CHAPTER 6

EXPERIMENTAL INVESTIGATION

6.1 Overview

This chapter has two sub sections. The first section deals with the experimental investigation required to generate the pulsatile flow through an elastic tube in two stages. In the stage-1, pulsatile flow through elastic tube is generated using heart-lung machine and ECG waves synchronized with the pulsatile simulator. Since this attempt proved to be unsuccessful in developing the physiological pulse pressure, the existing test rig is replaced with the pulsatile duplicator setup in the stage-2 to generate the required pulse pressure. The variation in pulse pressure is investigated for normal and stenosed conditions (25%, 50% and 75%) and the results are compared with those obtained numerically. Further, in the second section, clinical study taken up is discussed which demonstrates the variation in carotid flow during change of postures such as sleeping, standing and inclined head-down position.

6.2 Experimental Investigation – Stage:1

The previous experimental investigations have utilized several methods like hot-wire anemometer (Clark, 1980), Laser & Doppler techniques (Gijsen et al., 1999), (Siouffi et al., 1998), (Levesque et al., 1986), including the most advanced Particle Image Velocimetry Techniques ((Eguchi et al., 2003),(Kaminsky, 2007)) to capture the velocity profiles while pressure is measured using pressure transducers (Ji et al., 2008),(Pontiga, Gaytán, 2005)). In the above studies, the test specimen representing the artery is chosen to be flexible in some studies and rigid in others for the simple straight orientation under both normal and abnormal conditions with stenosis or
aneurysms. Some of the materials used for simulating the arterial function are transparent silicone or latex (flexible) and acrylic resin (rigid) for normal conditions and Plexiglas, Polyvinyl Alcohol Hydrogel for abnormal conditions such as stenosis/aneurysms, respectively. The fluid chosen is water combined with glycerol and other additives such that the fluid properties are similar to that of blood. In a majority of the past studies ([Ji et al., 2008], [Shalman et al., 2001]), nature of flow is found to be steady while in very few cases, pulsatile flow is obtained through a programmable pump.

As observed in [Canić et al., 2001], inlet and outlet pressure was measured through pressure transducer in normal latex tube with high frequency Doppler probe to measure the flow anywhere within the tube and experimental results were compared with the numerical simulation. Similar approach has been adopted in the present study to measure the pressure in normal elastic tube at inlet and outlet in addition to inlet flow through stationary Doppler probe and the same was done under stenosed conditions. However, due to lack of high frequency ultrasound Doppler probe, used at various sections flow at various sections within the tube couldn’t be measured. More importantly any probe used at the stenosis is expected to disrupt the flow behavior. However, it is presumed that PIV would have been a better option; unfortunately there is no access to such a facility.

A novel approach is utilized to generate the pulsatile flow through the test specimen using Heart Lung Machine (HLM) as a part of the stage-1 and the investigation is carried out at CATH LAB, Kasturba Hospital, Manipal University, Manipal. To simulate the pulsatile blood flow through the elastic artery, a simple straight silicone tube is considered as the test specimen.
6.2.1 Description of the setup

The schematic diagram of the closed circulatory loop as shown in the Fig-6.1 consists of Heart Lung Machine (HLM) serving as a pulsatile flow pump, silicone tubing serving as the flow conduit, two compliance chambers (C1 and C2), flow resistance (R), pressure transducers at inlet and outlet (P1 and P2) respectively, and reservoir. HLM is used generally during an open-heart surgery to take over the functions of the heart and lungs. The use of the machine allows the surgeon to carefully stop the heart such that, the vital organs continuously receive the blood and oxygen. Thus, surgery can be carefully performed without interference from bleeding or the heart's pumping motion. In the present work, the HLM consists of four pumps with control module and other accessories from Stockert-Shiley (SKU: ES0015). The pressure transducers fixed at the inlet and outlet of the test specimen will measure the systolic/diastolic pressure through pressure transducer (BD DTX Plus DT6012, Becton Dickinson, USA).

