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Duplex Ultrasonographic Studies on Peripheral Arterjes in Patients Having Coronary Artery Disease

Bhaduri, Joydeep

University of Rajshahi

DUPLEX ULTRASONOGRAPHIC STUDIES ON PERIPHERAL ARTERIES IN PATIENTS HAVING CORONARY ARTERY DISEASE

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BY

Joydeep Bhaduri
MBBS ((Raj). DMUD (USTC)

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INSTITUTE OF BIOLOGICAL SCIENCES
UNIVERSITY OF RAJSHAHI
RAJSHAHI-6205, BANGLADESH

Declaration

I do hereby declare that, the research work submitted as a thesis titled "DUPLEX ULTRASONOGRAPHIC STUDIES ON PERIPHERAL ARTERIES IN PATIENTS HAVING CORONARY ARTERY DISEASE" to the Institute of Biological Sciences, University of Rajshahi, Bangladesh for the degree of Doctor of Philosophy (PhD) is the result of my own investigation and observation.

I further declare that this thesis or part of the thesis has not been concurrently submitted elsewhere for any degree or diploma.

(Joydeep Bhaduri)

Certificate

This is to certify that Joydeep Bhaduri is the author of the thesis titled "DUPLEX ULTRASONOGRAPHIC STUDIES ON PERIPHERAL ARTERIES IN PATIENTS HAVING CORONARY ARTERY DISEASE". He worked under our supervision.

This thesis has not been previously submitted for the award of any degree or diploma.

We are forwarding this thesis to be examined for the degree of Doctor of Philosophy (PhD) to the Institute of Biological Sciences, University of Rajshahi, Bangladesh.

Supervisors

Prof. Dr. K.A.M. S.H. Mondal
Professor
Institute of Biological Sciences
University of Rajshahi,
Rajshahi, Bangladesh.

Prof. Dr. Quazi Tarikul Islam
Professor of Medicine
Popular Medical College
Dhaka, Bangladesh

Dr. Md. Rais Uddin Mondol
Associate Professor and Head
Cardiology Department
Rajshahi Medical College
Rajshahi, Bangladesh.

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Abstract

Coronary artery disease (CAD) is the disease of arteries inside the heart which supply blood to heart muscle. It is characterized by narrowing of lumen of artery due to deposition of lipid materials inside the arterial wall by a process called atherosclerosis. Atherosclerosis is a systemic disease and once the processes have been started it can affect any arteries of the human body. So, arteries other than coronary arteries may be involved by atherosclerotic process simultaneously with CAD. This study was designed to see the extent of involvement of peripheral arteries with coronary arteries involvement, to see the relation of PAD involvement with severity of CAD, to see the role of common risk factors in synchronous PAD and CAD patients, When peripheral arteries are involved by atherosclerotic process it is called peripheral Arterial disease (PAD).

The study was conducted at Inpatient Unit of Cardiology Department of Rajshahi Medical College Hospital. Total 210 patients was included in the study who had definite evidence of CAD, proved by coronary angiogram.

Duplex ultrasonography was performed on both sided major carotid and major lower limb peripheral arteries of each patient to see haemodynamic status any structural change. Both sided common carotid and internal carotid arteries, femoral arteries, popliteal arteries anterior tibial arteries and posterior tibial arteries, total 12 segments of peripheral arteries of each patients were examined. Intima Media Thickness of both common carotid

arteries and internal carotid arteries were measured. Evidence of plaques or any stenosis was assessed from B mode image and from spectral Doppler analysis by measuring the peak systolic velocity (PSV), end diastolic velocity (EDV), pulsatile index (PI) and resistance index (RI).

Data regarding cardiovascular risk factors, physical examination of cardiovascular system, finding in echocardiography, coronary angiography, fasting lipid profile and Duplex ultrasound findings of peripheral arteries were collected.

Total 43 (20.4 %) patients were found having stenosis in peripheral arteries, among which 26 in carotid system, 15 in lower limbs and 2 in both carotid and lower limb arteries. Twenty seven had critical (>50%) stenosis with haemodynamic change and 16 had (<50%) non critical stenosis with no haemodynamic change.

The study revealed that most of the patient of PAD were asymptomatic and were diagnosed for the first time after enrollment in the study. Patients with synchronous PAD and CAD were found older, greater smoking history, were more likely to be diabetic, hypertensive, higher BMI and trend towards high total cholesterol and high serum triglyceride level. Carotid intima media thickness was found had a linear correlation with number of coronary vessels involvement and significantly associated with number of total PAD patient and carotid artery involvement.

ABBREVIATION

ABI	:	Ankle/brachial index
ACC	:	American College of Cardiology
AHC	:	American Heart Association
ASNC	:	American Society of Nuclear Cardiology
BMI	:	Body mass index
CAC	:	Coronary artery calcification
CACS	:	Coronary artery calcium score
CAD	:	Coronary artery disease
CCA	:	Common Carotid Artery
CK-MB	:	Creating kinase myocardial bound
CCTA	:	Coronary Computed Tomography Angiography
CIMT	:	Carotid Intima-media thickness
CT	:	Computed tomography
DM	:	Diabetes mellitus
EBCT	:	Electron beam tomography
ECA	:	External Carotid Artery
ECG	:	Electrocardiogram
EDV	:	End diastolic velocity
EF	:	Ejection Fraction
ESC	:	European Society of Cardiology
ETT	:	Exercise tolerance test
HD	:	Ischaemic heart disease
HDL	:	High Density Lipoprotein

Hz	:	Hertz
IC	:	Intermittent claudication
ICA	:	Internal Carotid Artery
IMT	:	Intima-media thickness
IVUS	:	Intravascular Ultrasound
LAD	:	Left anterior descending artery
LCX	:	Left circumflex artery
LMCA	:	Left main coronary artery
MHz	:	Megahertz
MI	:	Myocardial Infarction
MR	:	Magnetic Resonance
MRA	:	Magnetic Resonance Angiography.
LDL	:	Low Density lipoprotein
PVD.	:	Peripheral Vascular Disease
PSV	:	Peak systolic velocity
PI	:	Pulsatility index
RI	:	Resistance index
RCA	:	Right coronary artery
SPECT MPI	:	Single-photon emission computed tomography myocardial perfusion imaging
TASC	:	Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease
Tc 99m	:	Technetium 99m
TG	:	Triglyceride
WHO	:	World Health Organization

CHAPTER 1

INTRODUCTION

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INTRODUCTION

1.1 INTRODUCTION

The heart (Fig-1) is a muscular organ located in the middle compartment of the mediastinum in the chest which pumps blood to all other organs of the body for their nourishment and to lung for exchange of oxygen and carbon-di-oxide. Blood is pumped through a network of arteries called the arterial system. Blood provides the body with oxygen and nutrients, and also assists in the removal of metabolic wastes (Hall 2011; Moore *et al.*2014).

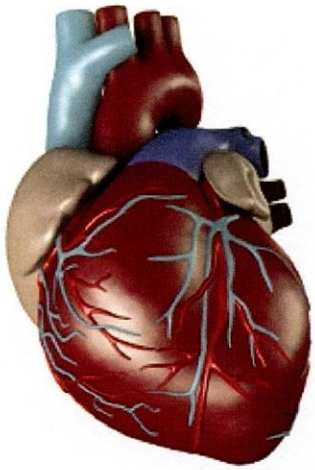
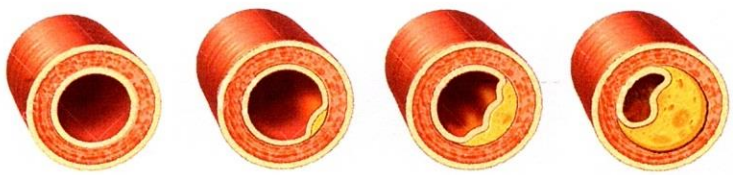


Fig 1: Image of a human heart -External view.

The heart itself also needs oxygen-rich blood to survive like every other organ or tissue in our body. The arteries which convey blood to heart itself

are called coronary arteries. The coronary arteries run along the surface of the heart and provide oxygen-rich blood to the heart muscle. The aorta, just at point of emerging from heart, branches off into two main coronary arteries, right and left. These coronary arteries branch off into smaller arteries, which supply oxygen-rich blood to the entire heart muscle.

A good patency of lumen is essential for proper supply of blood. Deposition of lipid materials on arterial wall may cause thickening of wall which cause narrowing of lumen and decrease in blood supply. When demand exceeds the supply, lack of oxygen cause injury to muscle cell, a condition called ischemia (Federick and Mitchell 2014).



