^{*} 100$$

1.5 Research objectives

Modelling vasodilation of FMD experiment as shown below,
Brachial artery $\Rightarrow$ system
Shear stress $\Rightarrow$ input
Vasodilation(FMD%) $\Rightarrow$ output,
then we estimate the transfer function of this system which gives the performance of endothelial function of the artery. We are actually estimating endothelial function as amount of NO released per unit of shear stress applied in the artery

We observed that the normalised parameters(transfer function) are better consistent across the 5 consecutive days and also within a day of FMD experiment than the FMD% value. Then we validate the above system model for FMD

1.6 Literature Review

[Ma *et al.* (2021)] discusses about the parameter $\frac{mFMD\%}{eFMD\%}$, where $mFMD$ = measured FMD%, $eFMD$ = expected FMD%. The expected FMD% is calculated by the change in diameter equation,

$$\Delta d = \frac{2(P - P_{basal})}{\overline{E}} * \frac{(1-\nu^2)d_{basal}^2 D_{basal}}{D_{basal}^2 - d_{basal}^2}$$

where Δd is the change of inner diameter relative to the baseline diameter following a fluctuation of blood pressure $(P - P_{basal})$, ν is the poisson ratio. D_{basal}, d_{basal} are the outer and inner diameter in the baseline time interval which are measured before the experiment to calculate $eFMD\%$

$\overline{E}$ is the average arterial young's modulus. Response of $\overline{E}$ to NO is modelled as,

$$\overline{E} = E_v e^{a(\overline{c_w} - c_b)} + E_f$$

where E_v = basic elasticity of VSM, a is the response of VSM to NO, $\overline{c_w}$ is the NO concentration which is assumed to be linearly proportional to the concentration of L-NMMA and nitroglycerine before the experiment, E_f is the average Young's modulus of the collagen and elastin fibers, which is equal to $0.1MPa$, E_f and a are found from best fitting model. In vivo fitting result is $E_v = 9.8*10^5 Pa$; $a = -0.45$. Now one can use this to find Δd from pressure readings and estimate expected FMD% as below,

$$eFMD\% = \frac{\Delta d_{max} - \Delta d_{pre}}{d_{basal} + \Delta d_{pre}}$$

where d_{pre} is the arterial diameter at just before the cuff inflation. Now the parameter$\frac{mFMD\%}{eFMD\%} * 100$ is estimated, $mFMD\%$ and $eFMD\%$ differ in value due to the assumption that the subject is totally healthy. Hence, this ratio provides the health index of that person

Now the key aspect of this ratio is the normalization with expected FMD% for that person. This was the motivation for this work to normalise the measured FMD% with wall shear stress which is the main input to FMD system and we assess endothelium function alone by this transfer function model. A similar idea is briefly explained in [Guo and Kassab (2009*a*)]

1.7 Organization of the thesis

Chapter 2 gives an overview of the experimental procedures, theory and methods followed to estimate FMD% and shear stress values for the 4 subjects

Chapter 3 gives the analysis of the FMD experiment results with the estimated parameters and evaluates its significance

Chapter 4 concludes the work and gives the future scope for this work

CHAPTER 2

MATERIALS AND METHODS

In this chapter, we will discuss the theory behind shear stress calculation, FMD normalization with shear stress and the FMD experimental procedures

2.1 Shear stress calculation

2.1.1 Assumptions

In order to evaluate the shear stress on arterial wall, we make the following assumptions to make the calculations easier but also we will be as close to real life value as possible

- Blood is a Newtonian fluid, i.e., viscosity is constant with changing shear stress. We will account for this and take the best possible value for viscosity
- Blood flow is Laminar
- Blood flow is Incompressible, i.e., density is the same

The last assumption, i.e., density of blood being constant is almost true as given in [Vitello *et al.* (2015)] which says it is nearly equal to density of water. In the region of blood vessel bents, rough vessel inner surface, the density of blood varies significantly. But in the region of brachial artery, the arterial wall is not rough and there are no bents and so constant density for blood is a good assumption

As discussed in [Team (2015)], the majority of the body's vessels have laminar blood flow. Despite the fact that blood flow in arteries is pulsatile, laminar blood flow is silent. When there are some obstructions (or) constriction in blood vessel, the blood flow collapses and there is formation of small eddies which lead to flow in directions other than parallel to the long axis of the vessel. Such current eddies lead to turbulence flow, which is noisy. Also, higher blood flow velocity leads to turbulence, for example, blood flow in ascending aorta is turbulent

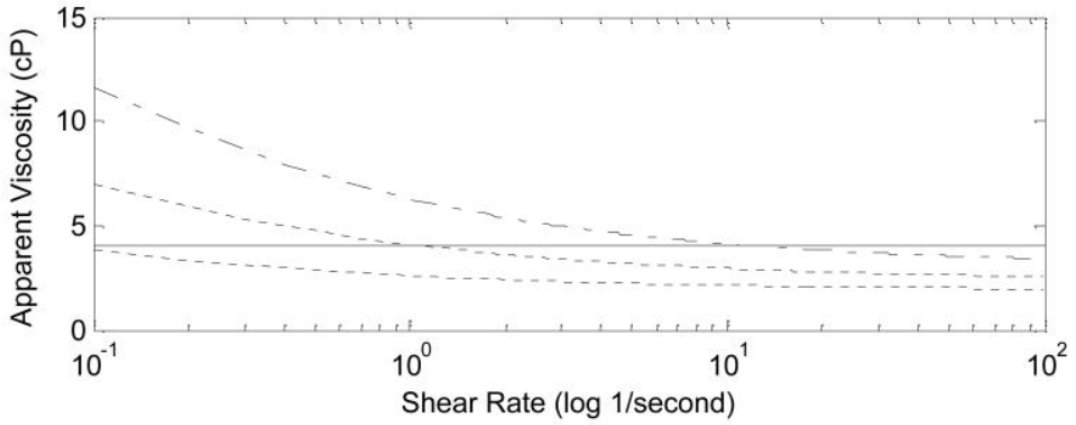

Figure 2.1: Variation of Apparent viscosity with shear rate of blood for hematocrit values of 20%, 32%, 45% in the blood. source : [Sprague (2010)]

In our case the blood flow is turbulent near the cuff due to inflation in the lower arm during FMD experiment but we take the ultrasound reading in upper arm of brachial artery far from the point of inflation of cuff and so blood flow is almost laminar.

2.1.2 Viscosity of Blood

The viscosity of blood(μ) changes with shear rate and so blood is non-newtonian fluid. In article [Sprague (2010)], the blood apparent viscosity model is plotted against shear rate as shown in figure 2.1 for various hematocrit levels of the blood model which is the major determinant of blood viscosity. As given in [Patel and Drummond (2010)], the normal range of hematocrit level is $33\% - 45\%$ in healthy humans and average shear rate is around 50 to 700(s^{-1}). So from the figure 2.1, we can say that viscosity do not change much in this range of operation and $\mu = 4cP$, is a good approximate for blood viscosity. More details on blood viscosity in brachial artery can be found in [Dammers *et al.* (2003)]

2.1.3 Velocity Profile Equation

Now, even though blood flow is pulsatile, we consider the flow to be steady for infinitesimally small time interval and proceed with the estimation of Shear stress. Now, consider the artery as a cylindrical pipe model with

L = Length of pipe, ΔP = pressure difference between two ends of pipe, R =

Radius of pipe, μ = Viscosity of blood. For laminar and incompressible flow of newtonian fluid, the flow profile will be parabolic as shown in figure 2.2 and the velocity at distance r from the centre is described by the equation (2.1). For more details regarding these equations can be found in [Vlachopoulos *et al.* (2011)] and [Ostadfar (2016)]

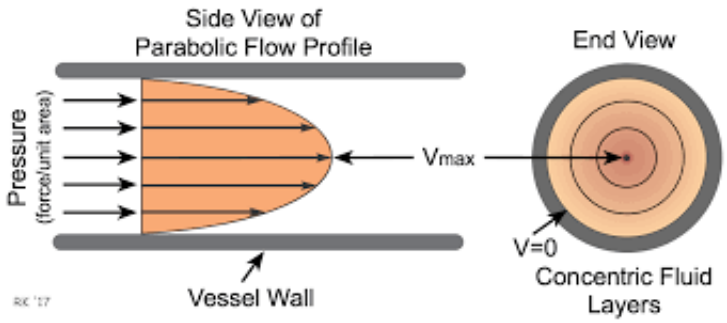

Figure 2.2: Parabolic flow profile of blood through artery for laminar and incompressible flow. source : [Reisfeld (2021)]

$$V(r) = \left(\frac{\Delta P}{4\mu L}\right)\left(R^2 - r^2\right) \tag{2.1}$$

After some manipulation,

$$\Rightarrow \boxed{\frac{\Delta P}{L} = \frac{4\mu V_{max}}{R^2}} \tag{2.2}$$

where V_{max} represents the peak velocity in the centre of vessel.

From Newton's law of Viscosity for newtonian fluid,

$$\boxed{\text{Shear stress}(\tau) = \mu\frac{dV}{dr}}$$

where V is the velocity of blood at a distance r from the axis of the vessel.

$$\tau = \frac{r\Delta P}{2L}$$

We are interested in wall shear stress, i.e., at endothelium,

$$\text{So, } r = R,\ \tau_{wall} = \frac{R(\Delta P)}{2L} \tag{2.3}$$

From the equations (2.2) and (2.3),

$$\boxed{\tau_{wall} = \frac{2\mu V_{max}}{R}} \tag{2.4}$$

Now since we are doing this for infinitesimally small time-interval, we write the equation 2.4 as,

$$\boxed{\tau(t) = \frac{2\mu V_{max}(t)}{R(t)}} \tag{2.5}$$

2.2 FMD normalization with Shear stress

As we will see in chapter 3 that FMD% is not consistent for a person through the 5 consecutive days and there are more variations, so we are unable to conclude a person's vascular health using FMD% precisely. To get rid of other parameters in the brachial vasculature that involve in FMD%, we take into consideration, the **wall shear stress** of brachial artery applied by the blood which is the main reason for the release of NO, the major vasodilator

2.2.1 Rate of release of NO

An interesting article [Kanai *et al.* (1995)] speaks about the NO release rate upon shear stress. The transient NO peak release rate is found to be increasing linearly with wall shear stress which was calculated by multiplying the peak NO concentration (in nanomoles per milliliter) times the flow rate (in milliliters per second) as shown in figure 2.3

But the overall NO peak concentration in blood remains unchanged with shear stress because the released NO is consumed entirely by Vascular Smooth Muscle (VSM) cells for vaso-dilation and some by the blood erythrocytes So we could say that the effect of shear stress is completely shown in NO release which is reflected in relaxing VSM cells of the arterial wall and dominate the arterial dilation, which is the principle of flow-mediated dilation (FMD) [DH *et al.* (2011)] and hence is captured by FMD% value. Concentration distribution of NO in endothelium upon shear stress in thoracic aorta is discussed in [Liu *et al.* (2014)].

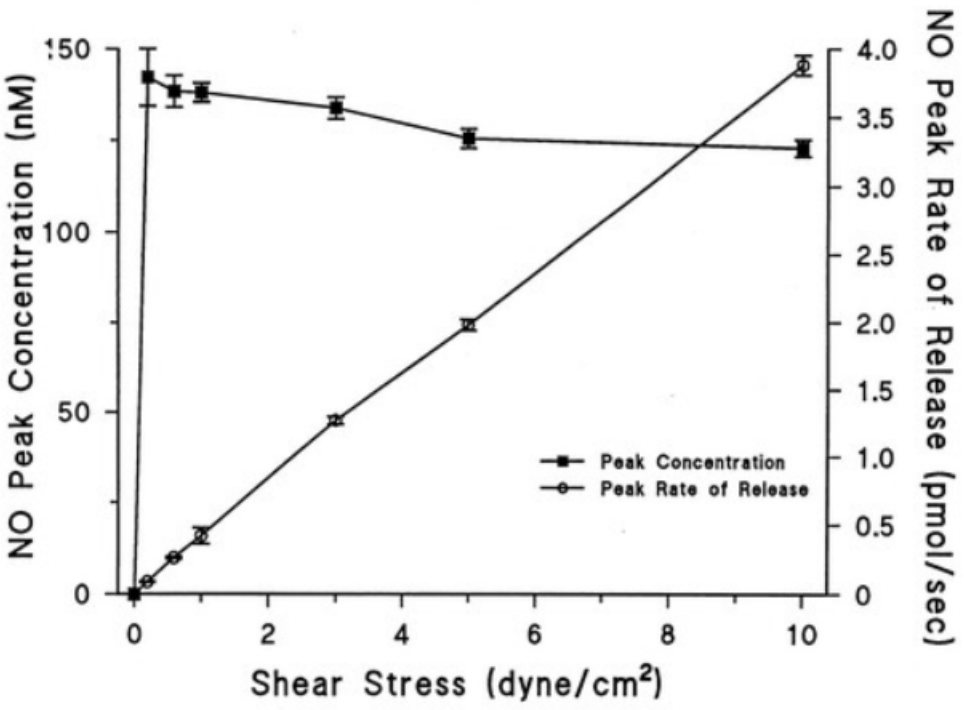

Figure 2.3: NO release rate and NO peak concentration with wall shear stress. source : [Kanai *et al.* (1995)]

2.2.2 Transfer Function parameters

We are interested in assessing the endothelial function, which is quantified by the amount of NO produced upon shear stress and not by magnitude of shear stress. Hence, we would like analyze the parameter which gives us the amount of NO produced per unit of shear stress. So, we normalize the FMD% ($\propto$ amount of NO released) value with the shear stress estimated.