![Fig-6.1: Schematic representation of circulatory loop](image-url)
Fig - 6.2: Experimental test rig setup for pulsatile flow (Different views)

A – Reservoir
B – Compliance chamber
C – Test tube
D – Ultrasound transducer
E – Sonosite Doppler machine
F – Heart Lung Machine
G – Pressure Monitor
H – Pulsatile simulator
The pressure waves are recorded for a few cardiac cycles until the flow stabilizes. The velocity is measured using Ultrasound Doppler technique through M-Turbo ultrasound machine (Sonosite). A high-frequency Doppler probe, L-38n used during vascular application having bandwidth of 10-8 MHz for a depth of 6cm is used as probe to measure the velocity at various locations on the test specimen. The B-mode images will provide radial distension and M-mode will provide the velocity spectrum. The test specimen is an elastic tube of medical grade silastic used in perfusion pump during Cardio-pulmonary bypass and the specifications of the tube are 1/2”(I.D)×3/32”(Tube thickness)×12”(Length of tube), while the rest of the other flow tubing is of PVC to complete the circuit.

The fluid pumped through the flow circuit is a mixture of water and dairy whitener with proportion of 0.6:0.4, having density and viscosity of 1050 kg/m$^3$ and 0.0035 Pas. To obtain the exact proportion, several trials with proportions like 25:75, 30:70, 40:60, 50:50 were conducted, so as to match the required density of blood and viscosity is measured using Brooke’s field viscometer. The following equation is used to obtain the density of dairy whitener water solution (DWWS):

$$Density \ of \ DWWS = \frac{Weight \ of \ DWWS}{Weight \ of \ water} \times \ Density \ of \ water$$

The details of the experimental setup to develop the pulsatile flow through test specimen are shown in the Fig - 6.2. Out of four working roller pumps in HLM, one of them is used to pump the fluid from the reservoir through the test specimen. The commercially available plastic containers are used as the compliance chambers in this setup.
6.2.2 Results and Discussion

Initially, several trials are conducted to maintain systolic and diastolic pressure within the test specimen with and without using compliance chambers. The pressure wave and velocity spectrum obtained without and with the compliant chambers are shown in the Fig-6.3 and Fig-6.4, respectively. The pressure wave patterns and velocity spectrums in both the cases are different from the physiologically observed behavior. Pressure of 127/55 mmHg is developed within the test tube without the compliance chamber in the flow circuit.

![Fig-6.3: (a) Pressure wave (b) velocity spectrum obtained without compliance chambers](image)

![Fig-6.4: (a) Pressure wave (b) velocity spectrum obtained with compliance chambers](image)
The systolic pressure is within the permissible physiological range, but the diastolic pressure is very low when compared with the required 80 mmHg. The pressure wave has a sharp peak followed by a small rise without forward flow as observed in the Fig-6.3(a). The velocity spectrum is non-uniform similar to half sinusoidal wave with a peak systolic velocity of 40 cm/s and diastolic velocity of 14 cm/s as observed in the Fig-6.3(b). There is significant rise in the diastolic pressure after including the compliance chamber in the flow circuit.

Even though the systolic pressure is within the physiological range, but the difference between systolic and diastolic pressure is very less and wave form is highly unstable with non-uniform range as shown in the Fig-6.4(a). The velocity spectrum obtained represents a uniform band of unsteady sharp spikes (Fig-6.4 (b)) without any traces of peak velocity as that required in pulsatile flow. Both the cases are unsuccessful in developing the required physiological pressure of 120/80 mmHg. Further, PULSATILE FLOW CONTROL-28H1724 module also known as pulsatile simulator from Stockert-Shiley is added to the flow circuit to refine the pressure wave developed. This simulator controls the rotation of the roller head in the HLM by generating a square wave.

![Fig-6.5: (a) Pressure wave without ECG synchronization (b) Pressure wave with ECG synchronization](image)
The wave pattern can be refined based on heartbeat, flow rate, pulse cycle to develop the desired flow pattern. But, this square wave pattern fails to generate the required pattern of pulsatile flow. Therefore, the square waves generated from pulsatile simulator have to be synchronized with the ECG waves to obtain the desired pulsatile flow pattern. The ECG waves obtained from a healthy volunteer using Philips M8001A Intellivue MP20 bed side monitor are synchronized with the pulsatile simulator. ECG synchronized pressure waves are compared with unsynchronized ECG pressure waves as shown in the Fig-6.5.