2: Development of atheromatous plaques causing gradual narrowing of arterial lumen.

Coronary artery disease (CAD) or coronary heart disease is reduced blood flow through coronary arteries leading to ischemia of heart muscle. The most common cause is luminal narrowing of coronary arteries due to obstructive atherosclerotic lesion (Fig 2). Atherosclerosis is a progressive inflammatory disorder of the arterial wall where a lesion is formed in intimal layer (the innermost layer) of arterial wall, called Atheroma or Atheromatous plaque. Atheromas are composed of lipoproteins, extracellular

matrix, calcium, vascular smooth muscle cells, inflammatory cells and new blood vessels (Newby *et al.* 2010; Federick and Mitchell 2014). Developmental process of atheromatous plaques is called atherogenesis. The build up of an atheromatous plaque is a slow process, developed over a period of several years. A complex series of cellular events occurs within the arterial wall in response to a variety of local vascular circulating factors. It

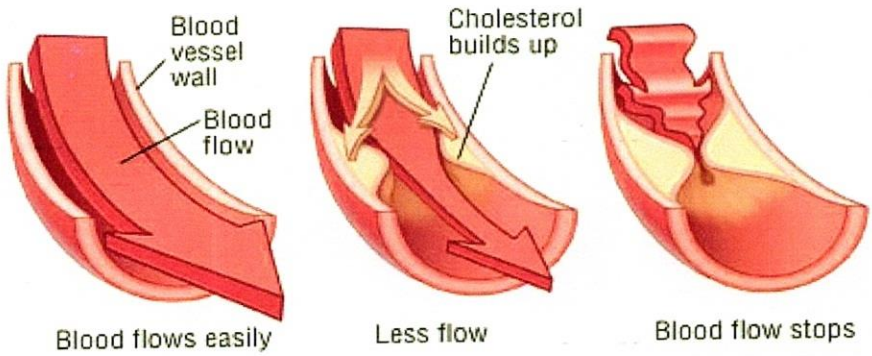


Fig 3: Effect on blood flow due to atheromatous plaques formation

protrudes into vessel lumens resulting progressive narrowing of the coronary artery lumens, compromising blood flow to the musculature of the heart (Fig 3). During stress or exercise, oxygen requirement of heart muscle increases abruptly. Coronary blood flow must increase to fulfill these extra demands (Hoit and Walsh 2008). When the demand of blood exceeds the supply, ischemic injury begins which is called myocardial ischemia – an imbalance between the supply and demand of the heart for oxygenated blood. This typically occurs at approximately 70% fixed occlusion. At this

degree of stenosis which is called Critical stenosis, patients develop symptoms (Federick and Mitchell 2014). Complete blockage results in deficient oxygenation and nutrient supply to the heart tissues, leading to damage, death and necrosis of the tissue, which is known as Myocardial Infarction (MI).

Besides mechanical obstruction, an atheromatous plaque may rupture leading to vessels thrombosis (Federick and Mitchell 2014).

Atherosclerosis may affect any artery of body but the major targets of atherosclerosis are coronary arteries, carotid arteries, and arteries of lower extremities. So, symptoms involved are related to arteries supplying heart, brain and legs. When they involve heart they produce Ischaemic Heart Disease (IHD). When they involve brain they produce Cerebrovascular Disease and in case of legs or arms they produce Peripheral Arterial Disease (PAD).

IHD presents as one or more of the clinical syndromes like i)Myocardial infarction (MI), ii)Angina Pectoris, iii) Chronic ischemic heart disease (IHD) or heart failure and iv) Sudden Cardiac Death (Federick and Mitchell 2014). This may be symptomatic or asymptomatic, may occur with exertion or at rest, depending on obstruction severity and the rapidity of development. It remains clinically silence until they become large enough to impair arterial perfusion or until ulceration or disruption of the lesion resulting thrombotic occlusion. In most cases there is a long period of silence, slow progression of coronary lesions before symptoms appear. Unfortunately the sudden death may be the first symptoms. So, it acts as a silent killer.

It was thought that CAD was the leading cause of death and morbidity in much of the industrialized world. But different studies in last several years show that the incidence of CAD is declining in developed countries and progressively increasing in developing countries. In 1997 Murray CJ and Lopez AD stated that cardiovascular diseases would be expected to be the main cause of death globally within the next 15 years, owing to a rapidly increasing prevalence in developing countries and eastern Europe (Murray and Lopez 1997). In another study, Martiniuk et *al.* (2007) stated that number of people ≥60 years of age may be double by 2025 and be triple by 2050 globally. The proportion of this aged population is likely to increase more in the Asian-Pacific region. Thus, half of the world's cardiovascular burden is predicted to occur in this area.

Fifteen years already have been passed since the forecast done by Murray and Lopez (1997) and now it seems that the forecast was almost correct. According to a report published by American Heart Association on the heading "Heart Disease and Stroke Statistics -2013 Update" it was seen that from 1999 to 2009, death rates attributable to cardiovascular disease declined by 32.7%. In the same 10-years period, the actual number of cardiovascular disease deaths per year declined by 16.7% (Go *et al.* 2013). On the other hand according to World Health Organization (WHO), CAD is now an emerging epidemic in Indian subcontinent. The risks of CAD in Indians are 3-4 times higher than white Americans, 6 times higher than Chinese and 20 times higher than Japanese. The same pattern of CAD is observed among all south Asians which include persons of India, Bangladesh, Pakistan and Sri Lankan origin (Das 2013). It was also

observed that prevalence of CAD in Bangladeshis immigrant was higher than non-Bangladeshis in USA (Silbiger *et al.* 2011).

Enough population-based data are lacking in Bangladesh. So, the exact incidence and prevalence rate of coronary artery disease in Bangladesh is not known. However, every cardiologist and physician will tell from his or her personal experience that Bangladesh has been facing a big burden due to morbidity and mortality of coronary artery disease (Haque 2006, Sayeed *et al.* 2010).

It is a general saying that coronary artery disease is a disease of rich people, and people from lower income groups are usually not affected by this particular problem. However , this is not anymore true in the perspective of Bangladesh. It is affecting every socioeconomic group. It may be because of expenses involved and it is only the affluent group that comes to seek medical advice (Sayeed *et al.* 2010). Most unfortunate part of the problem is that, it is increasingly affecting younger groups in their 40s. This is an age when they are at the peak of their career and have a lot of duties to their families and a lot to contribute to the society and country. This premature nature of the disease was quite unheard in this country only 25 years ago (Haque 2006).

1.2 STATEMENT OF THE PROBLEM

Treatment of coronary artery disease is very expensive, hence comes the question of preventing it in the first place. Prevention of coronary artery disease requires identification and treatment of risk factors. A number of risk

factors for CAD have been identified. Some of them are constitutional and therefore less controllable and some are acquired which are potentially preventable by changing life style, food habit and behaviors. But no single parameter has been established yet to define the magnitude of risk or to predict the probability of having CAD. Measurement of Body Mass index (BMI), waist hip ratio, waist thigh ratio, sub scapular to triceps skin fold thickness ratio were tried to predict the risk of CAD but not very well established (Haque 2006).

Constitutional risk factors include age, gender and genetics. Acquired risks factors which are potentially amenable to intervention are hyperlipidemia, hypertension, tobacco smoking and diabetes. These are the risk factors which accelerate the process of atherosclerosis. However 20% of all cardiovascular events are seen in patients who do not suffer from hypertension, hyperlipidemia or diabetes and do not have the history of smoking. So other factors are thought to be involved in risk which include inflammation (Ridker 2007), hyperhomocysinemia, (Guthikonda and Haynes 2006), Metabolic syndrome, (Meerarani et al. 2006) and also factors affecting hemostasis (Crose and Libby 2007; Meadows and Bhatt 2007).
Fifty percentage of patient dying suddenly because of CAD have no preceding history of coronary heart disease. So, early diagnosis is important. If treatment is started early enough, dreadful complications of the disease can be halted. Resting electrocardiogram, Stress test, Nuclear stress test are used to find the ischemic status of cardiac muscle.

A resting 12-lead electrocardiogram (ECG) is obtained on patients with suspected CAD. Electrocardiographic results are normal in approximately

hypoperfusion is detected on peak stress images compared with resting images. Resting nuclear cardiac images may also be abnormal signifying profound myocardial ischemia at rest or an irreversible myocardial scar consistent with past MI (Rimmerman 2010).