The block diagram in figure 2.4, represents model of the system involved in FMD with its input as shear stress and output as FMD%, to represent the vasodilation. Essentially we are going to analyse the transfer function of this system in order to assess the endothelial function of brachial artery. Now, we know that shear stress changes with time even within a single pulse and the question arises as to which value to use. It is best to use the shear stress averaged over baseline period, the maximum shear stress during post-deflation period of FMD which accounts for FMD% value. Now we will define few shear stress notations,

- τ_{max} = Shear stress at the time instant of maximum diameter in post-deflation period
- τ_{basal} = Averaged shear stress over the baseline period

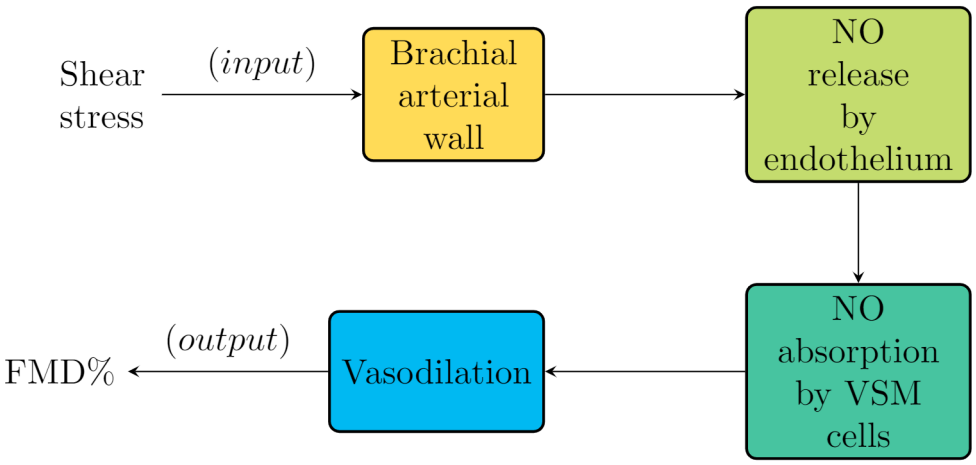

Figure 2.4: System involved in FMD

- $\Delta\tau = \tau_{max} - \tau_{basal}$
- $\tau_{avg} = \frac{\tau_{max}+\tau_{basal}}{2}$

To know about post-deflation and baseline period, one may refer to section 2.3. The 4 different parameters used to represent the system transfer function are,

- Parameter 1 = $\frac{FMD\%}{\Delta\tau}$
- Parameter 2 = $\frac{FMD\%}{\tau_{avg}}$
- Parameter 3 = $\frac{FMD\%}{\tau_{max}}$
- Parameter 4 = $\frac{FMD\%}{\tau_{basal}}$

We took 4 different parameters for analysis because it is not very clear that which one of the shear stresses acts as input to the system. So, the best evaluated parameter will be used

2.3 FMD Experiment

For the analysis of result we need Diameter, peak blood flow velocity data for every instant of time and we will get those results by FMD using imaging system

2.3.1 Experimental procedure

For the FMD experiment 4 non-hypertensive subjects were taken in the age of 20's. The Instrument used for the experiment is *Ultrasonix machine*

- Continuous measurement of peak blood flow velocity and arterial diameter is taken using simultaneous live duplex ultrasound
- Blood pressure is checked twice before and after the entire FMD procedure
- B-mode images with a linear probe of $\geq$ 7.5 MHz should be used. The highest MHz available should be used, given tissue depth considerations
- Baseline diameter is examined before cuff inflation for a period of 2 minutes after 10 minutes of supine rest
- Lower arm cuff is placed distal to the imaged artery as shown in figure 2.5, with cuff pressure exceeding systolic pressure by $>$ 50mmHg and inflated for 5 minutes. It is called as the intervention period

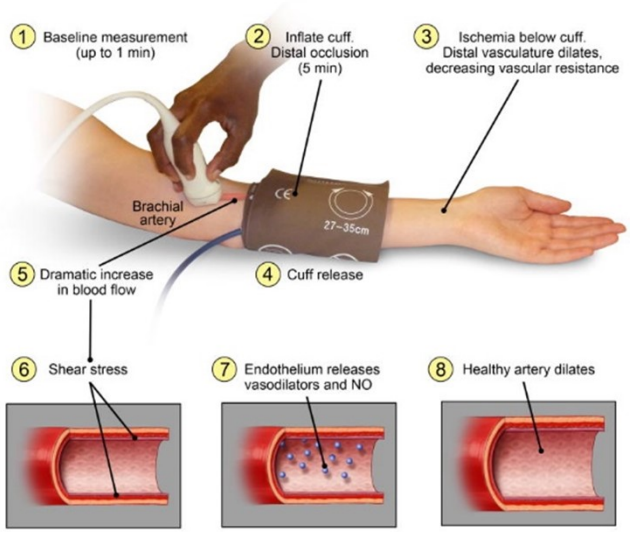

Figure 2.5: FMD procedure

- After intervention period, the cuff is deflated and the pressure is released
- Post-deflation diameter is monitored continuously from deflation for 3 minutes.
- Endothelium-dependent vasodilation is analyzed using `FMD studio` which is,
  - Continuous edge-detection
  - Blood vessel wall tracking
  - Blood flow velocity tracking software
- Automated mathematical algorithms are used to calculate the peak diameter(D_{max}) in post-deflation period and the baseline diameter(D_{basal} = average end diastolic diameter in baseline period for 20 good pulse cycles). It also calculates the recovery diameter(D_r = average end diastolic diameter in last 20s of post-deflation period)
- The FMD% is calculated as relative change

$$FMD\% = \left(\frac{D_{max} - D_{basal}}{D_{basal}} \right) * 100$$

- The shear rate is calculated for every instant $= \frac{V_{max}(t)}{R(t)}$ and it is averaged for 20 good pulse cycles in baseline period($= SR_{basal}$), and also the post-dilation shear stress at time instant of maximum diameter($= SR_{max}$) is calculated in the software
- Images during FMD experiments, photo of ultrasonix screen, images of edge detection and flow velocity capturing of FMD studio, Output screen of FMD studio are provided in the figures 2.6, 2.7, 2.8, 2.9, 2.10

Figure 2.6: FMD Experiment with ultrasonix

Figure 2.7: FMD Experiment with ultrasonix

Figure 2.8: FMD Experiment with ultrasonix

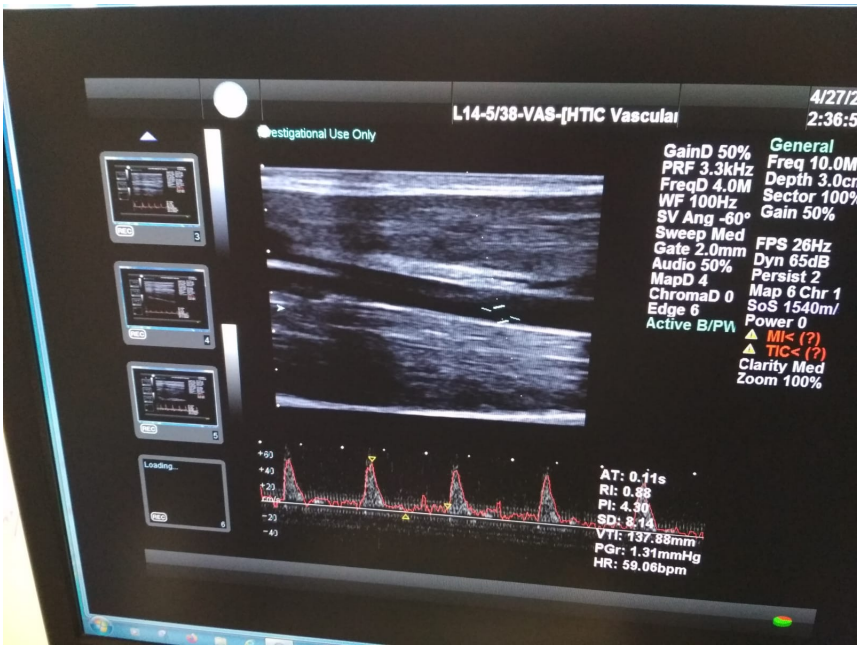

Figure 2.9: Ultrasonix screen during FMD experiment

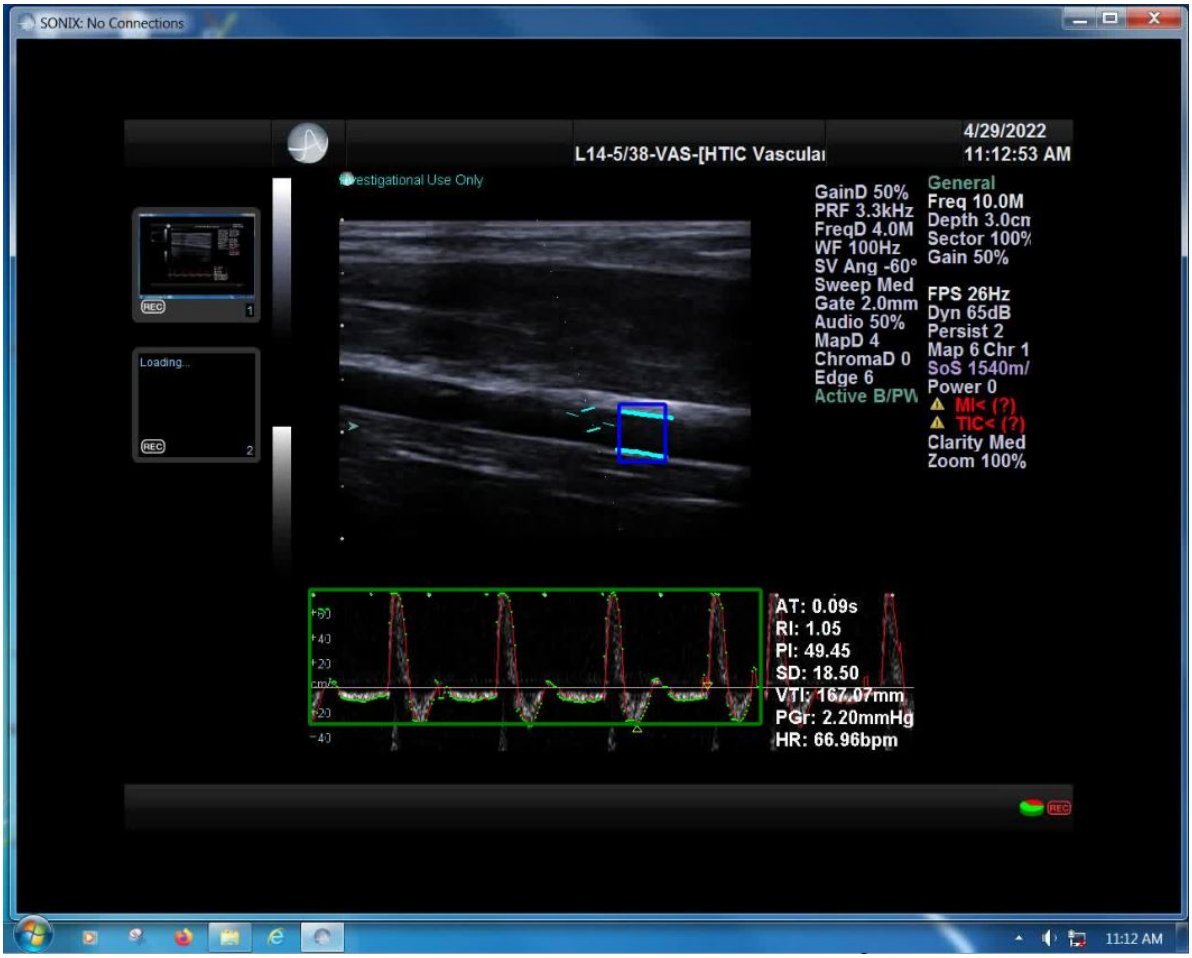

Figure 2.10: Analysis of experiment using `FMD studio`. It shows the edge detector algorithm for the artery and blood flow velocity capturing at the centre of artery in `FMD studio`