The pressure developed without ECG synchronization and using compliance chamber is 116/105 mmHg, without any traces of forward diastolic flow as shown in Fig-6.5(a). The magnitude of systolic and diastolic ECG synchronized pressure wave obtained is found to be approximately 126/86 mmHg as shown in Fig-6.5(b). However, the wave patterns in both the cases do not match with the physiologically observed pulse cycle. To avoid the discomfort caused to the volunteers in taking the ECG waves, artificial ECG ANOLG STIMULATOR is used to mimic the realistic ECG wave, and it synchronizes well with the rotation of the roller head. However, with several trials and redesign of compliance chambers, the obtained pressure wave form and values are not close to the realistic physiological pulse wave.

**6.3 Experimental Investigation – Stage:2**

Since the stage-1 setup had inherent problems in obtaining the required physiological pulse, an alternate approach of using pulsatile duplicator is used to obtain the physiological waveform. The experimental investigation is continued at Device Testing Laboratory, Biomedical Technology Wing, Sree Chitra Tirunal
Institute for Medical Sciences and Technology, Trivandrum to simulate the pulsatile flow using a pulse duplicator through the test specimen (Bhuvaneshwar et al., 1996). The pulse duplicator simulates the function of the left side heart by generating the pulsatile flow. The test fluid is a glycerin water mixture with a density of 1080 kg/m$^3$ and viscosity of 3.5×10$^{-3}$ Pas similar to that of blood.

### 6.3.1 Description of setup

The schematic diagram of pulse duplicator is shown in the Fig-6.6. The different hardware components of the pulse duplicator system are; the left ventricular chamber (LV), Collapsible rubber balloons (B1 and B2), Manometer cuff (MC) for regulating the pressure in the balloon B1, Three way solenoid valve (SV), Air reservoir (AR), Pressure regulator (PR), and Compressed nitrogen (CN) for driving the balloon B1, Mitral and Aortic valves (MV and AV, respectively), Atrial reservoir (AR), Systemic resistance (SR), Compliance chambers (Ch1 and Ch2), Ultrasonic flow probe (UFP) and Vascular Chamber (VC). T1 and T2 are the pressure transducers at inlet and outlet of test specimen, respectively.

The pulsatile flow is accomplished by periodic expansion and contraction of a collapsible rubber balloon B2, which is fitted inside a rigid transparent left ventricular chamber (LV). The balloon B2 is periodically inflated by compressed nitrogen, (stored in a tank CN) to produce the pumping action of left ventricle, and deflated by venting the gas to atmosphere, to produce the ventricular filling action. This cyclic operation is achieved with the help of a three-way solenoid valve (SV) controlled from a computer.
The filling pressure of the balloon is maintained by a pressure regulator (PR), which can be varied to change the stroke volume of the (mock) ventricle, and the air reservoir (AR) helps to supply the required quantity of air within a short period of time. The air volume in upper balloon B1 is changed by a hand pump (MC) to control the compliance of the LV chamber, in order to adjust the rise time of the ventricular pressure. A three-way solenoid valve is actuated to influence the inflation and deflation cycles. Other hydraulic elements, i.e., compliance chambers (Ch1, Ch2) and resistances (Rh1, Rh2, SR) are used to simulate the systemic impedance.

With the present experimental setup, it is possible to obtain a pulse rate from 40 beats per minute (BPM) to 150 BPM and a systolic duration from 20% of the cardiac cycle to 50% of the cardiac cycle. The instrumentation system used in the pulse duplicator is developed with modular data acquisition components and the
software interface is created in LabVIEW graphical programming language (M/s. National Instruments, USA). The flow rate is measured using an ultrasonic flow meter (T106, M/s.Transonic Systems Inc, USA) by placing the flow probe close to inlet as shown in the Fig-6.7. The disposable blood pressure transducers (BD DTX Plus DT6012, Becton Dickinson, USA) are used for pressure measurement at inlet and outlet of test specimen (Fig-6.6). These interchangeable transducers are excited at 5V to get a sensitivity of 25 μV/mm Hg. The fluid is pumped through the test specimen at a heart rate of 72BPM and mean cardiac output of 3.5LPM. The mean aortic pressure is maintained at 100 mmHg and systolic duration is maintained between 28% to 32% of the cardiac cycle. A constant flow rate of 3.5 LPM is maintained at inlet for normal and stenosed conditions. The normal and stenosed test tubes used in the present test rig is shown in the Fig- 6.8.