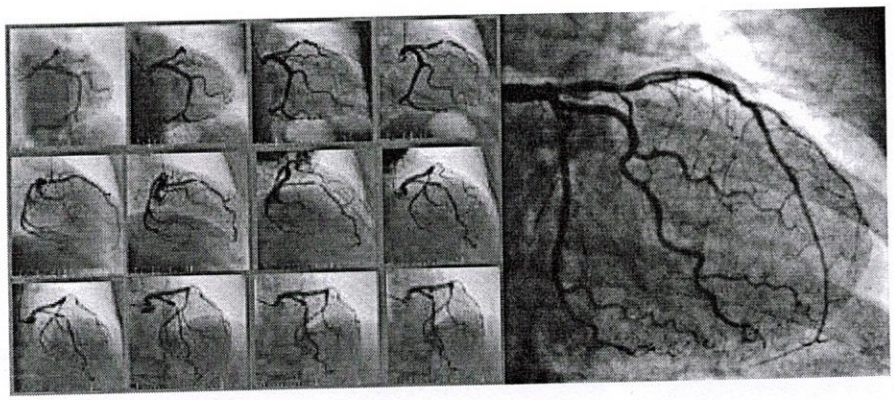


Fig 4: Images of Coronary angiogram

The only way of assessing the definite risk factor of CAD could be the direct visualization of the degree of blockage in coronary artery which is possible only by coronary angiography (Fig 4). Coronary angiography is a minimally invasive procedure to access the coronary circulation and blood filled chambers of the heart using a catheter. Contrast agent is injected directly into the coronary arteries. X-Ray imaging is recorded during the contrast injection. It remains the gold standard for determining the presence of obstructive CAD. But coronary angiography is hazardous, expensive, and not easily available and has many risk factors. It is associated with adverse events like vascular complications (1.6%), arrhythmia (0.3%), stroke (0.1%) and myocardial infarction (< 0.05%) and death (0.12%) (Nieman *et al.* 2001; Sun and Ng 2010). So, it can not be a suitable method for screening procedure of cardiac risk assessment unless there is definite indication. The

American Society of Nuclear Cardiology (2012) recommends that patients without cardiac symptoms or high-risk markers for a heart problem should not have a coronary catheterization to screen for problems. Definite indication for suspected or known case of CAD includes new onset angina, unstable angina, evaluation before a major surgical procedure, silent ischemia, positive ETT and atypical chest pain or coronary spasm. (Kern 2003).

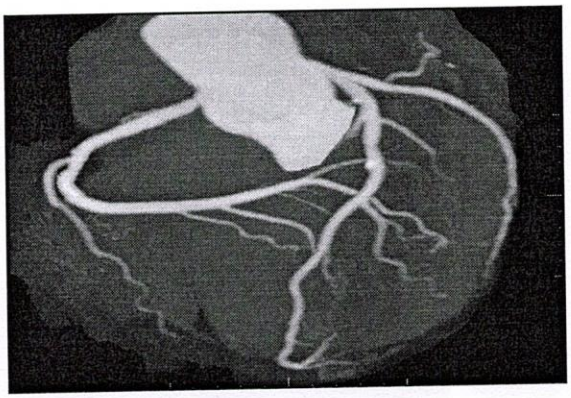


Fig 5: An image of a CT angiogram

Multislice computed tomography angiogram (CT Angiogram) is a promising noninvasive method of detecting coronary artery disease (Fig-5). When negative, this test possesses a high negative predictive value. The positive predictive value is also high, but exact stenosis quantification can be complicated. The sensitivity is 87.5% for single vessel disease, 84.0% for double vessel disease and 69.5% for triple vessel disease (Shirin *et al.* 2012). In patients at high risk for developing coronary disease, who have unclear results with treadmill or other testing, or who have symptoms

suspicious of coronary disease, CT angiography is an excellent next step in the diagnosis (Bryg 2014). But it is not yet being widely used in Bangladesh. It can be carried out only in some specialized tertiary level hospital situated in big cities.

1.3 PURPOSE OF THE STUDY

High-resolution B-mode ultrasonography has been shown to be a valid and reliable method for detecting initial structural atherosclerotic changes in the arterial walls. Ultrasonography is safe, easily available, low cost, and hazard free technique but it can be used only on major peripheral arteries, not in coronary arteries. As the coronary arteries are very small (5mm or smaller), and hidden deep within the chest they are difficult to track by the technique of ultrasonography. However, Intravascular Ultrasound (IVUS) of coronary arteries can be done by cardiac catheterization to visualize the coronary arteries, but not yet available in Bangladesh. It is performed in USA, Europe, Japan and in other developed countries but there are many limitations and risks (Tuzcu 2008). It will be not be very cost effective procedure in Bangladesh. But if there is a good correlation between progression of atherosclerosis in peripheral artery and that in coronary artery, ultrasonography can be a suitable imaging modality to assess the risk for having coronary artery disease. Atherosclerotic change of arterial wall and narrowing of lumen of arteries other than arteries of heart and brain is called peripheral arterial disease or PAD. As atherosclerosis is a systemic disease affecting the entire arterial tree some scientist believe that CAD and PAD occur synchronized. Sabeti *et al.* (2007) reported more than 50% of

the CAD positive patients could have active progressive plaque in peripheral arteries.

Measurement of the intima-media thickness (IMT) of the common carotid artery has been established as an index of early-stage atherosclerosis in some studies, and the results were strongly correlated with the presence of CAD. (Bots *et al.* 1997; Polak *et al.* 1993; O'Leary *et al.* 1991). Unfortunately no work has been done so far in Bangladesh in this field. This led to the present study. In this study attempt was taken to tried to find out the relationship between atherosclerosis in carotid arteries and incidence of coronary heart disease in our population and we also extended our research by seeing the haemodynamic status of lower limb major arteries, so that they will be clinically useful as more accurate marker for prediction of coronary artery blockage.

The relationship between atherosclerosis in carotid arteries and incidence of coronary heart disease in our population and the hemodynamic status of peripheral arteries may be clinically useful as more accurate marker for prediction of coronary artery blockage.

1.4: RESEARCH QUESTIONS

1) What is the structural and hemodynamical changes in peripheral arteries of coronary artery disease patients?

2) What is the occurrence of PAD in CAD patients?

3) Is there any relation to the extent of involvement of both diseases?

4) Is there any risk factor involved for development of PAD in CAD patients(synchronous PAD and CAD)?

1.5 OBJECTIVE OF THE STUDY

1.5.1 General Objective

To see the structural change and haemodynamic status of major peripheral arteries (carotid arterial system, and major lower limb arteries) of patients having coronary artery diseases.

1.5.2 Specific Objectives

1. To measure the intima media thickness of both common carotid and internal carotid arteries.
2. To measure the peak systolic and end diastolic velocity of blood flow through the both common carotid and both internal carotid arteries..
3. To detect any narrowing of carotid vessel and assess the severity of narrowing by B mode image.
4. To measure the peak systolic velocity of blood flow through the both Common femoral, Superficial femoral, Popliteal, Anterior Tibial and Posterior Tibial arteries.
5. To find out the pulsatility index (PI) and resistance index (RI) in above mentioned limb arteries.
6. To observe the shape of waveform in above mentioned arteries.
7. To estimate degree of stenosis by compiling all the information obtained from above mentioned arteries.
8. To compare the coronary involvement pattern and status of above mentioned arteries on duplex sonographic findings.
9. To assess the role of common risk factors in developing PAD.
10. To formulate/recommend guidelines for early detection and prevention of coronary heart disease.

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

LITERATURE REVIEW

2.1 ULTRASONOGRAPHY

2.1.1 Definition and Basic Principle

Ultrasonography is a medical diagnostic imaging technique where very high frequency sound waves are directed at tissues in the body to produce images of anatomical structures. When sound travels from one place to another, it passes in form of waves. Number of waves in a unit of time is called frequency (Fig-6). Ultrasound refers to sound waves with a frequency too high for humans to hear.

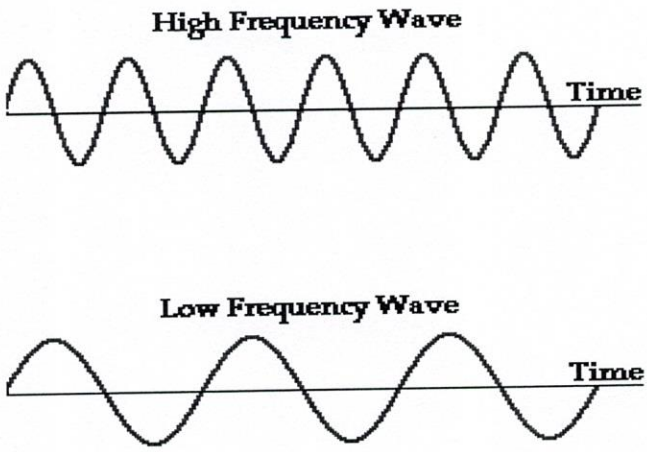


Fig 6: Frequency of sound is the number of wave per unit of time.