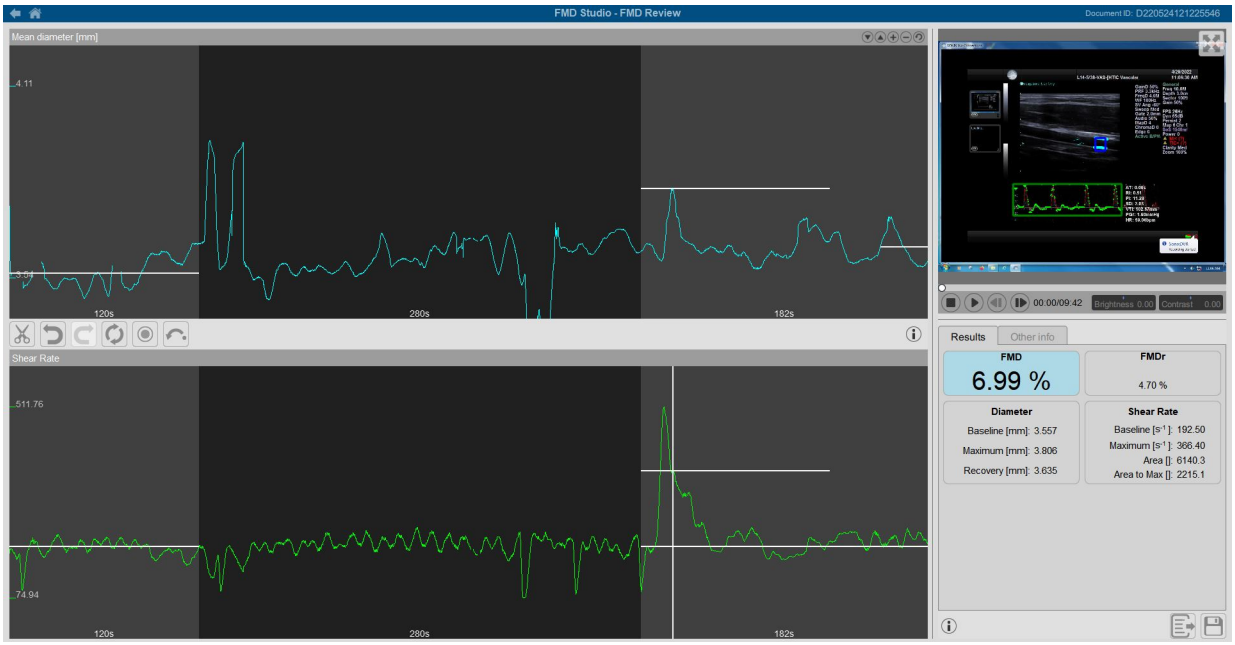

Figure 2.11: Output screen after analysis from `FMD studio`, showing values of $D_{basal}, D_{max}, D_r, SR_{basal}, SR_{max}$ and time varying plot of diameter and shear rate

2.3.2 Steps followed for reliable measurements

- Subjects must be fasted for ≥ 3 hours
- Subject should avoid exercise for $\geq$ 24 hours if he/she is not exercising regularly
- If subject is exercising regularly, then it is continued as per his/her regular schedule
- Subject should not consume alcohol or food/drinks that contain caffeine or are rich in polyphenols for ≥ 12 hours
- No smoking or any tobacco consumption prior to measurement (> 6 hours)
- Subjects were given 100ml of water and are asked to take 10 minutes rest before the start of experiment
- Careful history should be taken regarding the use/timing of any drugs. Drug withdrawal may be required, but this depends on the protocol and is not always feasible (in patients) or required
- Premenopausal women should be assessed in a standardized part of the menstrual cycle (especially when performing repeated measures) or the menstrual phase should be annotated
- Subjects may neither speak nor sleep during assessments
- Be aware of possible disturbance of data due to cardiac arrhythmia
- The operator should be a well-trained sonographer and the same operator should handle a particular subject
- Do not apply excessive pressure on the brachial artery with the tonometer probe. Do not attach cuff loosely or too tight

2.4 Conclusion for calculations

We can now estimate the shear stress values using equation 2.5,

$$\boxed{\tau_{max} = 2\mu * SR_{max}} \tag{2.6}$$

$$\tau_{basal} = 2\mu * \frac{\int_T \frac{V_{max}(t)}{R(t)}\, dt}{T}$$

where T is the baseline time interval,

$$\boxed{\tau_{basal} = 2\mu * SR_{basal}} \tag{2.7}$$

Now after performing the experiment we will be estimating the parameters using the equations 2.6, 2.7 and evaluating its consistency

CHAPTER 3

RESULTS AND DISCUSSION

In the plots, normalised parameters are represented as below,
FMD/ΔTau = $\frac{FMD\%}{\Delta\tau}$
FMD/Avg_Tau = $\frac{FMD\%}{\tau_{avg}}$
FMD/Max_Tau = $\frac{FMD\%}{\tau_{max}}$
FMD/Baseline_Tau = $\frac{FMD\%}{\tau_{basal}}$
which were discussed in 2.2

3.1 Experiment Results

After readings were taken from the FMD experiments for 4 subjects as detailed above, we then calculated the Shear stress and 4 normalised FMD parameters as discussed above in 2.2.2. The detailed measurement result is given in the table 3.1. In table 3.1, Basal Diameter = D_{basal}, Maximum Diameter = D_{max}, Recovery Diameter = D_r ; Basal Shear rate = SR_{basal}, Shear rate at instant of maximum diameter = SR_{max}, the detailed description of these were discussed above in 2.3. As you could see, there are some negative FMD% data which do not contradict the theory behind FMD, i.e., vasodilation but the ultrasound probe is very sensitive that even small changes in the position of artery changes the angle of probe w.r.t artery and hence the image of artery. So, the perspective of image changes sometimes and it is easily identified in our analysis. There are some subjects with negative FMD values which are unexplained theoretically, but in our case negative FMD% is explained with small angle changes in probe. Hence, some wrong FMD results are not considered for parameter estimation and its analysis

Subject ID	Day	Diameter (in mm)			FMD%	Shear rate (in s^{-1})	
		Basal	*Maximum*	*Recovery*		*Basal*	*Maximum*
1	1A	3.36	3.57	2.86	6.38	168.75	458.38
	1B	3.24	3.44	3.39	6.14	176.53	665.62
	2A	3.28	3.55	3.51	8.02	144.45	440.23
	2B	3.33	3.6	2.92	8.08	155.76	522.3
	3A	3.13	3.25	3.28	3.9	237.91	375.41
	3B	3.42	4.02	3.62	17.34	203.69	446.28
	4A	3.34	3.52	3.62	5.25	156.96	296.27
	4B	3.25	3.53	3.37	8.7	190.61	408.9
	5A	3.56	3.81	3.64	6.99	192.5	366.4
	5B	3.45	3.45	3.26	-0.18	175.97	138.61
2	1A	3.86	3.99	3.6	3.51	143.19	464.37
	1B	4.02	4.18	4.09	4.06	165	263.8
	2A	3.86	4.12	3.94	6.77	143.2	364.06
	2B	3.9	3.91	3.82	0.33	170.38	179.71
	3A	3.66	3.77	3.73	3	150.64	309.09
	3B	3.46	3.53	3.4	2.13	192.77	320.61
	4A	3.86	3.95	3.75	2.31	186.31	306.31
	4B	3.56	3.65	3.6	2.55	168.63	304.2
	5A	3.91	3.88	3.96	-0.61	128.9	254.51
	5B	3.51	3.64	3.65	3.72	87.42	120.25
3	1A	4.12	4.45	4.17	7.94	118.39	416.77
	1B	4.02	4.09	3.72	1.63	109.16	268.7
	2A	3.9	4.1	4.07	5.27	111.73	260.63
	2B	4.34	4.16	3.96	-4.27	105.68	158.13
	3A	3.77	4.12	4.24	9.45	169.59	385.51
	3B	3.69	3.83	3.74	3.55	155.17	185.79
	4A	3.62	3.73	3.32	3.09	109.36	230.1
	4B	3.96	4.04	3.73	1.93	125.5	184.38
	5A	3.91	3.96	4.15	1.2	140.23	203.45

	5B	4.03	4.13	4.2	2.65	69.79	77.78
4	1A	2.27	2.56	2.24	12.4	96.73	290.43
	1B	2.23	2.44	2.33	9.24	115.11	283.65
	2A	2.63	2.98	2.42	13.15	87.41	167.19
	2B	2.63	2.87	2.79	9.02	94.06	250.69
	3A	2.27	2.58	2.4	13.72	150.95	517.81
	3B	2.49	2.77	2.66	11.55	73.45	311.29
	4A	2.22	2.54	2.45	14.57	192.66	481.89
	4B	2.36	2.5	2.4	5.83	109.21	224.33
	5A	2.3	2.46	2.51	7.16	193.79	602.66
	5B	2.47	2.52	2.25	2.05	132.43	369.26

Full subject details with the 4 estimated parameter values averaged over all 5 days are provided below

Subject ID	: 1
Height	: 176 cm
Weight	: 68 kg
BMI	: 22 kg/m^2
Age	: 22
Average SBP	: 112 mmHg
Average DBP	: 65 mmHg
Pulse Rate	: 59 bpm
Average D_{basal}	: 3.32 mm
Average τ_{basal}	: 1.45 Pa
Average FMD	: 7.87%
Average $\frac{FMD}{\tau_{max}-\tau_{basal}}$	: 4.18%
Average $\frac{FMD}{\frac{\tau_{max}+\tau_{basal}}{2}}$	: 3.19%
Average $\frac{FMD}{\tau_{max}}$	: 2.28%
Average $\frac{FMD}{\tau_{basal}}$	: 5.5%

Subject ID	: 2
Height	: 178 cm
Weight	: 110 kg
BMI	: 34.7 kg/m^2
Age	: 26
Average SBP	: 118 mmHg
Average DBP	: 71 mmHg
Pulse Rate	: 78 bpm
Average D_{basal}	: 3.78 mm
Average τ_{basal}	: 1.3 Pa
Average FMD	: 2.57%
Average $\frac{FMD}{\tau_{max}-\tau_{basal}}$	: 2.75 %
Average $\frac{FMD}{\frac{\tau_{max}+\tau_{basal}}{2}}$	: 1.3 %
Average $\frac{FMD}{\tau_{max}}$	: 1 %
Average $\frac{FMD}{\tau_{basal}}$	: 2 %

Subject ID	: 3
Height	: 170 cm
Weight	: 70 kg
BMI	: 24.2 kg/m^2
Age	: 25
Average SBP	: 120 mmHg
Average DBP	: 73 mmHg
Pulse Rate	: 73 bpm
Average D_{basal}	: 3.9 mm
Average τ_{basal}	: 0.99 Pa
Average FMD	: 4.36%
Average $\frac{FMD}{\tau_{max}-\tau_{basal}}$	: 3.45%
Average $\frac{FMD}{\frac{\tau_{max}+\tau_{basal}}{2}}$	: 2.47%
Average $\frac{FMD}{\tau_{max}}$	: 1.78%
Average $\frac{FMD}{\tau_{basal}}$	: 4.2%

Subject ID	: 4
Height	: 157 cm
Weight	: 46 kg
BMI	: 18.7 kg/m^2
Age	: 23
Average SBP	: 99 mmHg
Average DBP	: 70 mmHg
Pulse Rate	: 86 bpm
Average D_{basal}	: 2.36 mm
Average τ_{basal}	: 1.03 Pa
Average FMD	: 9.5%
Average $\frac{FMD}{\tau_{max}-\tau_{basal}}$	: 5.4%
Average $\frac{FMD}{\frac{\tau_{max}+\tau_{basal}}{2}}$	: 5.1%
Average $\frac{FMD}{\tau_{max}}$	: 3.45%
Average $\frac{FMD}{\tau_{basal}}$	: 10.2%

3.2 Variability and Repeatability of parameters

The main objective of this experiment is to evaluate the consistency and repeatability of the normalised parameters for the 5 consecutive days. The evaluation methods we adopt to illustrate it are the following:

- Coefficient of Variation
- Mean Absolute Deviation
- Standard Deviation
- Repeatability coefficient

Coefficient of Variation(COV) is a statistical measure of relative dispersion of the data about its mean.

$$COV\% = \frac{\sigma}{\mu} * 100$$

where σ = standard deviation, μ = mean of the data set taken for the 5 days. COV is best regarded for the evaluation of extent of variability of the data around

its mean. The COV% for the estimated normalised parameters and FMD are plotted against the subjects in figure 3.1 for comparison We could see that nor-