Fig - 6.7: Test specimen setup
(1) Silastic tube, (2)Ultrasonic flow meter , (3) - (4) Inlet and outlet pressure transducer
The different percentage of stenosed models is prepared by using the medical grade PVC tubings: $\frac{1}{2}$" (O.D), $\frac{3}{8}$" (I.D), $\frac{1}{16}$" (Wall thickness), 2.5" (Length) of PRO FLOW make. The material properties are provided in the Appendix C. The length of stenosis was assumed to be 6D. Based on the percentage of area reduction, 25%, 50% and 75% stenosis geometry was obtained. With the repeated blowing of hot air and controlled constriction of outer wall of PVC tube, the stenosis shape was obtained. The measured pressure at the inlet and outlet and flow rate waveforms at inlet, recorded for five cardiac cycles are described for normal and stenosed conditions.

6.3.2 Results and Discussion

The pressure waveforms measured at the inlet and outlet and the flow rate spectrum are shown in the Fig-6.9 (a). Due to technical reasons, the noise obtained through the ultrasound transducer could not be filtered resulting in moderate oscillations. Even after several attempts, the noise factor was hard to trouble shoot.
However, the wave pattern obtained is similar to the clinical observations. The flow waveform is similar to the clinically observed pattern (Refer Fig-6.9 (b)). The physiological pressure of (120/80) mmHg is maintained and the waveform pattern corresponds well with the clinically observed waveform. The pressure lag between the inlet and outlet pressure wave demonstrates the compliance introduced by the test specimen, which adds to extra flow received at the time of diastole stored and during systole.

Fig - 6.9: (a) Experimental pressure and flow waveform in normal tube
(b) Clinical observation of flow duplex waveform
The stenosed tube element could be assumed to behave analogous to an RC element in an electrical circuit, the compliance of the tube representing the capacitor element \((C)\) and the resistance offereded by the stenosis representing the resistance element \((R)\). The compliance is change in volume or area of the tube representing artery for a given change in pressure and depends on geometry and material property of the elastic tube (McVeigh et al., 2002). So, this RC component generates a delay between the inlet and outlet pressure signals, while also generating a pressure drop across the element, which can be noticed from the different sets of pressure waveforms for varying stenotic conditions. The difference at the peak inlet and outlet pressures in normal case is insignificant, but this difference will gradually increase due to the inclusion of the stenosis. Fig-6.10 describes the pressure waveforms for 25% stenosis condition. Difference of less than 5% is observed at the peak inlet and outlet waveform, which gives a clear understanding that there is no significant disturbance for the normal flow.

![Fig-6.10: Comparison of pressure wave forms in 25% stenosis](image-url)
Fig-6.11: Comparison of pressure wave forms in 50% stenosis

Fig-6.12: Comparison of pressure wave forms in 75% stenosis
Fig-6.13: ΔP comparison of pressure waveform between inlet and outlet

Further, with 50% stenosis, the gap between the peak inlet and outlet pressure increases by 10-12%, as shown in the Fig-6.11. But for 75% condition, the peak systolic inlet and outlet gap increases by more than 35%, as shown in the Fig - 6.12. Also, the early and later part of inlet pulse cycle increases by 15-20%. It can be observed that the influence of narrowing becomes significant for the stenosis above 75% as the complexity in the flow pattern begins from this stage of narrowing. Fig-6.13 compares the ΔP waveform between measured inlet and outlet pressures for normal and various stenosed conditions. The above mentioned percentage variation for different stenosed conditions and significance of the increased stenosis can be clearly observed.
Fig-6.14: Comparison of inlet pulse pressure for different conditions

Fig-6.15: Comparison of outlet pulse pressure for different conditions
The pulse pressure obtained from the measured inlet and outlet pressures in normal and different stenosed cases are compared and shown separately in Fig 6.14 and Fig.6.15 respectively. The presence of stenosis will increase the disturbance in flow disturbance in the downstream end, while in the upstream side, it leads to pressure buildup. The outlet pressure comparison for normal and stenosed condition reveals that there are no noticeable pressure differences. However, in contrast, at the inlet, with the increased in severity of stenosis, a significant increase in the pressure difference can be observed.