The unit of frequency is Hertz(Hz). One hertz is number of complete sound waves produced each second. One megahertz (MHz) = 1000000 Hz or 10^6 Hz = 10^6 waves per second. Normal human capability of hearing is sound having frequency between 20 Hz and 20,000 Hz. (Fig -7). Sound which has frequency above 20,000 Hz is called ultrasound and they can not be heard by human ear. (Palmer 1999; Wikipedia, 2015a). Diagnostic Ultrasound involves mainly frequency in the 1 MHz to 20 MHz range.

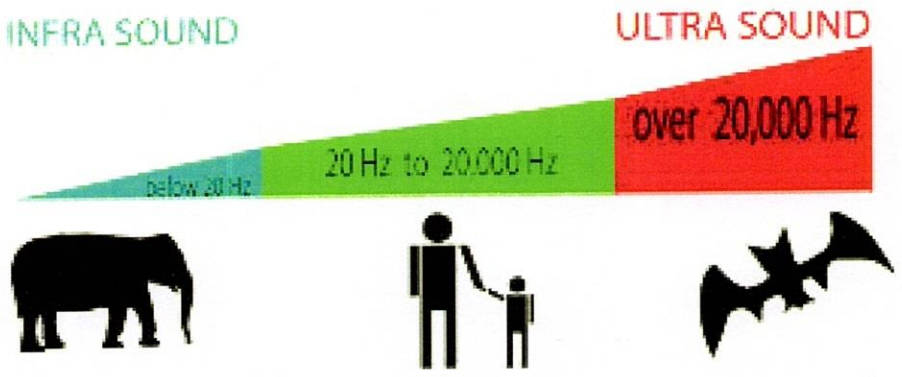


Fig 7: Human ear can hear only sound which has frequency range between 20Hz to 20 MHz (Green colour in picture). Sound above 20Mhz is ultrasound (Red Colour in picture) can be heard by bat.

In ultrasonography, a pulse of ultrasound is sent into tissue using an ultrasound transducer which is commonly called as ultrasound probe. The sound reflects from parts of the tissue. These reflected sound or echoes are detected and converted into light energy. Then by a highly sophisticated computerized system, by computing the information a two dimensional map of all the tissues are made and displayed as an image on a electronic monitor

which is called B Mode ultrasound. B-mode stands for brightness mode and provides structural information utilizing different shades of gray or different brightness. Thus a virtual image of the organ is produced on computer screen.

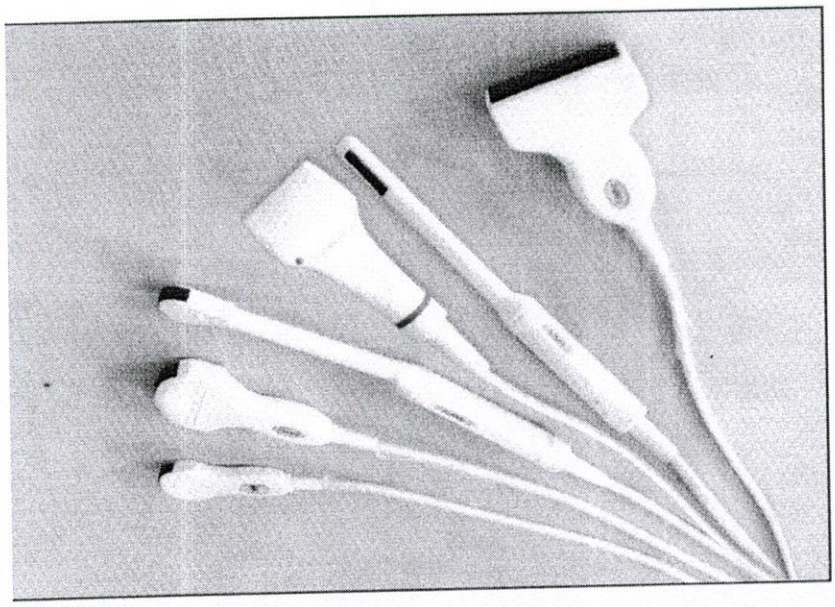


Fig 8- Different types of transducer

Different values of frequency are used to image a particular organ. The transducer determines the frequency of the ultrasound within the pulses. An ultrasonic scanner usually has a range of transducer with different design and characteristics, specific for target organ (Fig-8). Frequency is inversely proportional to the penetration of sound. The higher the frequency, the lesser the penetration is. So superficial structures such as muscles, blood vessels, tendons, testes, thyroid, parathyroid glands and the neonatal brain are imaged at a higher frequency (7 to 18 MHz). Deeper structures such as liver, gall bladder, spleen, pancreas, kidneys and other organs in abdominal cavity

are imaged at a lower frequency (1 to 6 MHz). Frequencies between 3 and 7.5 MHz are generally used for peripheral vascular imaging (Lunt 2000). Compared to other methods of medical imaging, ultrasonography has several advantages. It provides images in real-time (rather than after an acquisition or processing delay), it is portable and can be brought to a sick patient's bedside, it is substantially less expensive, and the most important is that it is free from hazards of ionizing radiation. It is also suitable for patients who are claustrophobic in closed rooms for MRI or CT Angiogram machines (Silver 2015).

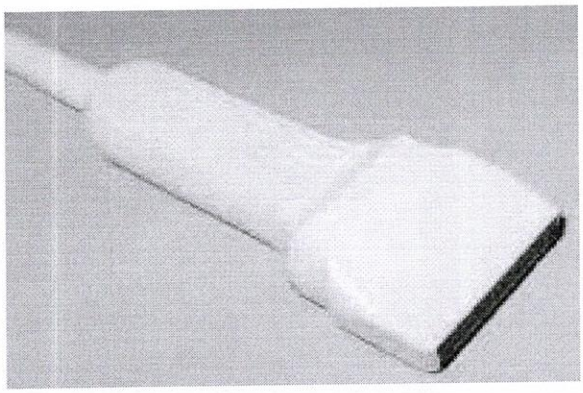


Fig 9: Linear Transducer for vascular ultrasound scans

2.1.2 Ultrasound Images of blood vessels

Conventional ultrasound images are obtained by holding a probe on the skin surface. For carotid and limb vascular scanning a high frequency linear array probe is normally used (Fig-9). A rectangular image is displayed in monitor

with the skin surface at the top, the vertical axis showing depth into the body. The horizontal axis showing position along the probe. When imaging blood vessels the lumen of blood vessels show anechoic and the vessels wall becomes echogenic. The intima and adventia produce parallel echogenic lines with an intervening echo void that represents media.The probe can either be placed along the vessel to produce a longitudinal scan or across the vessel to produce a transverse scan (Fig-10).

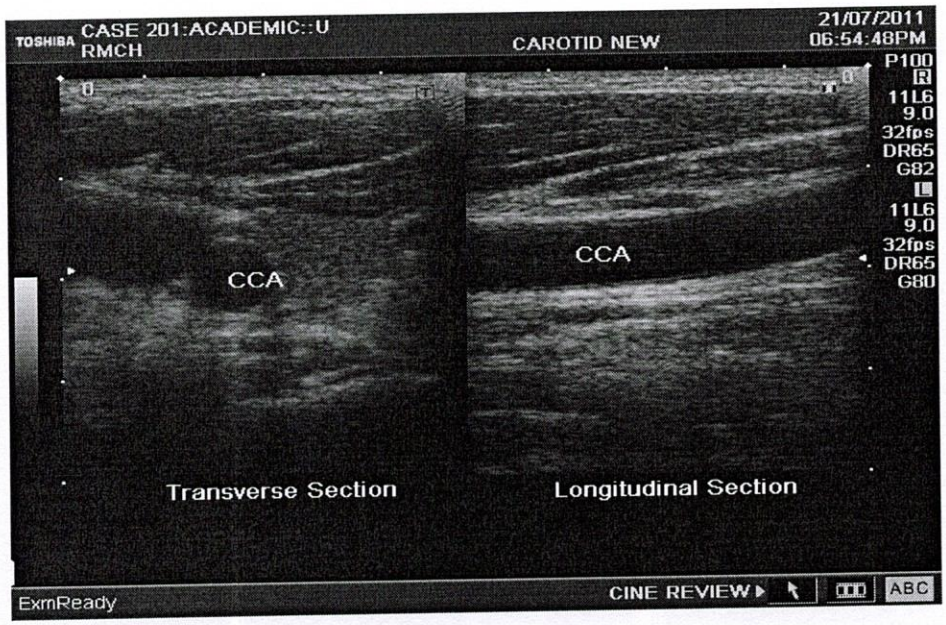


Fig-10: Transverse (on left side) and Longitudinal (on right side) images of Common Carotid Artery (CCA).