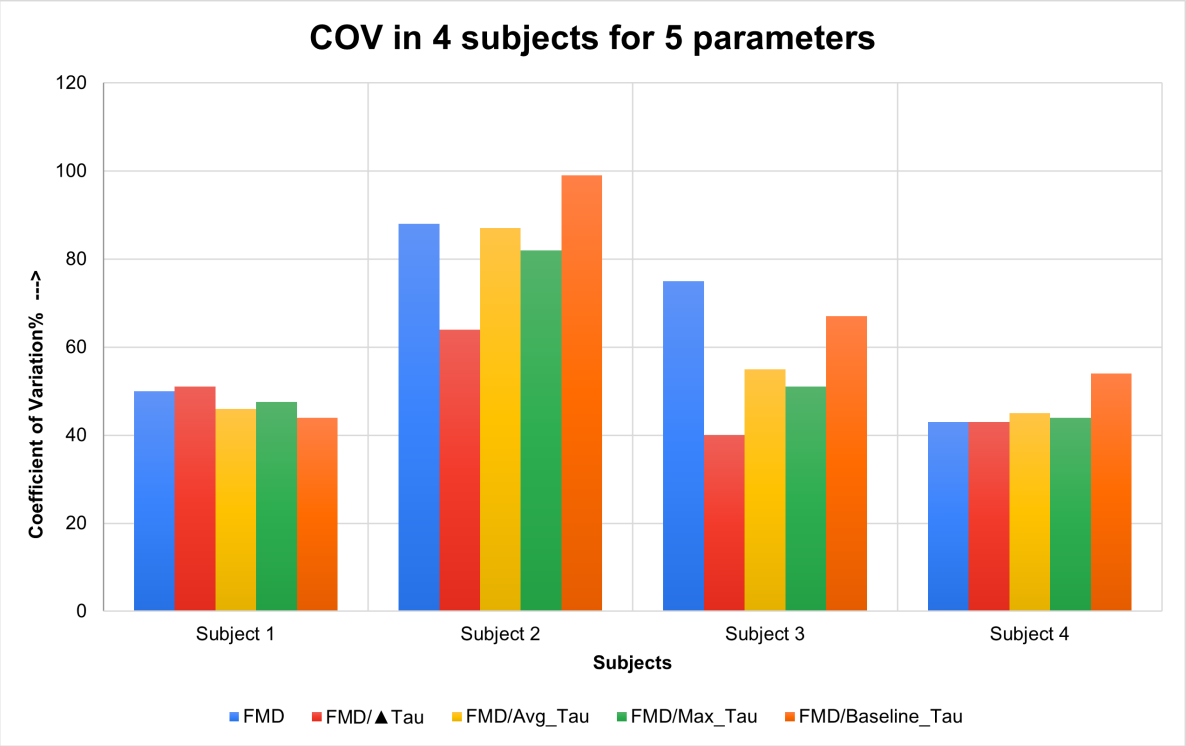

Figure 3.1: COV% of parameters for the 4 subjects

malised parameters have lower COV when compared to FMD%, except the $\frac{FMD\%}{\tau_{basal}}$ parameter. Particularly, $\frac{FMD\%}{\Delta\tau}$ term has very low COV when compared to FMD%

Mean Absolute Deviation(MAD) is a measure of average absolute deviation of data around its mean but it is not relative.

$$MAD = \frac{\sum_{n=1}^{N} |x_i - \mu|}{N}$$

where x_i's are the observations, N = no. of observations, μ = mean of the data set. It gives an essence of how varied is our data with its mean, similar to standard deviation. MAD of normalised parameters and FMD% are plotted against the subjects in figure 3.2

We observe again that normalised parameters gives lesser deviation when compared to FMD's mean absolute deviation

Standard Deviation(SD) is a measure of root mean square variation of observations with mean. SD of normalised parameters and FMD% are plotted in figure 3.3 for comparison

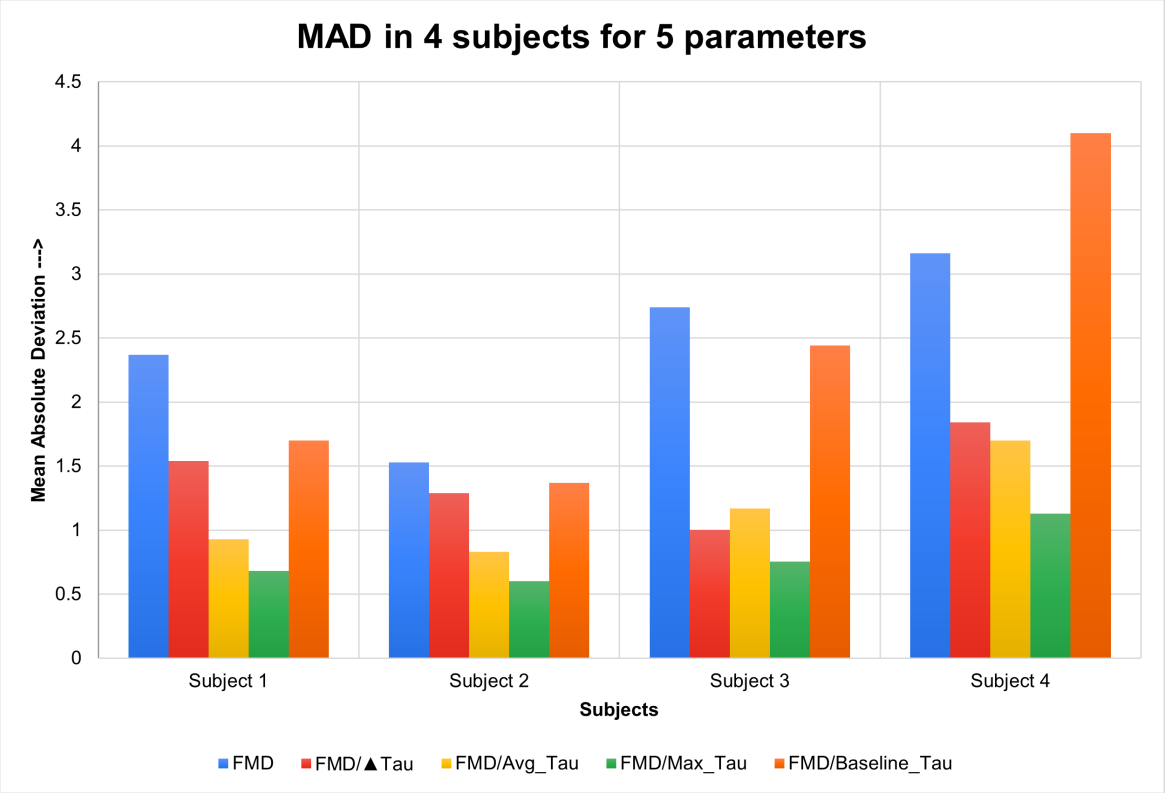

Figure 3.2: MAD of parameters for the 4 subjects

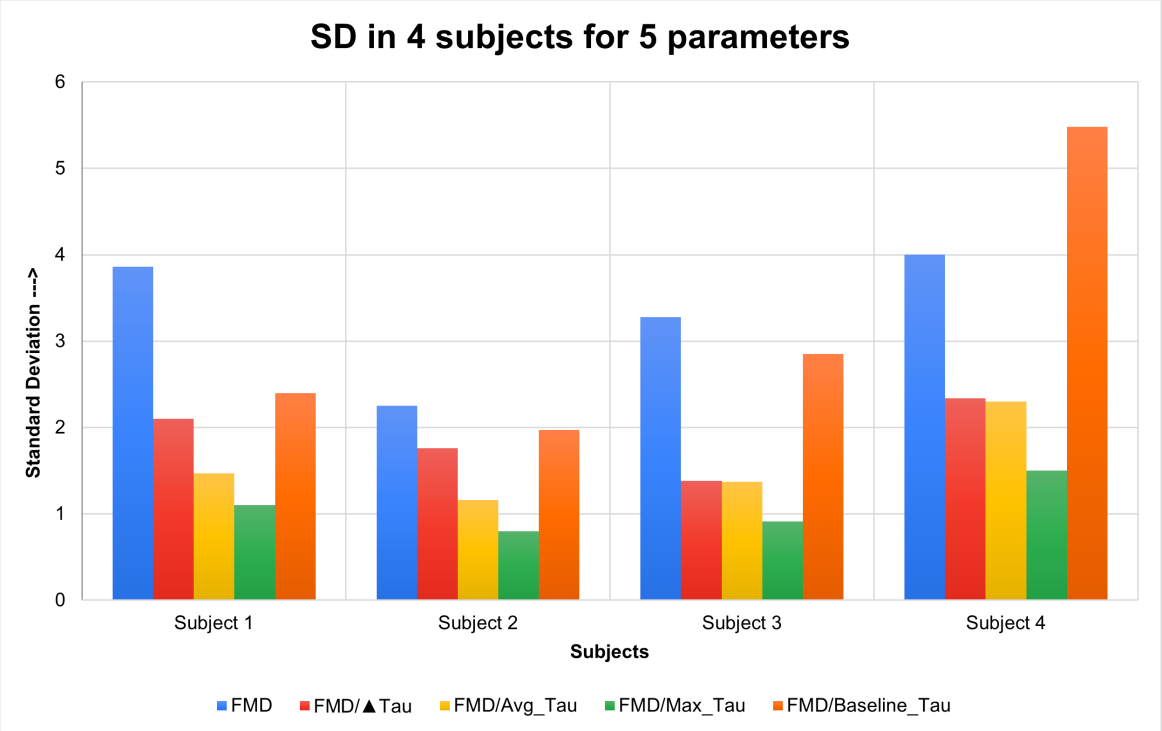

Figure 3.3: SD of parameters for the 4 subjects

It is also observed that SD is better for the normalised parameters than FMD

The last evaluation is done with repeatability coefficient(RC), which tells us about definite range of the parameter value around its mean with 95% probability.

$$\text{Range} = \text{Mean} \pm \text{RC}$$

where $\text{RC} = 1.96 * \sqrt{2} * \sigma$, σ represents standard deviation of the data set. The repeatability ranges for different parameters are plotted against the subjects in figures 3.4, 3.5, 3.6, 3.7, 3.8

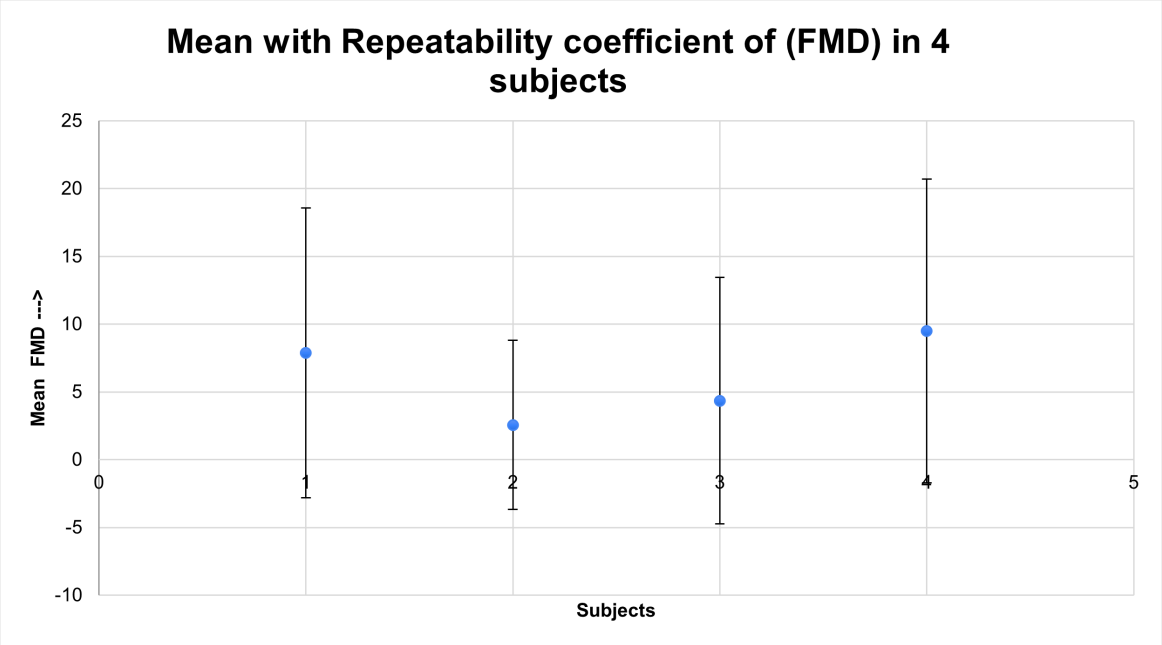

Figure 3.4: Range of FMD% values from RC for the 4 subjects

Now by carefully observing the figures 3.4, 3.5, 3.6, 3.7, 3.8 , we see that the range for FMD, $\frac{FMD\%}{\tau_{basal}}$ values are large compared to all other parameters. So, we see a shorter range for other 3 normalised parameters