The comparison of pulse pressure and time lag between pulse pressure at inlet and outlet is shown in the table-6.1. With reference to the Fig.6.14 and Fig.6.15, the maximum value of pulse pressure at inlet and outlet during peak systole is mentioned in the table-6.1 for normal and stenosed conditions with constant flow rate at inlet. Experimentally obtained time lag is also mentioned for different conditions. As observed in (Alberto Figueroa C, 2006) and (Torii et al., 2006, 2009), the pulse pressure adopted in normal and mildly stenosed (>50%) tube is 40mmHg. The time lag depends on pulse wave velocity and length of the tube. In (Alberto Figueroa C, 2006), time lag was 0.024s for pulse wave velocity of 5.25m/sec and 126mm tube length. In the present study, length of tube is 250mm, relative pulse wave velocity is 12m/sec and phase lag is 0.021s.

<table>
<thead>
<tr>
<th></th>
<th>Pulse pressure at Inlet (Pa)</th>
<th>Pulse pressure at Outlet (Pa)</th>
<th>Time lag (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41.508</td>
<td>39.098</td>
<td>0.020</td>
</tr>
<tr>
<td>25% stenosis</td>
<td>41.521</td>
<td>39.124</td>
<td>0.021</td>
</tr>
<tr>
<td>50% stenosis</td>
<td>45.722</td>
<td>39.353</td>
<td>0.024</td>
</tr>
<tr>
<td>75% stenosis</td>
<td>54.341</td>
<td>39.247</td>
<td>0.030</td>
</tr>
</tbody>
</table>
Fig-6.16: Percentage variation among experimental trials during peak systole for different flow conditions

Fig-6.17: Percentage variation among experimental trials during end diastole for different flow conditions
The pulse pressure for normal and 25% stenosed conditions at inlet and the
time lag between the pulse pressure at inlet and outlet are similar to the physiological
observation with minor variation. With the increase in severity of stenosis the time
lag also increases. Thus, there will be time delay for flow to reach the peak systole.
However, with the increased severity of stenosis, the pulse pressure and time lag
increases linearly. The pulse pressure increases significantly at inlet by more than
10% and 25% in addition to 8% and 20% increase in time lag between the pulse
pressures for 50% and 75% stenosed conditions, respectively. However, there is no
increase in the pulse pressure at the outlet, and it remains constant without much
significant variation with increased severity of stenosis. The percentage variation
among various experimental trials is estimated separately during peak systole and
end diastole as shown in the Fig-6.16 and Fig-6.17, respectively and it is found to be
insignificant as the variation is found to be within 1%.

6.3.3 Comparison of experimental results with numerical simulation

The results obtained experimentally can be compared with the CFD
simulation results for normal and stenosed cases. The model of normal and stenotic
cases is generated based on the geometry of the test specimen in CATIA V5R19 and
meshing is carried out in ANSYS WORKBENCH as described in the Fig- 6.18.
The fluid is assumed to be Newtonian, and incompressible with density and viscosity
as observed in the experimental study. Grid independency check is performed using
steady state analysis by adopting first order high resolution scheme before simulating
transient conditions in ANSYS CFX (Refer Appendix B: Mesh Comparison).
Maximum velocity and average pressures are evaluated at the throat section for
different grid sizes and compared. Optimum grid size for normal model, 25%, 50%
and 75% stenotic models are found to be 25700, 26500, 26200 and 27000 hexahedral elements. For the steady state analysis constant uniform velocity is applied at the inlet. In the transient analysis, the time varying pulsatile velocity is applied at the inlet based on the flow rate calculated experimentally and pulsatile pressure at the outlet for normal and stenotic cases. The numerically obtained inlet and outlet pressures are compared with the experimental results.

Fig-6.18: CFD model description

![Diagram showing CFD model description](image)

Fig-6.19: Pressure contours in normal and stenosed tube during peak systole
Transient CFD analysis is performed by adopting second order high resolution scheme for five pulse cycles to allow the flow to stabilize. Fig-6.19 shows the pressure contours during peak systole in normal, 25%, 50% and 75% stenosed conditions. In the normal condition the fluid flows without any obstruction and pressure varies from very large value at inlet, and reduces gradually towards distal side as observed from the Fig.6.19. The highlighted region locates the stenosed region meant for comparing the difference in the pressure distribution in partial narrowing and normal conditions. As the percentage of stenosis increases, the restriction offered for the flow also increases (Jung et al., 2004). The pressure contours in stenosis is characterized by very large pressure build up in upstream of stenosis, significant drop at the throat, and disturbed flow nearer to the stenosis stabilizes once again in the downstream end as discussed in the chapter-5.