However, only anatomical study is not always enough to come to in a conclusion whether the vessel is stenosed or not. The B-mode image may not provide sufficient resolution to indentify the plaque and to determine the degree of narrowing. Even though atherosclerotic plaques and areas of calcification can be identified, the degree of stenosis cannot be accurately

measured with this technique due to complex plaque structures and calcification (Allan and Gallagher 2006). Haemodynamic study gives more accurate information regarding arterial stenosis. At sites of narrowing there will be a local increase in the velocity of speed of blood flow. The narrower the vessel, the higher the velocity will be. The Degree of stenosis can be assessed by measuring velocity change and calculating appropriate velocity ratio of blood through blood vessels. This is reasonable as a result of the simple continuity equation for flow. If a tube of cross sectional area A_1, with mean velocity v_1, narrows to an area of A_2, then to maintain the continuity of flow, the mean velocity must increase to v_2, as in following equation:

$$A_1 v_1 = A_2 v_2$$

Doppler US is another advancement in the field of diagnostic US to study velocity status of blood through major blood vessels. It can also provide information about flow direction. Thus a hemodynamic study is possible by Doppler US (Carter 2005; Thrush and Hartshorne 2009).

2.1.3 Doppler Ultrasound

In conventional B mode ultrasonography, ultrasonic echoes returning from a static organ like liver, kidney etc, towards the probe come back with the same frequency. That means the frequency of transmitted sound and the frequency of reflected sound is same for a stationary organ. However, if the target is moving towards or away from the probe, the echoes return with a higher or lower frequency. This was discovered by an Austrian scientist Christian Andreas Doppler in 1842 and the phenomenon is known as Doppler Effect after his name. The change of frequency is known as Doppler

Shift. The scanner detects any change in frequency. As the frequency change is directly proportional to the velocity of moving object from where the sound is returning, the machine can calculate the actual speed of the object. Thus the scanner calculates the speed of the blood by calculating the Doppler shift. So the Doppler technique is applied to measure velocity of blood flow through a blood vessel. Thus the status of vascularity, amount of blood flow can be known which is helpful for the diagnosis of arterial block. (Evans *et al.* 1989)

2.1.4 Duplex Ultrasound

Duplex ultrasound is the combination of conventional B mode ultrasound and applying the Doppler Effect. B mode ultrasound uses sound waves that bounce off blood vessels to create pictures and Doppler ultrasound records frequency change of sound reflecting off red blood cell to measure their speed and other aspects of how they flow. Thus simultaneously the Duplex Ultrasound shows the structure of blood vessels and the velocity information of red blood cells through the vessels. (Sudheendra 2014)

2.1.5 Analyzing Doppler Signals

Two types of techniques are used to get the information regarding blood flow. One is colour Doppler which produces array of Red-Yellow-Blue colour to indicate the direction of flow, known as colour Doppler and another is graphic presentation known as spectral Doppler, giving the information of velocity profile.

2.1.5.1 Colour Doppler

In colour Doppler option when the transducer detects any Doppler shift, it produces a range of colour, depending upon the direction of flow in relation to direction of transmitted sound beam. By convention, velocities towards the transducer are displayed red and velocities away from the transducer are displayed blue in colour Doppler. If there is no Doppler shift, no colour is produced, indicates no flow. So presence of colour indicates presence of flow which is very much useful to detect any flow of blood and also identification of blood vessel. Thus the use of color can shorten the examination time by aiding in the identification of arteries.

Color Doppler alone can be used to estimate the stenosis. It does provide a rough index of degree of stenosis although not as precise. Normal laminar flow appears as a region of homogeneous colour. Stenosis results in the production of a high velocity jet and an abrupt change in the color flow pattern. This is identified as either aliasing or desaturation (whitening) of the color display at the site of stenosis. At the poststenotic region a mosaic pattern is seen indicating turbulent flow. Presence of a bruit at the site of narrowing and turbulence at poststenotic region are indicative of >50% stenosis. Complete absence of color and evidence of collateral arteries proximal to the site of obstruction suggest total occlusion.

However, color should not be used without the simultaneous application of spectral analysis. Best results are obtained when the arteries of interest are first identified by color and spectral analysis is then used to quantify the velocity changes across the detected lesion. (Gerhard Herman *et al.* 2006, Hatsukami *et al.* 1992)

2.1.5.2 Spectral Doppler

A Spectral Doppler signal is analyzed into its frequency components in order to give a display of the velocities of the blood cells at each component. The consecutive velocity spectra are displayed as side by side grey shade lines. In this way a spectral display or spectrogram is built up.

Measurement can be obtained from spectrogram by placing a cursor on the relevant point of interest. A variety of calculation can be performed by the system computer, for example maximum velocity in systole or velocity at the end period of diastole, which are usually expressed as peak systolic velocity (PSV) and end diastolic velocity (EDV) respectively. The shape of waveform also is informative. Usually two types of shape of waveform are observed. One is triphasic and the other one is biphasic. Biphasic flows are seen in vessels of low resistant pattern Carotid, vertebral, renal arteries show biphasic spectrum (Fig-11). They are characterized by a steep upslope in systole and then a forward flow throughout diastole without any reverse flow and no wave below the baseline.

Triphasic flows are seen in vessels of high resistance pattern as found in extremity arteries of resting individual (Fig-12). Three phases of flow is seen. Initially they have tall, narrow and sharp systolic peaks indicating a high velocity flow at the beginning of cardiac cycle (1^{st} phase). Then it is followed by a reversed flow in early diastole, showing the wave in spectrum below the base line (2^{nd} phase), and lastly by a progressive forward flow in the late diastole (3^{rd} phase) (Hatuskami *et al.* 1992).