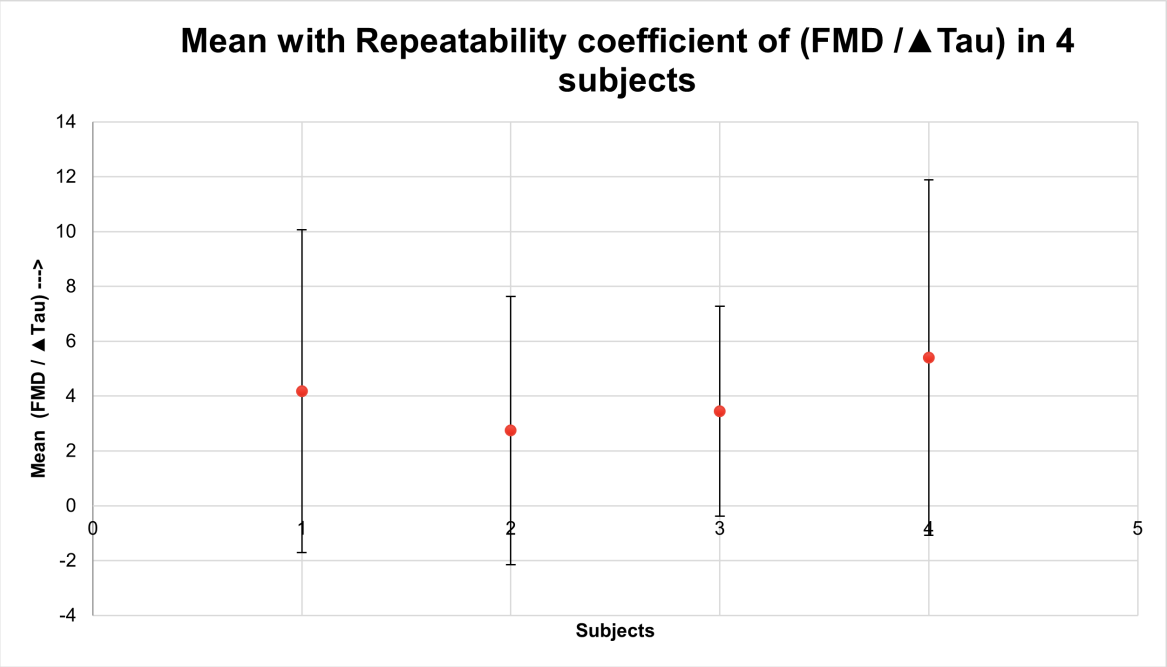

Figure 3.5: Range of $\frac{FMD\%}{\Delta\tau}$ values from RC for the 4 subjects

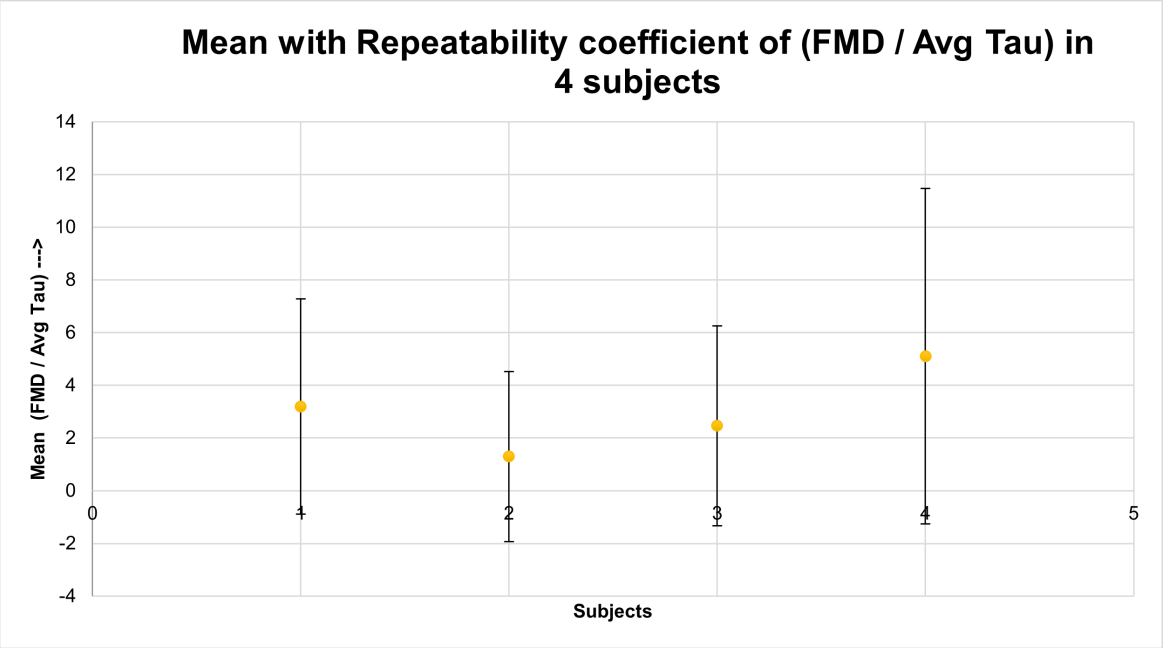

Figure 3.6: Range of $\frac{FMD\%}{\tau_{avg}}$ values from RC for the 4 subjects

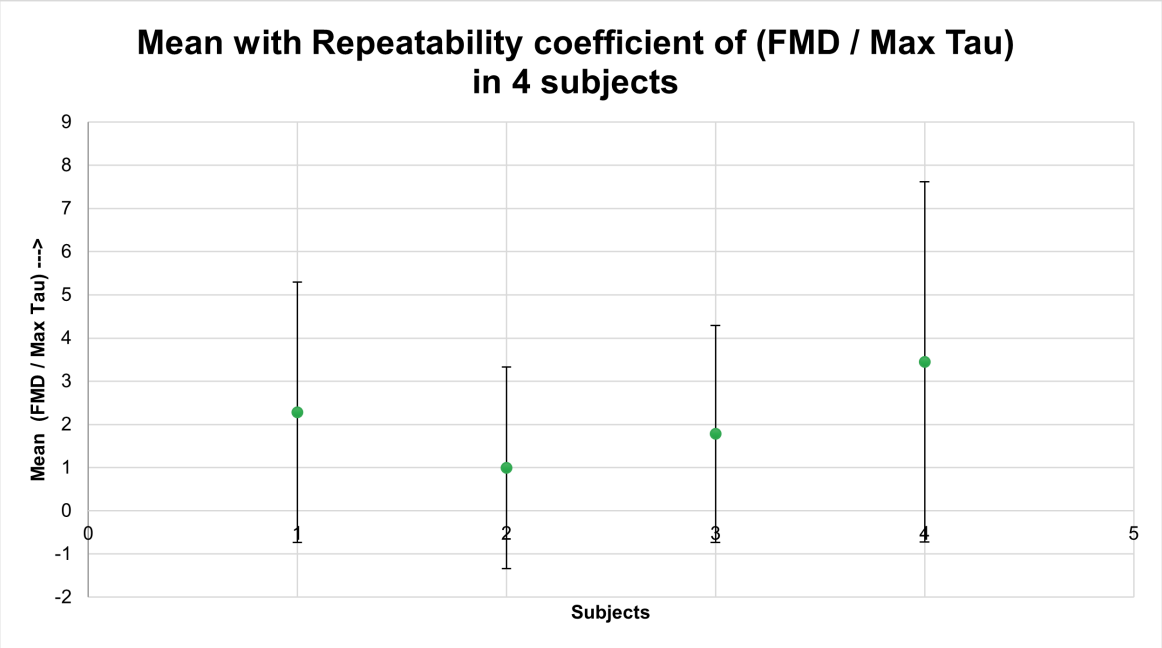

Figure 3.7: Range of $\frac{FMD\%}{\tau_{max}}$ values from RC for the 4 subjects

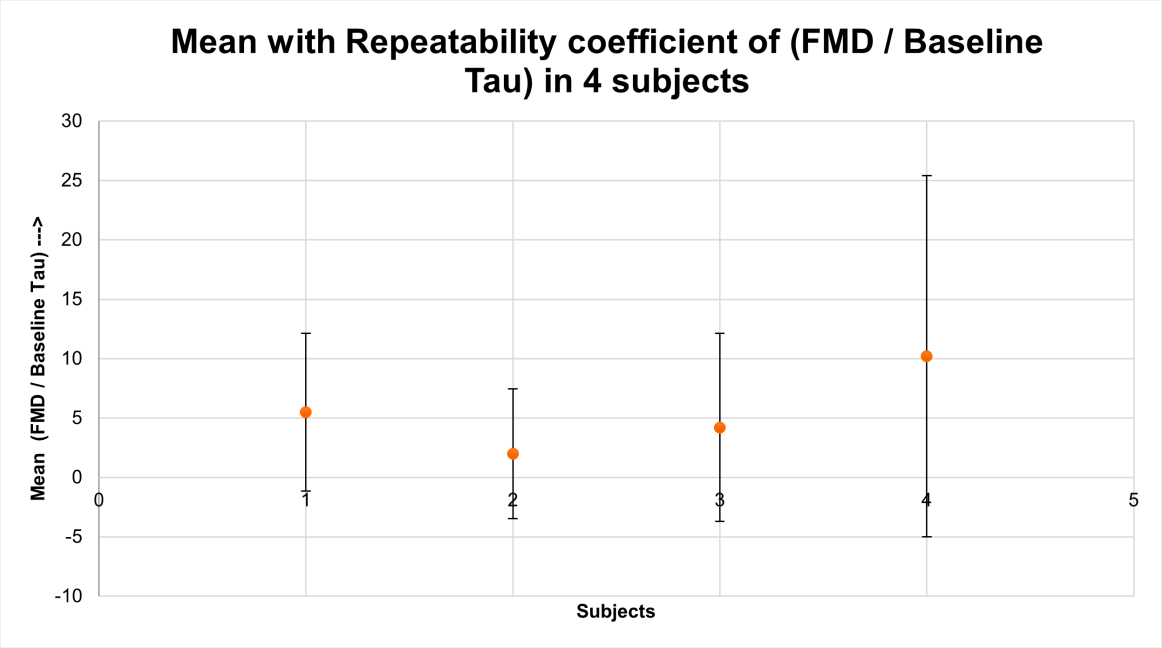

Figure 3.8: Range of $\frac{FMD\%}{\tau_{basal}}$ values from RC for the 4 subjects

3.3 Interday Analysis

The experimental results we have obtained is for 5 consecutive days and we are curious to know about the behaviour of the parameters over these days. So we found the average of morning and evening data for each day and plotted it against the days for each parameter in figures 3.9, 3.10, 3.11, 3.12, 3.13