The difference between normal and 25% stenosis is negligible without any significant flow changes in the downstream of the stenosis. The moderate variation in pressure distribution can be clearly seen in 50% stenosis condition, without large restriction for the flow. However, significantly large pressure variation is demonstrated in 75% with considerable pressure drop at the throat site and very high pressure build up in the upstream side. The constriction at the throat site increases the velocity, resulting in flow jet formation at the core and flow separation along the boundary in downstream end. The flow further regains the normal flow stream after moving to a few lumen diameter lengths from the throat site ((Li et al., 2009),(Ai et al., 2010)). The influence of stenosis for the flow restriction is very clear from the pressure contours. This brief discussion is further extended for comparing the numerical results with the experimental values.
Fig-6.20: Comparison of computed pressure with experimental pressure for normal case

Fig-6.21: Comparison of computed pressure with experimental pressure for 25% stenosis case
Fig-6.22: Comparison of computed pressure with experimental pressure for 50% stenosis case

Fig-6.23: Comparison of computed pressure with experimental pressure for 75% stenotic case
Fig. 6.20 compares the computed inlet and outlet pressure with the experimental values for normal condition. The broader width of pressure variation at inlet and outlet in addition to the minor lag as observed in numerical simulation when compared with experimental values is due to the computation limitations. Similarly, for different stenotic cases such as 25%, 50% and 75% conditions, comparisons are depicted as shown in the Fig-6.21, 6.22 and 6.23, respectively.

Table - 6.2: Comparison of pulse pressure and time lag between experimental and numerical results for various conditions

<table>
<thead>
<tr>
<th></th>
<th>Pulse pressure at Inlet (mmHg)</th>
<th>Pulse pressure at Outlet (mmHg)</th>
<th>Time lag (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>41.508</td>
<td>40.652</td>
<td>39.098</td>
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<tr>
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<td>41.521</td>
<td>40.462</td>
<td>39.124</td>
</tr>
<tr>
<td>50% stenosis</td>
<td>45.722</td>
<td>44.847</td>
<td>39.353</td>
</tr>
<tr>
<td>75% stenosis</td>
<td>54.341</td>
<td>52.858</td>
<td>39.247</td>
</tr>
</tbody>
</table>

Table-6.2 describes the comparison of pulse pressure and time lag between pulse pressure at inlet and outlet for experimental and numerical simulation results. The difference of pulse pressure at inlet is less for normal and 25% stenosed condition, with marginal increase for 50% and 75% stenosed conditions. In contrast, the difference of pulse pressure at outlet is slightly large for normal and stenosed conditions. As observed from the Table-6.2, time lag in the numerical simulation results is quite less when compared with the experimental observation. However, the numerically obtained results agree well with the experimental values. The percentage of error estimation is also carried separately during peak systole and end diastole for normal and stenosed conditions as shown in the Fig-6.24 and Fig-6.25, respectively.
Fig-6.24: Comparison of percentage variation between experimental and numerical values during peak systole for different flow conditions

Fig-6.25: Comparison of percentage variation between experimental and numerical values during end diastole systole for different flow conditions
The comparison reveals that there is no significant difference in experimental and numerical results for normal and different stenosed conditions as the variation value is within 2% during both peak systole and end diastole.

6.4 Physiological Investigation

The objective of the physiological study is to understand the vascular behavior during change of posture, and this investigation is supported clinically through carotid Doppler scan performed on young, healthy 25 male volunteers belonging to the age group of 20-25 years. In this study, changes in carotid flow behavior for various postures such as sleeping, standing and head-down position are investigated. The blood flow velocities are measured using ultrasound Doppler in the common carotid arteries (CCA) of the volunteers, which are measured easily in standing and supine postures without causing any discomfort to the volunteers since cardiovascular and cerebral auto-regulation mechanisms adapt to these postures.