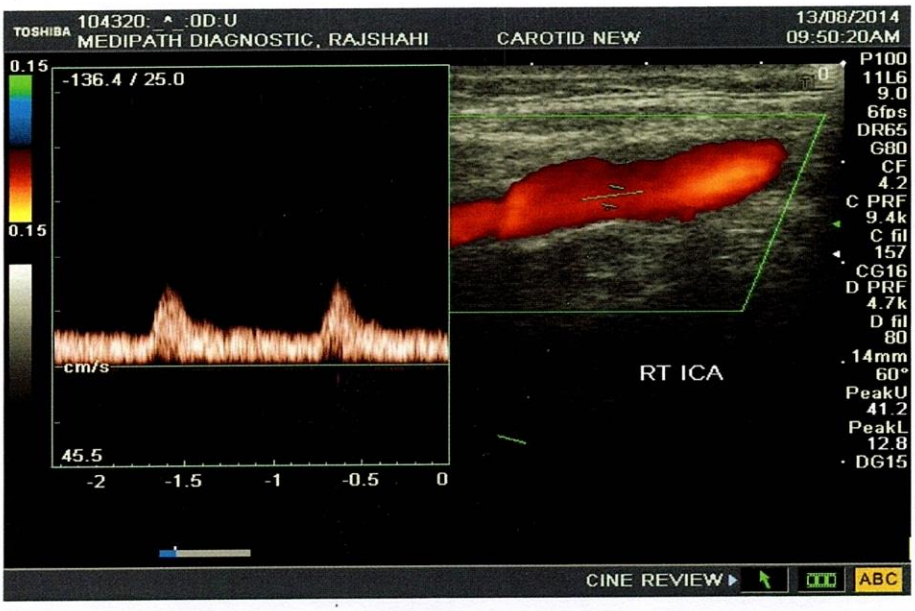


Fig.11: Biphasic flow pattern

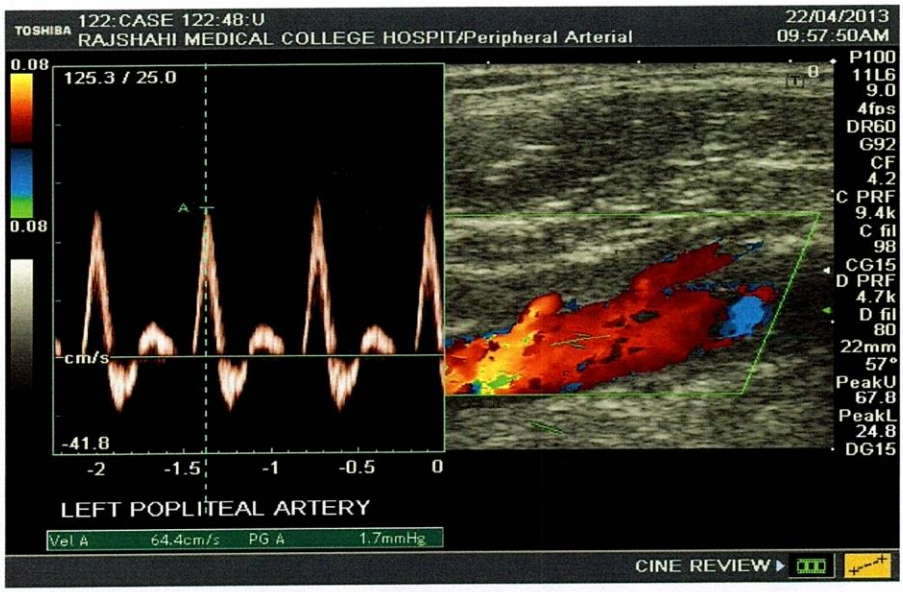


Fig. 12- Triphasic flow pattern.

2.1.5.3 Doppler Indices

Some indices are figured out from the waveform for the assessment of disease. Waveform indices are derived from a combination of a few dominant features of the waveform. In practice two classes indices are used, those related to the degree of diastolic flow and others related spectral broadening. (McDicken and Hoskins 2000) The common indices used are i) Resistance Index or resistive index (RI), ii) Pulsatility Index (PI), iii) A/B Ratio.

Resistance Index

Resistance Index is calculated as systolic velocity – diastolic velocity / systolic velocity. It is also known as Pourcelot index.

Pulsatility index

Pulsatility Index (PI) is peak to peak height of sonogram waveform / mean height over one cardiac cycle. The value is independent of probe angle to vessel. PI may prove to be a more reliable index for assessing distal rather than proximal disease.(Clifford *et al.* 1981)

Johnston *et al.* (1984) compared the use of four indices, the pulsatility index, height-width index, path length index, and a Laplace transform function index. The diagnostic accuracy of each index was determined from receiver operating characteristic curves. They concluded that all four indices were capable of detecting significant aortoiliac disease with approximately equal diagnostic accuracy of 90–95% but that pulsatility index had the advantages of simplicity and ease of calculation.

A/B Ratio:

It is defined as the ratio of two specified velocities.

2.1.6. Doppler US of Carotid Arteries

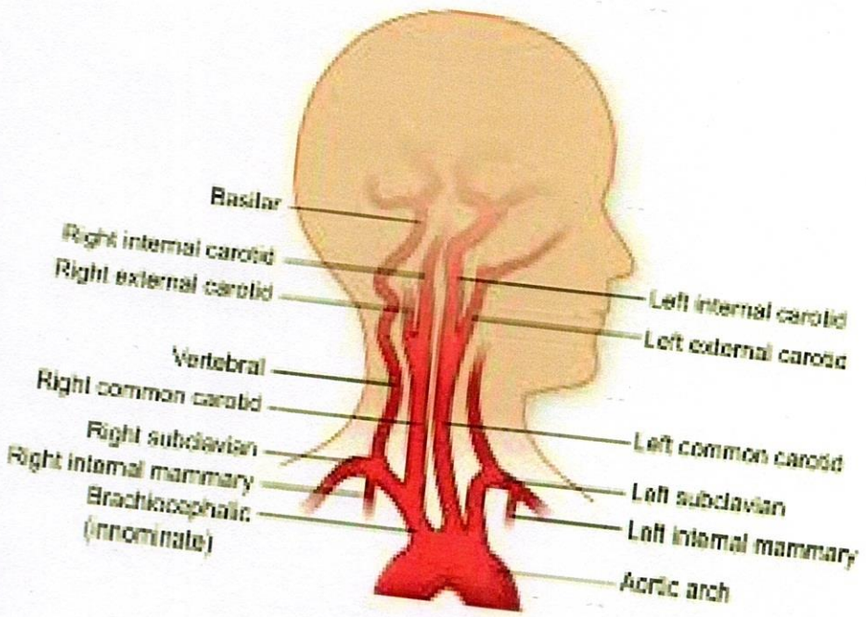


Fig-13: Carotid Vessels

The carotid arteries are major blood vessels in the neck that supply blood to the brain, neck, and face (Fig-13). There are two carotid arteries, one on the right and one on the left. In the neck, each carotid artery branches into two divisions: i) The internal carotid artery supplies blood to the brain. ii) The external carotid artery supplies blood to the face and neck. The carotid sinus, or carotid bulb, is a widening of a carotid artery at its main branch point.

Like all arteries, the carotid arteries are made of three layers of tissue: i) Intima, the smooth innermost layer, ii) Media, the muscular middle layer and iii) Adventitia, the outer layer. (Sinnatamby 2001). The measurement of the thickness of tunica intima and tunica media is called Intima-media thickness (IMT) which is an important criteria in assessment of the carotid artery.

Atheromatous plaque may slowly build up in the carotid artery wall, over decades. The growing plaque may eventually narrow the carotid artery and lead to a stroke.

Duplex ultrasonography is a useful diagnostic tool for assessing carotid artery disease. It has become the most common screening and diagnostic test to evaluate carotid artery stenosis. It has also been employed alone or together with other noninvasive techniques for definitive decision making prior to carotid endarterectomy.

American College of Cardiology Foundation/American Heart Association (ACCF/AHA) coordinated guideline 2011 recommended ultrasonography as the initial diagnostic test to detect hemodynamically significant stenosis to evaluate asymptomatic patients with known or suspected carotid stenosis. The same guideline recommended ultrasonography to detect carotid stenosis in patients who develop focal neurological symptoms. (Brott *et al.* 2011).

Initially a visual assessment of stenosis is on B mode and color Doppler image. It there is a plaque, the reduction in lumen area is calculated at the narrowest point of the artery with carotid plaque and expressed as percent reduction compared with the area of the original arterial cross section at the

site of the disease. Degree of stenosis can be measured by the cursor, measuring the area of the original arterial cross section and the area of stenosed colour filled residual lumen (Fig 14).

Most criteria for grading internal carotid artery stenosis rely on velocity measurements (Sergio *et al.* 2005, Persson *et al.* 1994). Stenosis in CCA of more than 50% can be inferred by the presence of a focally increased velocity followed by post stenotic turbulence. (Mitchell and Moneta 2005). Velocity criteria for ICA reported by many literature can be confusing, with apparently widely varying velocities being quoted for specific levels of stenosis. For diagnosis of a diameter stenosis by 60% or greater, Bluth *et al.* (1988) suggested a peak systolic velocity more than 130 cm/ sec and Carpenter *et al.* (1995) suggested velocities of 170cm/sec. For 70% diameter steosis, Robinson *et al.* (1988) suggested 225 cm/sec whereas Hood *et al.* (1996) suggested 130cm/sec. Robinson *et al.* (1988) also reported Internal carotid/Common Carotid arterial systolic velocity ratio as an useful measurement to determine the main level of stenosis, to distinguish 50% and 70% stenosis. Blood flow starts to decline at the level of 50% diameter stenosis and at 70% diameter stenosis clinical symptoms appear which is indicated for surgery. The ratio > 2 indicates 50% stenosis and > 3 indicates 70% stenosis.

Intima-media thickness is also an important criterion in assessment of the carotid artery.

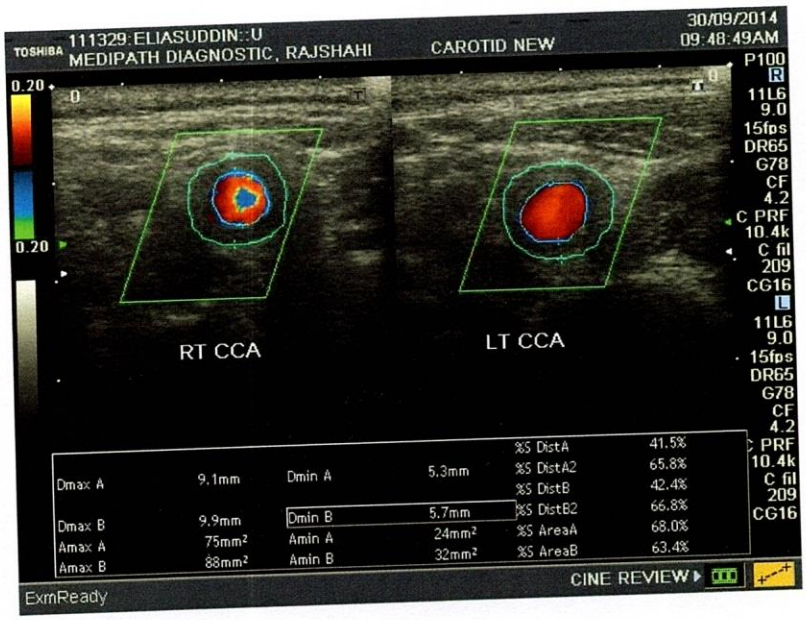


Fig 14 : Assessment of Carotid Stenosis on B mode and Color Doppler image.