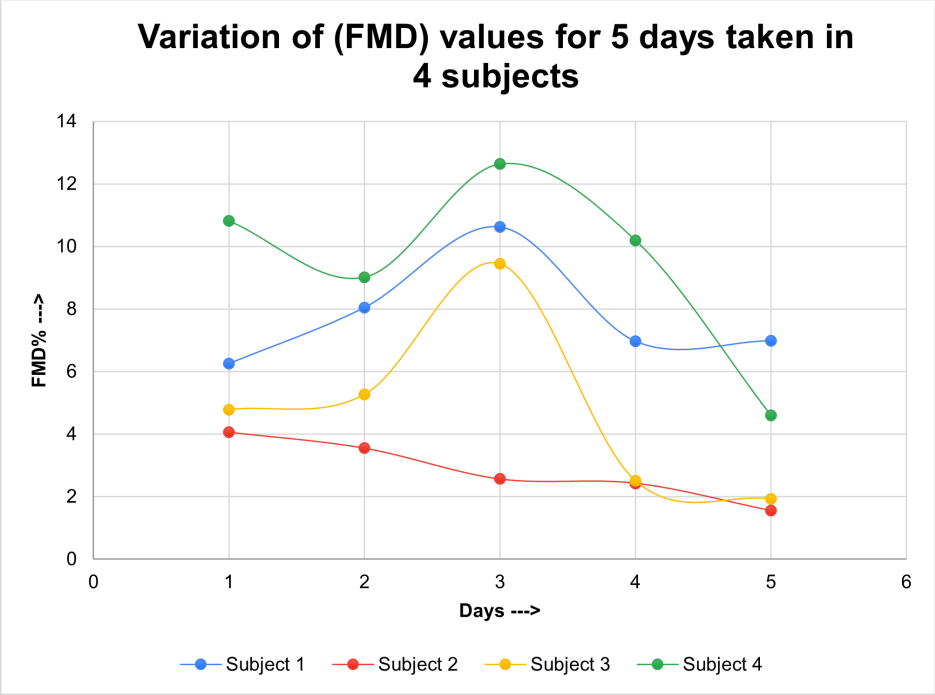

Figure 3.9: Variation of FMD% over the days

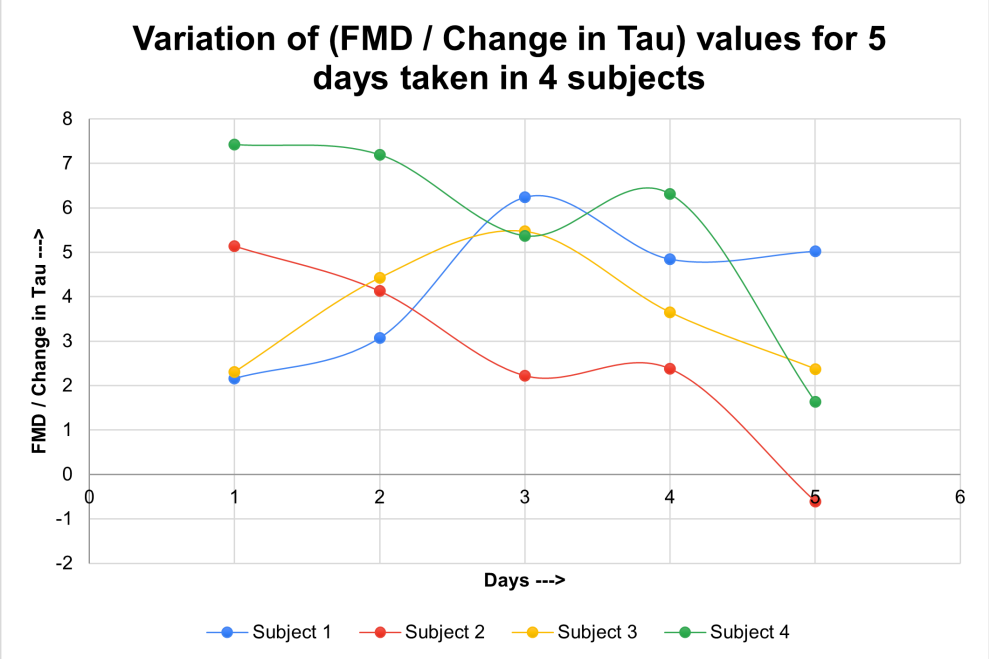

Figure 3.10: Variation of $\frac{FMD\%}{\Delta\tau}$ over the days

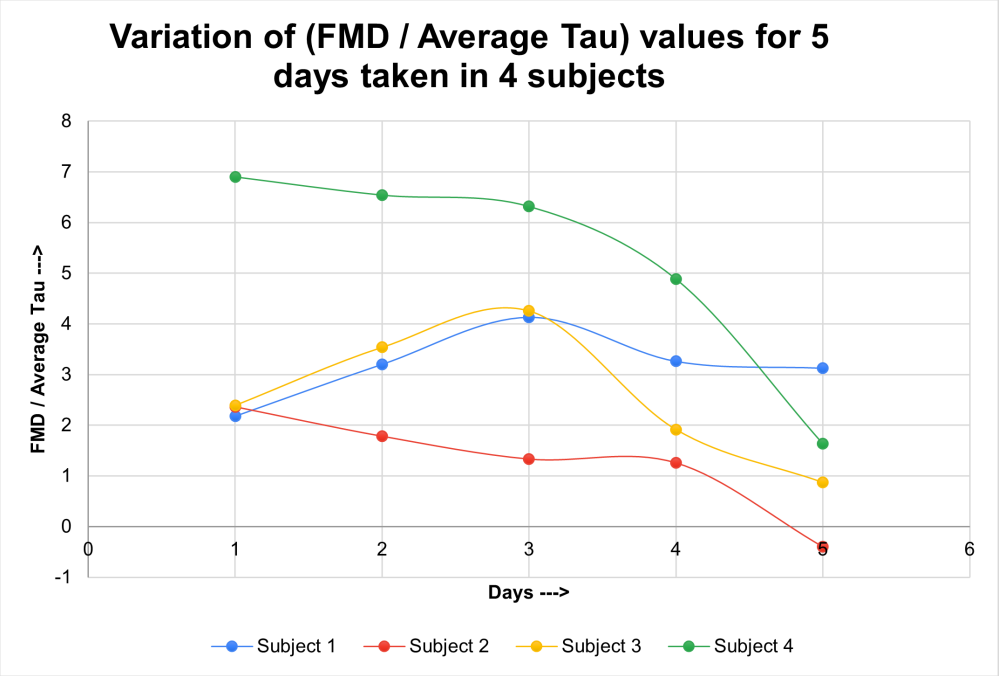

Figure 3.11: Variation of $\frac{FMD\%}{\tau_{avg}}$ over the days

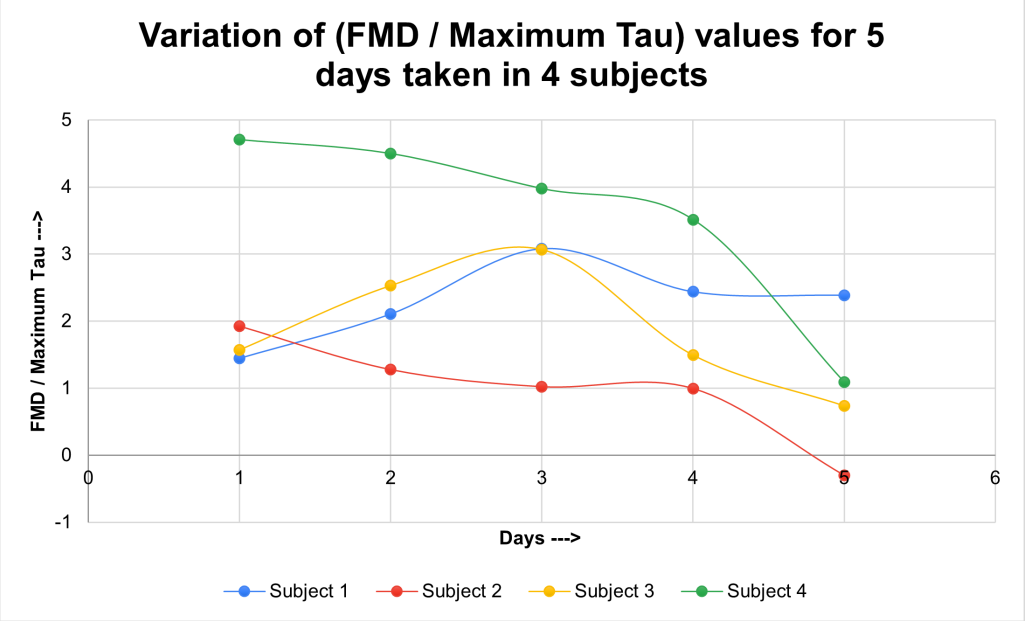

Figure 3.12: Variation of $\frac{FMD\%}{\tau_{max}}$ over the days

It is observed that the variations are huge in FMD and $\frac{FMD}{\tau_{basal}}$ when compared to other normalised parameters. There is a peaking observed on the 3^{rd} day in some of the parameters but the reason is not very clear

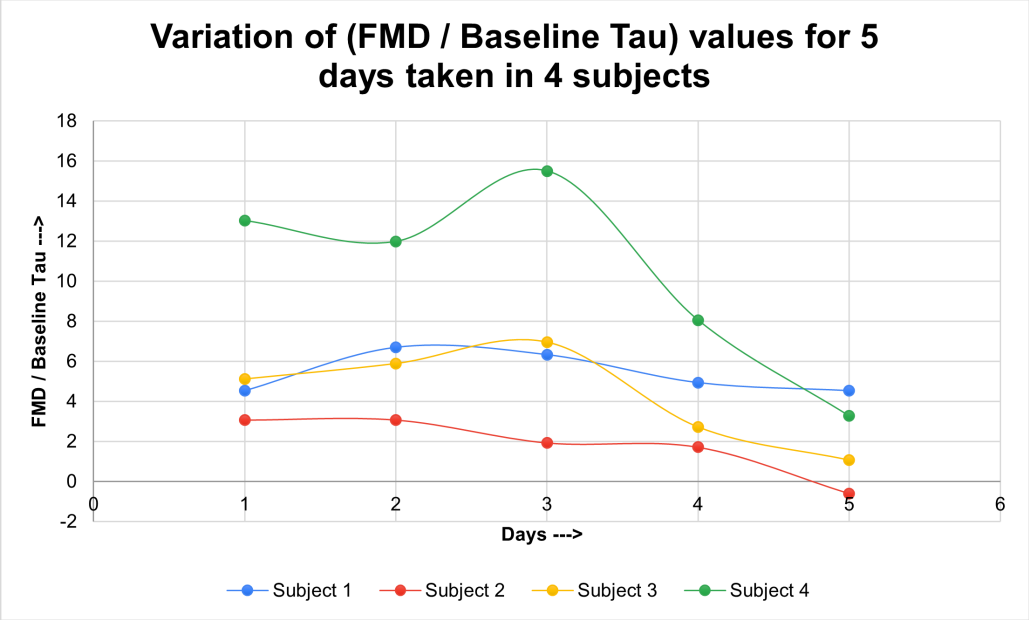

Figure 3.13: Variation of $\frac{FMD\%}{\tau_{basal}}$ over the days

3.4 Intraday Analysis

There are some interesting aspects in regards to morning and evening FMD data comparison. Figures 3.14, 3.15, 3.16, 3.17, 3.18 represent `Box plot` for morning and evening values of each parameter against the subjects. A Box plot is done for a dataset and it consists of :

- Minimum value
- First Quartile - Median of the data points to the left of the median
- Median
- Third Quartile - Median of the data points to the right of the median
- Maximum value

in the same order from bottom to top in box plot. The blue line inside the box is the median and the whiskers represent minimum and maximum values for corresponding subject and time within the day

To numerically prove that morning and evening values are statistically insignificant, a T-Test was conducted with the data sets in morning and evening for all 4 subjects in the 5 days. The resulting p-value is then plotted against the subjects for all the parameters in figure 3.19. If $p - value < 0.05$, we say that the data is statistically significant. But in the figure 3.19 all the values are greater than 0.05 and hence numerically it is clear that Morning data and evening data are not statistically significant