![Fig-6.26: Schematic diagram of carotid Doppler ultrasound flow waveform](http://pubs.rsna.org/doi/full)

However, in the unphysiological head down position, the volunteers suffered some discomfort, so velocity is measured for 30° inclined-head down posture. The time taken by individual volunteers during each posture change is 5-10sec.
Experiments were conducted starting with the supine posture followed by standing and then inclined head-down position. The time required during each change of posture was roughly 5-10s and time required to measure the carotid velocities in each posture was estimated to be 20s (including placing of ultrasound probe on the neck and measuring the velocities). Two different experiments are performed to measure the flow velocities in the CCA of all the subjects. In the first set of experiments, the PSV and the EDV are measured immediately after posture change without allowing any time interval. Schematic representation of carotid Doppler ultrasound measurement describing PSV and EDV is shown in the Fig-6.26.

In the second set of experiments, PSV and EDV are measured with a time interval of five minutes for each change of posture. The PSV and EDV measurements in sleeping position are taken as reference and compared with sitting and inclined position in both the experiments. Five min gap was provided assuming that the resultant postural change in heart rate and systemic blood pressure would stabilize within this time period, although as observed in Aaslid et al. (1988) and Greene and Lee et al.(2012), cerebral auto-regulation would occur within 5s in healthy volunteers. However, different studies such as Aaslid et al.(1988), Hughson et al.(2001), Reinhard et al.(2003), Azhim, A. et al. (2004, 2006) have quoted various time gaps and in the present study 5min time interval is chosen for data collection.

Depending on the individual behavior and nature of living, the carotid velocity is not a constant term and varies upon time to time. The typical peak systole and end diastole velocities measured through Carotid Doppler Ultrasound varies between 60-75cm/s and 10-15cm/s respectively. However, for each individual multiple
readings (3 sets) obtained for 3m, 5m, 10m exhibited similar behavior for different posture change. Hence for an individual volunteer, only single set of carotid velocity measurements are obtained for different postures rather than collecting multiple readings. Based on the advice from Statistical department, Manipal University as the volunteers are more in number and the variation in the velocity patterns are not similar, statistical analysis is preferred to analyze the physiological experiments. In the present study, the focus lies to understand and look for significant variation in carotid velocity during change of postures which has been clearly observed through individual measurements. Further, this analysis will help in concluding the significance of such experiments and exact hypothesis can be drawn for the changes in the flow behavior during various postures in no-time interval and time-interval experiments.

The statistical analysis is carried out using repeated measured ANOVA technique. This technique is used to observe the differences among the means for two or more values. The null hypothesis is always assumed that there is no significant difference in the means of the values being examined. The results obtained from the ANOVA technique will be in the form of graphs of mean variation of the values. In the results obtained, the p-value is considered to judge whether the analysis is statistically significant or insignificant. The p value is actually a calculated probability, which estimates the probability of rejecting the null hypothesis assuming that hypothesis is true. The hypothesis can be rejected by terming it as statistically insignificant when p-value is less than the significance level, which is often 0.05 or 0.001 and any p-value less than significance level is termed as statistically significant. All the measured data are reported as means and SD. The no-time
interval experiment measured values are shown in the table - 6.3. It is observed that there is a small decrease in both PSV and EDV while standing and slight increase in supine positions. However, there is slight dip in the PSV and rise in EDV values during inclined head down posture.

Table - 6.3: Comparison of No-time interval and time interval experiment measurements of PSV

<table>
<thead>
<tr>
<th></th>
<th>Peak systolic velocity (cm/s) (No -Time Interval)</th>
<th>Peak systolic velocity (cm/s) (Time Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>Supine</td>
<td>151.61</td>
<td>32.60</td>
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<tr>
<td>Standing</td>
<td>122.89</td>
<td>21.63</td>
</tr>
<tr>
<td>Inclined</td>
<td>147.81</td>
<td>36.83</td>
</tr>
</tbody>
</table>

Results from Repeated measures ANOVA indicates the significant difference in PSV as observed in No-time interval and Time interval data, $F_{(2,48)} = 39.118$ (p <0.001)

Table- 6.4: Comparison of No-time interval and time interval experiment measurements of EDV

<table>
<thead>
<tr>
<th></th>
<th>End Diastole velocity (cm/s) (No - Time Interval)</th>
<th>End Diastole velocity (cm/s) (Time Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Supine</td>
<td>25.82</td>
<td>6.21</td>
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<tr>
<td>Standing</td>
<td>20.47</td>
<td>4.74</td>
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<tr>
<td>Inclined</td>
<td>27.41</td>
<td>7.98</td>
</tr>
</tbody>
</table>