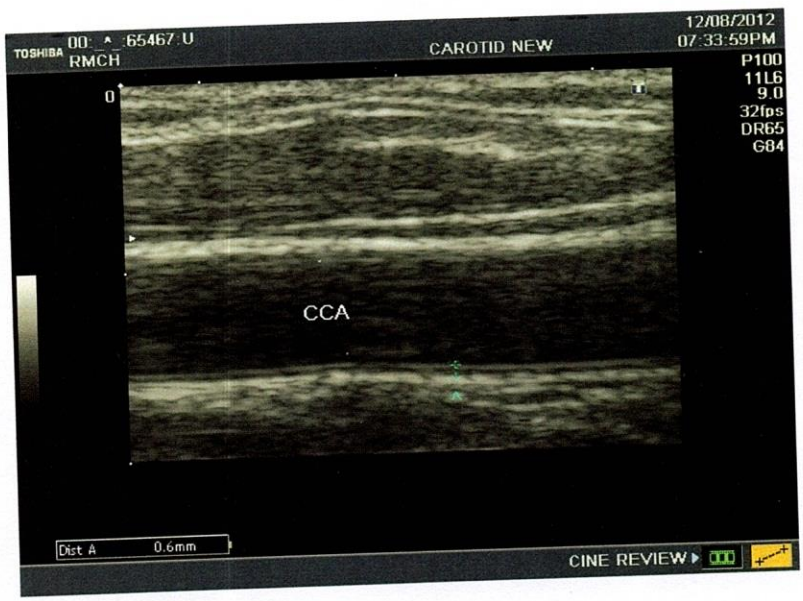


Fig 15: Measurement of CIMT showing CIMT is 0.6mm.

2.1.6.1 Intima-media thickness (IMT):

Intima-media thickness (IMT) is a measurement of the thickness of tunica intima and tunica media which is used to detect the presence of atherosclerotic disease and to track the regression, arrest or progression of atherosclerosis. Ultrasound IMT measurement was first proposed in 1984 in Milan by Paolo Pignoli (Pignoli *et al.* 1984). Later on carotid intima media thickness (CIMT) as an indicator of prognosis of atherosclerosis have been reported by many authors. The American Society of Echocardiography published a consensus statement on measurement of carotid IMT (CIMT) in 2007 (Stein 2007). IMT measurements can be done and ultrasound images can be obtained from the near and far walls of the right and left distal common carotid arteries, the carotid bifurcation and the proximal internal carotid arterial segments. CIMT in an individual patient is therefore often a composite of intima-media thickness measurements of various images of various segments and angles. For CIMT measurements, longitudinal images of the carotid arteries are obtained (Fig-15) in which the leading edges of the lumen-intima and media-adventitia interfaces of the arterial wall represent intima-media complex displayed as two bright white lines separated by a hypoechoic space (Bots *et al.* 1997). It is simple, safe, reliable and reproducible technique for evaluating patients risk for cardiovascular disease and cerebrovascular disease .

Blot *et al.* (1997) did a prospective follow up study to see association between common carotid IMT and myocardial infarction. The means duration of follow up was 2.7 years. They provided evidence that an increased common carotid IMT is associated with future cardiovascular and

cerebrovascular events. They also stated that the level of IMT does not reflect the presence of arterial stenosis in the CCA and the arterial blood flow in these subject was virtually normal.

Many investigators have evaluated associations between the risk factors and mean IMT. They found that IMT increases with age, sex, hypertension, diabetes, hyperlipidemia and many other factors (Davis *et al.* 2001, Sun *et al.* 2002, Zanchetti *et al.* 1998). Typically, normal common carotid CIMT at age 10 is approximately 0.4 to 0.5 mm, while from the firth decade of life onward this progresses to 0.7 to 0.8 mm or more. (de Groot *et al.* 2008 ; Naqvi and Lee 2014)

In a study on potentially healthy individuals who had no cardiovascular risk factors the upper limit of normal CIMT was 0.59 mm in those under 25 years to 0.95 in those aged over 65 years. An IMT greater than 0.8mm is indicative of atherosclerosis and increased risk of cardiovascular disease. (Jarauta *et al.* 2010, Salonen and Salonen 1991)

An increased cross-sectional carotid intima-media thickness was associated with unfavorable levels of established cardiovascular risk factors,(Heiss *et al.* 1991, Salonen and Salonen 1991, Walter 1992) prevalent cardiovascular disease,(O'Leary *et al.* 1991) and atherosclerosis elsewhere in the arterial system. (Bots *et al.* 1994, Polak *et al.* 1993).

2.1.7 Doppler US in lower limb vessels

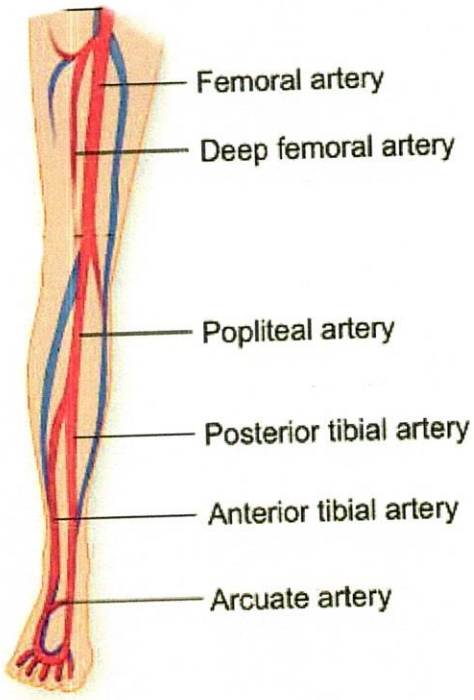


Fig 16: Arteries of lower limb.

The arteries of the lower limb (Fig-16) starts at inguinal ligament where the external iliac arteries continues around the side of the pelvis and become the commom femoral arteries. The common femoral artery runs from the inguinal ligament to its division into superficial and deep femoral arteries in the upper thigh usually at 3-6 cm distal to the inguinal ligament. The superficial femoral artery passes down-wards along the anteromedial aspect of the thigh lying anterior to the vein. In the lower third of the thigh it passes into the adductor canal, passing posteriorly behind the lower femur it enters the popliteal fossa and becomes the popliteal artery. Below the knee joint the

popliteal artery divides into the anterior tibial artery and the tibioperoneal trunk. The latter divides into the posterior tibial artery and the peroneal artery.

The anterior tibial artery passes forwards through the interosseous membrane between the fibula and tibia.

The posterior tibial artery passes down the deep medial aspect of the calf to pass behind the medial malleolus.

The first study of the peripheral arterial circulation by duplex scanning was published in 1985 (Jager *et al.* 1985a). The assessment of lower limb atheroma and degree of stenosis is not done on direct visualization of vessels on B mode image, rather it depends upon hemodynamic status. The assessment is done on the basis of velocity of flow and spectral waveform. (Legemate *et al.* 1989).Various criteria for assessment of degree of stenosis were reported by many authors based on velocity and pattern of waveform.

Normal velocities in lower limb arteries were considered 120mm/sec in the iliac segment, 90mm/sec in the superficial femoral segment and 70mm/sec in popliteal segment by Jager *et al.* (1985b). Strandness (1993) suggested normal ranges of peak systolic velocities were 119±22 cm/sec in the common external iliac arteries; 114±25 cm/sec in the common femoral artery; 91±14 cm/sec in the proximal superficial femoral artery; 94±14 cm/sec in the distal superficial femoral artery; and 69±14 cm/sec in the popliteal artery.