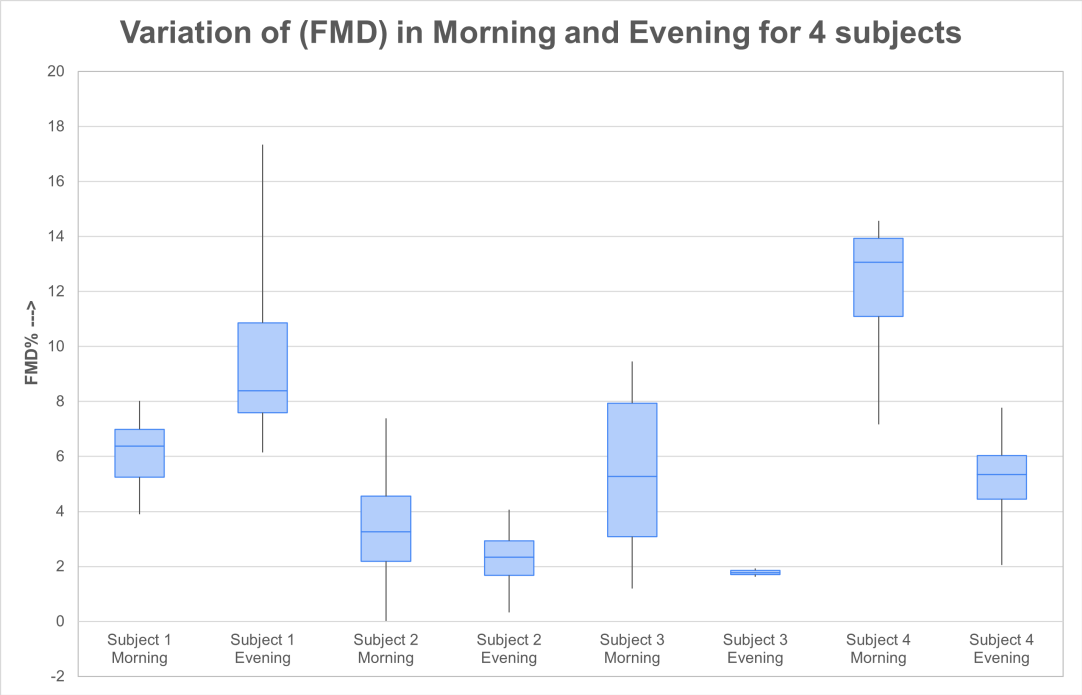

Figure 3.14: Box plot of FMD% values for Morning and Evening

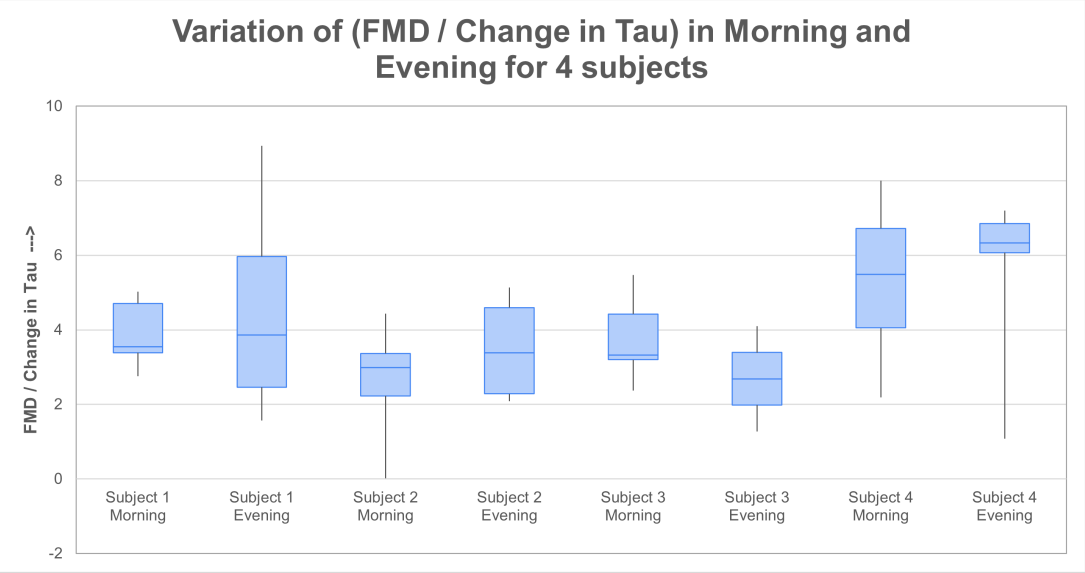

Figure 3.15: Box plot of $\frac{FMD\%}{\Delta\tau}$ values for Morning and Evening

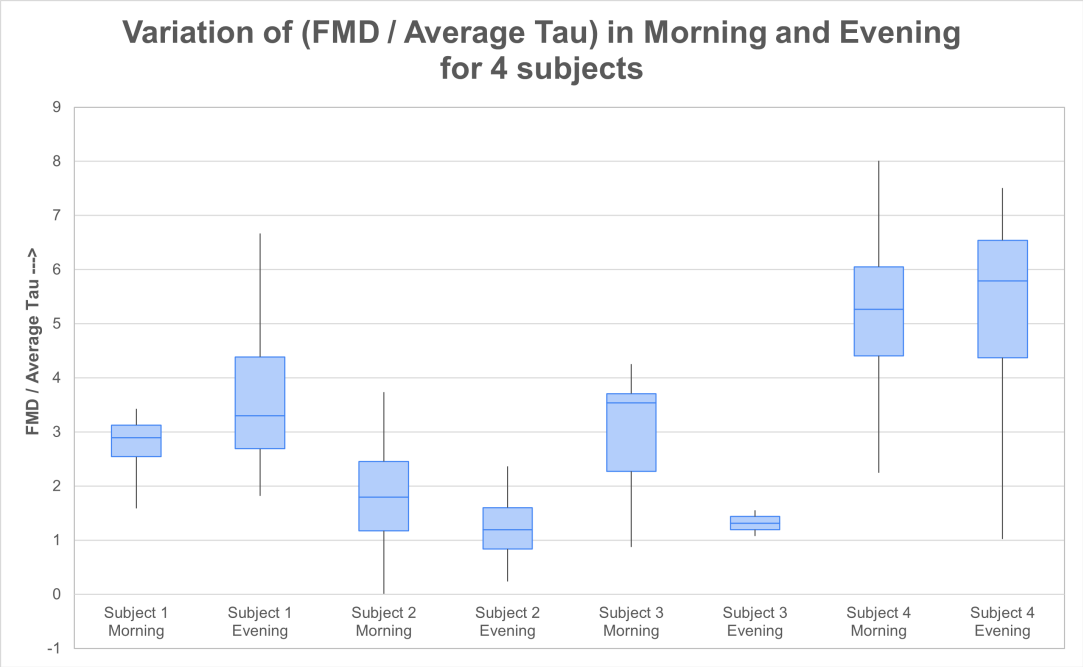

Figure 3.16: Box plot of $\frac{FMD\%}{\tau_{avg}}$ values for Morning and Evening

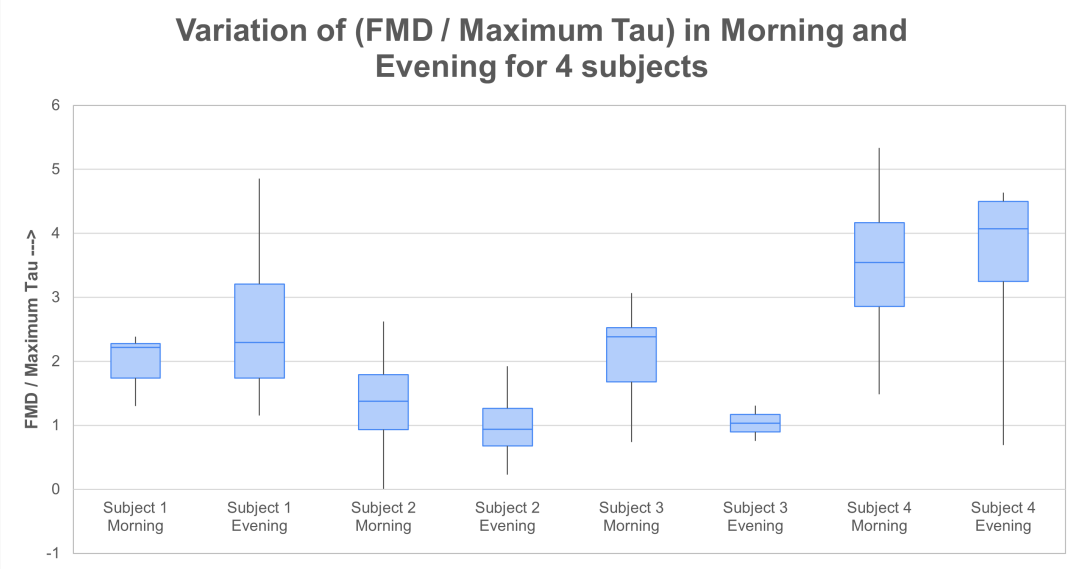

Figure 3.17: Box plot of $\frac{FMD\%}{\tau_{max}}$ values for Morning and Evening

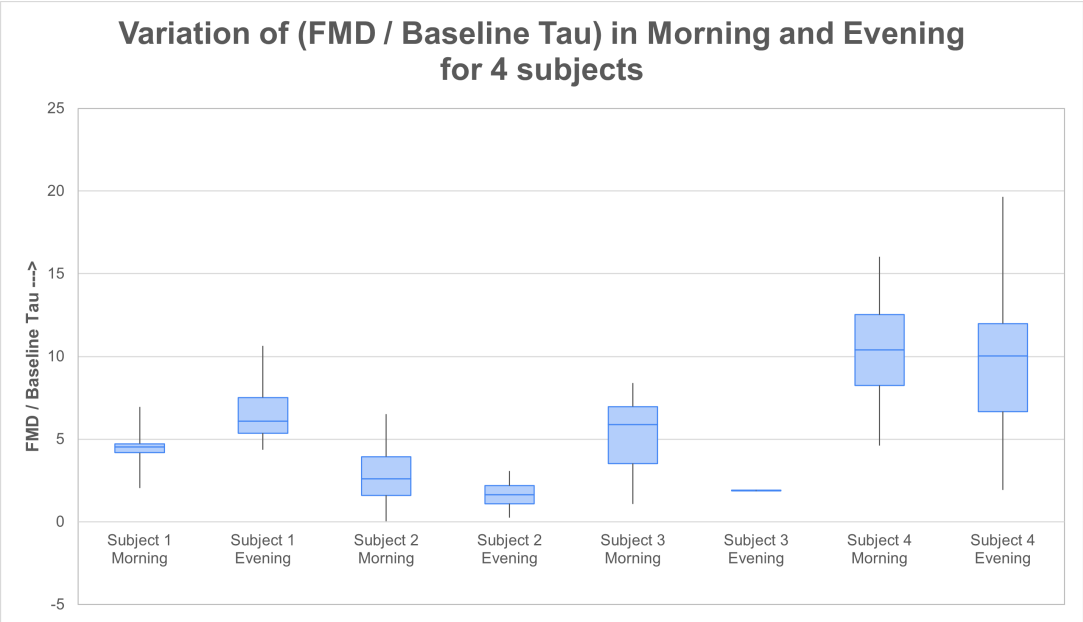

Figure 3.18: Box plot of $\frac{FMD\%}{\tau_{basal}}$ values for Morning and Evening

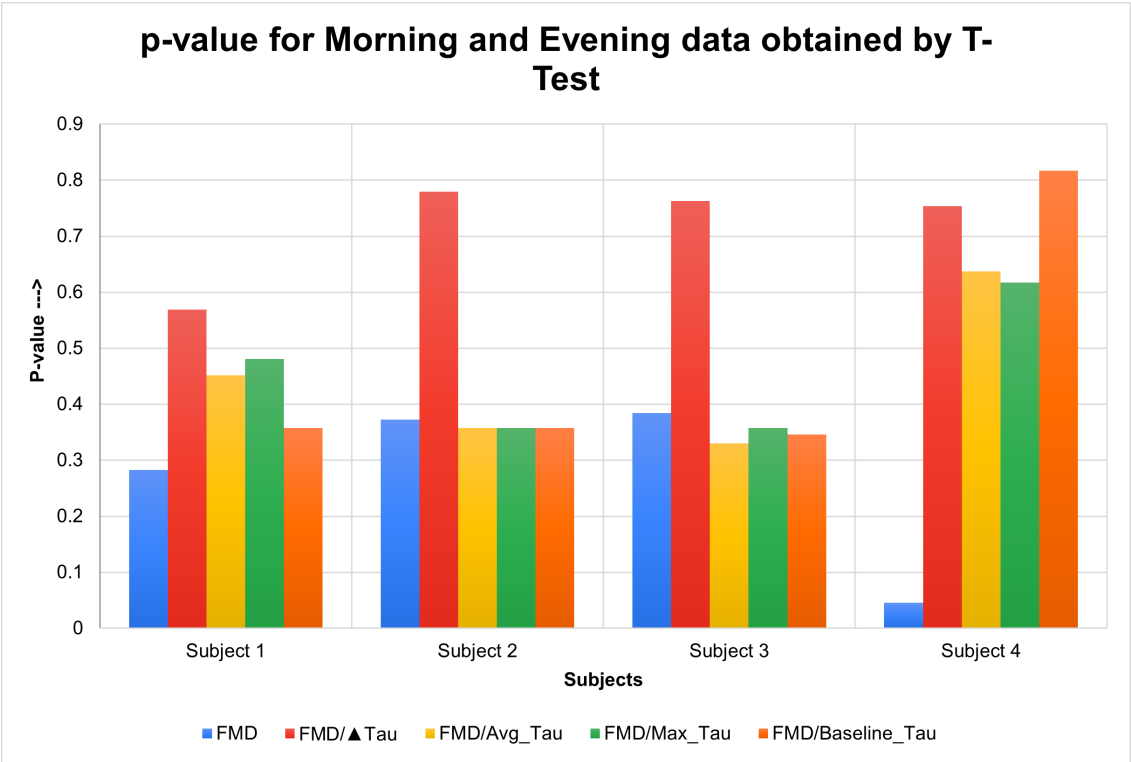

Figure 3.19: p-value of T-Test on Morning and Evening dataset for 5 days done for all parameters

3.5 Discussion

3.5.1 Statistical significance of the parameters

Even if the normalised parameters are having lesser variability and more consistency than FMD, one should also check for the statistical significance of those parameters with FMD% before concluding its usage so as to check for the extent of difference between the normalised parameter estimated and the FMD% values[Guo and Kassab (2009*b*)]. This is done by `T-Test` on each of the 4 normalised parameters with FMD values which gives the *p-value*.

$(1-(p-value))$*100 gives the probability for the claim, "Two data sets have statistically significant difference". So the parameters are compared with FMD% by T-Test and $(1-(p-value))$*100 probability are plotted against the parameters in the figure 3.20.