Results from Repeated measures ANOVA indicates the significant difference in PSV as observed in No-time interval and Time interval data, $F_{(2,48)} = 31.876$ (p <0.001)

In the time-interval experiment, the measurements are shown in the table - 6.4 and it is observed that there is a large decrease in both PSV and EDV in standing, and slight increase in the supine position. However, there is slight dip in PSV and rise in EDV in inclined-head down posture.
Fig-6.27: Variation of (a) Peak systolic velocity and (b) Diastolic velocity for different postures

(†p < 0.001 compared to no – time interval experiments, *p < 0.001 compared to sleeping, 
* p < 0.001 compared to sleeping)
The importance of afore mentioned experimental sets of time interval and no time interval and similar analysis for comparing effects during change of postures is determined by the significance level, representing p value with the significance level set to 0.001. It can be noticed that, there is a significant difference between time interval and no-time interval experiments (p < 0.001) during PSV and EDV instance of pulse cycle (Fig-6.27). In both the time interval and no-time interval experiments, there is a statistically significant difference (p < 0.001) in the flow velocity while comparing standing and supine postures i.e., the PSV and EDV decrease with change in posture from supine to standing as seen in Fig - 6.27(a) & (b). However, there is statistically insignificant difference when changing the posture from sleeping to inclined (p >0.001). During the change of posture from supine to inclined-head-down position, there is a further increase in the EDV (Fig-6.26(b)) and a slight decrease in estimated marginal means of PSV (Fig-6.27 (a)). The concept of gravity aiding the CCA flow in the inclined position and acting against the flow in standing posture can be clearly observed with the graphs obtained.

Similar type of studies conducted by (Azhim et al., 2004) discusses the arterial blood flow by Doppler ultrasound during supine, 90° head-down and 90° head-up at different levels of the body viz., in the carotid, brachial and femoral arteries of healthy volunteers simultaneously. It is observed that as compared to supine position, flow velocities in brachial and femoral arteries increase in head-up posture and decrease in head-down posture. It is also observed that the heart rate increased during posture change from supine to 90° head-up, while the flow velocity mildly reduced in carotid arteries.
However, since 70-80% of blood flow through the CCA is diverted to the brain via ICA and auto-regulation of ICA blood flow occurs immediately with change of posture, the relative change in flow rate is significantly lesser in CCA as compared to the brachial and femoral arteries (Azhim et al., 2006). This change probably reflects the change of flow in the external carotid artery (ECA), which normally has a high-resistance low-flow pattern as compared to the low-resistance high flow-pattern of ICA, and contributes to only 20-30% of CCA flow. The time taken for cerebral auto-regulation to normalize flow velocities after postural changes varies from person to person. In another study by (Savin et al., 1995), during $10^0$ head down tilt, the blood flow velocity increased by 13%, while there was a 18% decrease during standing position when compared to sleeping position as reference. It is observed that, cerebral blood flow is found to vary immediately but regulated to a steady-state within a few minutes. Similar observations were found in the (Aaslid 1988), (Reinhard et al., 2003) (Hughson et al., 2001)

In the ((Azhim et al., 2006), (Savin et al., 1995), (Aaslid 1988), it is observed that cerebral auto-regulation takes less than a five seconds (rather than minutes) in healthy subjects with no cardiovascular disease and uncompromised cerebro-vascular reserve. Therefore, the blood flow within the ICA hardly varies with the variation in posture in order to maintain the brain functioning during postural changes. The relatively small changes in CCA blood flow can thus be attributed to maintaining the ICA flow due to cerebral auto-regulation and gravity induced changes in ECA flow.

In the present study, it is observed that the difference in velocity change between supine, standing and inclined positions is less in the no-time-interval
experiment as compared to that for with-time-interval experiment in most of the subjects. The experiments on volunteers are carried out in the healthy volunteers. However, the similar study in different postures in patients with stenosis in carotid is not feasible due to the risks involved in stroke (plaque rupture). Also, there are studies (Akiyama et al., 1999)(White, Markus, 1997)(Duan et al., 2011), (Greene, Lee, 2012), which highlights that presence of stenosis in carotid bifurcation impairs the auto-regulation process. Hence, in the patients with high degree of stenosis during change of posture will certainly affect the auto-regulation.