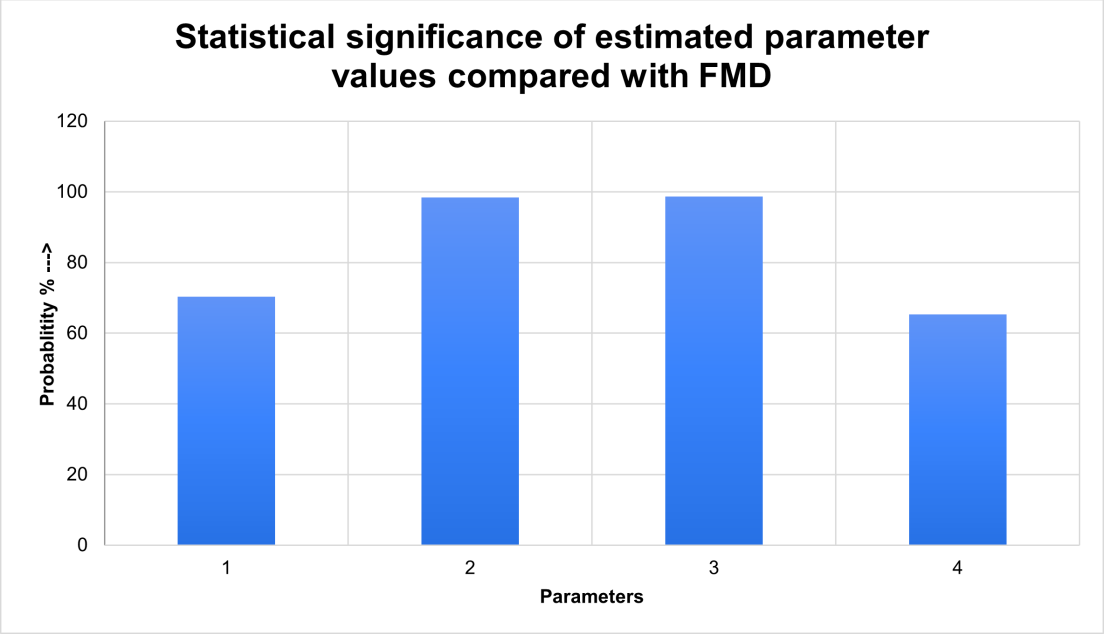

Figure 3.20: Probability of statistically significant difference of parameters compared with FMD. The parameter numbering are as discussed above in 2.2.2

As you could see from the figure 3.20, $\frac{FMD\%}{\tau_{avg}}$, $\frac{FMD\%}{\tau_{max}}$ are statistically significant compared with FMD with almost 100% probability

Whereas the parameters $\frac{FMD\%}{\Delta\tau}$, $\frac{FMD\%}{\tau_{basal}}$ are not very much statistically significant but are considerable with around 70% significant difference compared to FMD% values

3.5.2 Analysis of parameters

- In terms of **COV**, the normalised parameters shows better result as in figure 3.1. Especially, $\frac{FMD\%}{\Delta\tau}$ shows very low COV compared to FMD and hence has increased consistency

- In the case of **MAD** and **SD**, the normalised parameters are better than FMD as shown in figure 3.2, 3.3 and $\frac{FMD\%}{\tau_{max}}$ shows the best value

- In case of **RC**, also the normalised parameters are better with $\frac{FMD\%}{\tau_{max}}$ showing the lowest range

- But out of the estimated parameters, $\frac{FMD\%}{\tau_{basal}}$ shows poor COV, MAD, SD, RC. Rest all parameters are better than FMD with consistency

- In the inter-day analysis, normalised parameters have lower variations than FMD which is evident from the Y-axis scale shown in figures 3.9, 3.10, 3.11, 3.12, but $\frac{FMD\%}{\tau_{basal}}$ parameter variations is worse than FMD variations shown in figure 3.13

- In the intra-day analysis, we showed that morning and evening data sets do not have statistically significant difference and observe from figure 3.19 that $\frac{FMD\%}{\Delta\tau}$ has the highest p-value and larger when compared to any other parameter, indicating that there is almost no statistical difference between morning and evening for $\frac{FMD\%}{\Delta\tau}$ parameter

CHAPTER 4

CONCLUSION AND FUTURE SCOPE

4.1 Conclusion

- As discussed in 3.5.2, the COV, MAD, SD, RC are better for all the parameters except $\frac{FMD\%}{\tau_{basal}}$ when compared to FMD% across the days. It is due to the fact that $\frac{FMD\%}{\tau_{basal}}$ do not take post-dilation shear stress into consideration, which is a major factor of FMD system input
- In our analysis, COV% is the most important factor determining consistency of data across the days and p-value of T-test within the day given in figure 3.19, so the best value for these are given by $\frac{FMD\%}{\Delta\tau}$ and it is regarded as the best parameter for representing endothelial function
- $\frac{FMD\%}{\tau_{max}}$ has better COV, MAD, SD than $\frac{FMD\%}{\tau_{avg}}$
- Hence on the whole, considering consistency across the 5 days and within the day, we arrange the best evaluated parameters in decreasing order,

$$\frac{FMD\%}{\Delta\tau} > \frac{FMD\%}{\tau_{max}} > \frac{FMD\%}{\tau_{avg}} > FMD\% > \frac{FMD\%}{\tau_{basal}}$$

- $\frac{FMD\%}{\Delta\tau}$ can be now used for estimating the vascular age by considering its improved consistency across the days and within the day
- From the improved consistency of normalised parameters, we validate the FMD system model with input as shear stress and output as vasodilation
- However, since the system considered also contains blood, some haemodynamics of blood will also affect the transfer function, for example, hypertensive subject will have a different transfer function value when compared with normal subject with same vascular age

4.2 Future Scope

There could be improvement in the calculations of shear stress. For example, apparent viscosity was taken to be 4 cP considering the hematocrit concentration but it varies among the people with different arterial diameter and the variations are provided in the figure 4.1 by [Pries *et al.* (1992)]. This error is revealed in

the result 3.1 where average FMD% value is slightly higher for people with lower arterial diameter. Using this graph, one could model for estimating shear stress more accurately. Some more detailed discussion on viscosity is given in [Sprague (2010)].

Also the blood pressure data taken before and after FMD experiments could

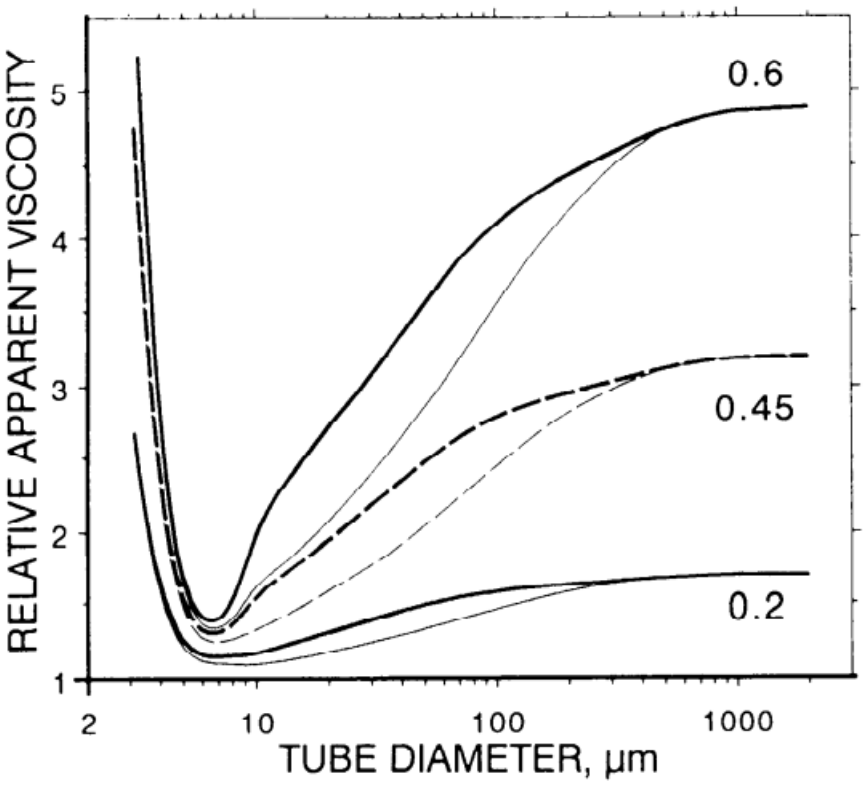

Figure 4.1: Apparent Viscosity Vs Diameter. The thick lines represent different hematocrit levels. Thin lines are to be neglected. source : [Pries *et al.* (1992)]

be used for remodelling the system as it would have some effect on the system during the post-deflation period and we will have a even better parameter that is consistent across the days and also consistent between different people, i.e., different people with same parameter value would be having exactly the same vascular age

REFERENCES

1. **Dammers, R.**, **F. Stifft**, **J. H. M. Tordoir**, **J. M. M. Hameleers**, **A. P. G. Hoeks**, and **P. J. E. H. M. Kitslaar** (2003). Shear stress depends on vascular territory: comparison between common carotid and brachial artery. *J. Appl. Physiol.*, **94**(2), 485–489.

2. **DH, T.**, **B. MA**, **P. KE**, **P. J**, **A. G**, **H. RA**, **P. B**, **W. ME**, **T. ME**, and **G. DJ.** (2011). Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol*.

3. **Finkler, B.**, **B. Eibel**, **W. Souza**, and **E. Barbosa** (2019). Arterial stiffness and coronary artery disease. *Journal of Cardiology Cardiovascular Therapy*, **14**.

4. **Guo, X.** and **G. S. Kassab** (2009*a*). Role of shear stress on nitrite and nos protein content in different size conduit arteries of swine. *Acta physiologica (Oxford, England)*, **197**(2), 99–106. ISSN 1748-1716. URL `https://pubmed.ncbi.nlm.nih.gov/19432590`. 19432590[pmid].

5. **Guo, X.** and **G. S. Kassab** (2009*b*). Role of shear stress on nitrite and NOS protein content in different size conduit arteries of swine. *Acta physiologica (Oxford, England)*, **197**(2), 99–106.

6. **Kanai, A. J.**, **H. C. Strauss**, **G. A. Truskey**, **A. L. Crews**, **S. Grunfeld**, and **T. Malinski** (1995). Shear stress induces atp-independent transient nitric oxide release from vascular endothelial cells, measured directly with a porphyrinic microsensor. *Circulation Research*, **77**(2), 284–293.

7. **Liu, X.**, **Z. Wang**, **P. Zhao**, **Z. Fan**, **A. Sun**, **F. Zhan**, **Y. Fan**, and **X. Deng** (2014). Nitric oxide transport in normal human thoracic aorta: Effects of hemodynamics and nitric oxide scavengers. *PloS one*, **9**, e112395.

8. **Ma, T.**, **X. Liu**, **Q. Ren**, **Z. Zhang**, **X. Sun**, **Y. Zheng**, **X. Deng**, **X. Yu**, and **Y. Fan** (2021). Flow-mediated dilation analysis coupled with nitric oxide transport to enhance the assessment of endothelial function. *Journal of Applied Physiology*, **131**(1), 1–14. URL `https://doi.org/10.1152/japplphysiol.00039.2021`. PMID: 33830813.

9. **Ostadfar, A.**, Chapter 1 - fluid mechanics and biofluids principles. *In* **A. Ostadfar** (ed.), *Biofluid Mechanics*. Academic Press, 2016. ISBN 978-0-12-802408-9, 1–60. URL `https://www.sciencedirect.com/science/article/pii/B9780128024089000016`.

10. **Patel, P.** and **J. C. Drummond**, Cerebral physiology and the effects of anesthetic drugs. 2010.

11. **Pries, A. R.**, **D. Neuhaus**, and **P. Gaehtgens** (1992). Blood viscosity in tube flow: dependence on diameter and hematocrit. *American Journal of Physiology-Heart and Circulatory Physiology*, **263**(6), H1770–H1778. URL `https://doi.org/10.1152/ajpheart.1992.263.6.H1770`. PMID: 1481902.

12. **Reisfeld, B.** (2021). Fluids and fluid flow. URL https://www.engr.colostate.edu/CBE101/topics/fluids_and_fluid_flow.html.

13. **Sprague, C. N. C. . M. R. R., B.** (2010).

14. **Team, P.** (2015). Physiology illustration: Laminar versus turbulent flow in blood vessels. URL https://www.physiologyweb.com/figures/physiology_illustration_Gxr9bSdjRij69DSavoTbjWtiQ44Pw3aM_laminar_versus_turbulent_flow_in_blood_vessels.html.

15. **Vitello, D. J.**, **R. M. Ripper**, **M. R. Fettiplace**, **G. L. Weinberg**, and **J. M. Vitello** (2015). Blood density is nearly equal to water density: A validation study of the gravimetric method of measuring intraoperative blood loss. *Journal of Veterinary Medicine*, **2015**, 152730. ISSN 2356-7708. URL https://doi.org/10.1155/2015/152730.

16. **Vlachopoulos, C.**, **M. O'Rourke**, and **W. Nichols**, *McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles*. CRC Press, 2011. ISBN 9781444128789. URL https://books.google.co.in/books?id=byrSBQAAQBAJ.