In a normal lower extremity artery, there is triphasic flow pattern. This triphasic waveform is characteristic of arteries supplying muscular bed, which has high peripheral resistance. During exercise or transient ischemia,

there is loss of triphasic pattern. Waveform can be affected by stenosis. Waveform change is noticed at the site of stenosis and also at proximal or distal to stenosis. If the vessels cannot be visualized in continuity, then a change in the waveform between two points is indicative of disease. The presence of triphasic velocity pattern excludes a pressure and flow reducing lesion proximal to the recording site. (Allan and Gallagher 2006). In occlusive arterial diseases, flow velocity is increased in the region where the lumen is narrowed. A spectral broadening is seen just beyond the stenosis indicating turbulence (Fig-17). Conversely, vascular resistance is decreased as a result of collateral circulation and vasodilatation in the distal part of the obstruction. As the disease progresses, the triphasic flow diminishes to a biphasic flow. This is due initially to the loss of elastic recoil caused by hardening of the arterial wall. If the disease progresses further, the flow loses its pulsatile nature to a monophasic signal with increased diastolic flow owing to regional vasodilatation. More distally the width of first systolic component is increased and overall height is decreased, known as dumping of the waveform (Fig-18). Proximal disease above the point of measurement results in loss firstly of the third component and then of the second component of the waveform (Zierler and Zierler 2005).

Cossman *et al.* (1989) described velocity criteria for the assessment of lower limb arterial stenosis. They divided lower limb arteries stenosis in to 4 groups. 1) Normal, 2) 0-49% stenosis, 3) 50-99% stenosis and 4) 100% stenosis or total occlusion based on the peak systolic velocity (PSV) and velocity ratio (VR). Velocity ratio is the ratio of PSV at the stenosis compared with the PSV 1-2 cm upstream.

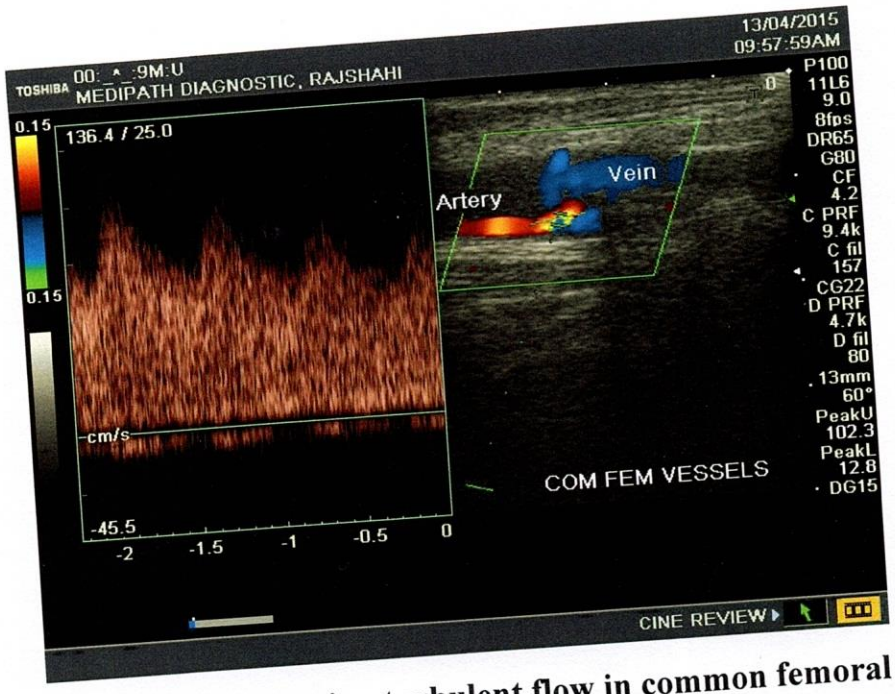


Fig 17 : Waveform indicating turbulent flow in common femoral artery.

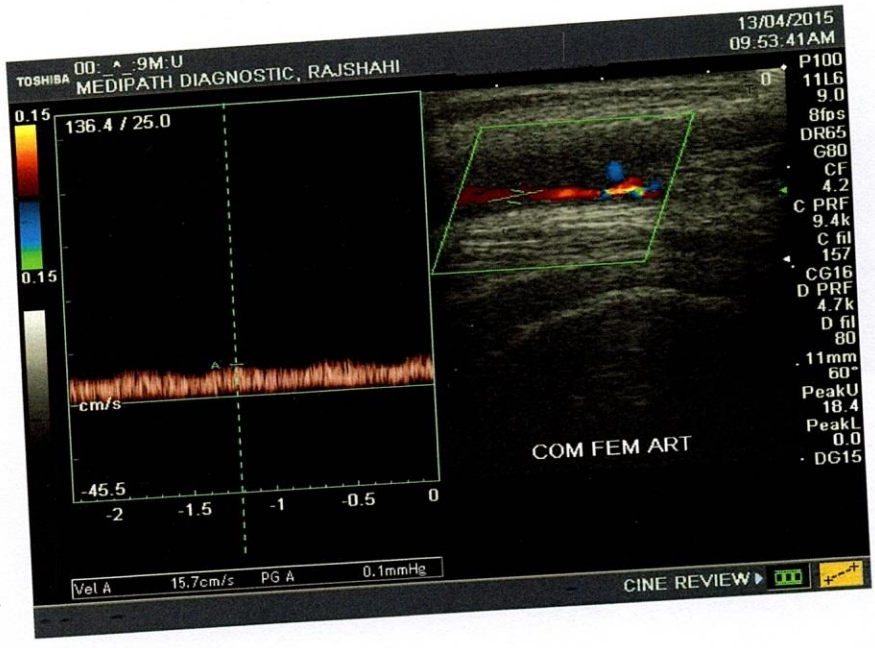


Fig 18 : Dumping waveform found in common femoral artery

According to Cossman *et al.* (1989) when PSV is < 150/ sec and VR is 1.5:1 it is considered normal. Degree of stenosis can be considered as follow.

Stenosis (%)	PSV (cm/sec)	VR
0-49	150-200	1.5-2:1
50-75	200-400	2-4:1
> 75	>400	>4:1
Occlusion	No flow	No flow

Stradness *et al.* (1993) classified the degree of arterial disease into 4 categories as 1) 1%-19% stenosis, 2) 20%-49% stenosis , 3) 50-99% stenosis and 4) Total occlusion. They suggested for determining the degree of arterial narrowing by comparing PSV change from one segment of the artery to next. The criteria of assessment as follow:

1%-20%% : Spectral broadening alone.

20%-49% : PSV increased by >30% but less than 100%

50%- 99% : PSV increased by >100 %

Total occlusion : No flow.

Using ultrasound, the degree of arterial disease in the lower extremities was classified into 4 categories according to Lau *et al.* (2011). 1) normal (0% stenosis), 2) 1-49% stenosis, 3) 50-99% stenosis, and 4) total occlusion (100% stenosis). They suggested use of wide range of 50%-99% because determining with precision the exact degree of stenosis is difficult, since the limb arteries are small. Diagnostic criteria for a hemodynamically important '50-99% stenosis' require that the peak systolic velocity is double at the lesion when compared with a more-proximal segment, and that it is greater than 200 cm/s, with evidence of turbulence demonstrated by colour Doppler ultrasonography.

2.2. ATHEROSCLEROSIS

2.2.1. Definition

Atherosclerosis derives its name from the Greek words 'athere' meaning accumulation of lipid and 'sclerosis' meaning hardening (Singh *et al.* 2002). It is a disease of arterial wall where there is accumulation of lipid, infiltration of macrophages, proliferation of smooth muscle cells, accumulation of connective tissue components and formation of thrombus. Atherosclerosis can affect any artery of body. Coronary arteries, Cerebrovascular arteries and lower limb arteries are most commonly affected.

2.2.2 : Pathogenesis of atherosclerosis

An atherosclerotic lesion is composed of three major components. The first is the cellular component comprised predominately of smooth muscle cells and macrophages. The second component is the connective tissue matrix and extracellular lipid. The third component is intracellular lipid that accumulates within macrophages, thereby converting them into foam cells. (Crowther 2005).

The earliest visible lesion of atherosclerosis is the fatty streak, which is due to an accumulation of lipid-laden foam cells in the intimal layer of the artery. With time, the fatty streak evolves into a fibrous plaque, the hallmark of established atherosclerosis. Ultimately the lesion may evolve to contain large amounts of lipid. If it becomes unstable, denudation of overlying endothelium, or plaque rupture, may result in thrombotic occlusion of the overlying artery. (Crowther 2005)

Atherosclerotic lesions develop as a result of inflammatory stimuli, subsequent release of various cytokines, proliferation of smooth muscle cells, synthesis of connective tissue matrix, and accumulation of macrophages and lipid (Fig 19).

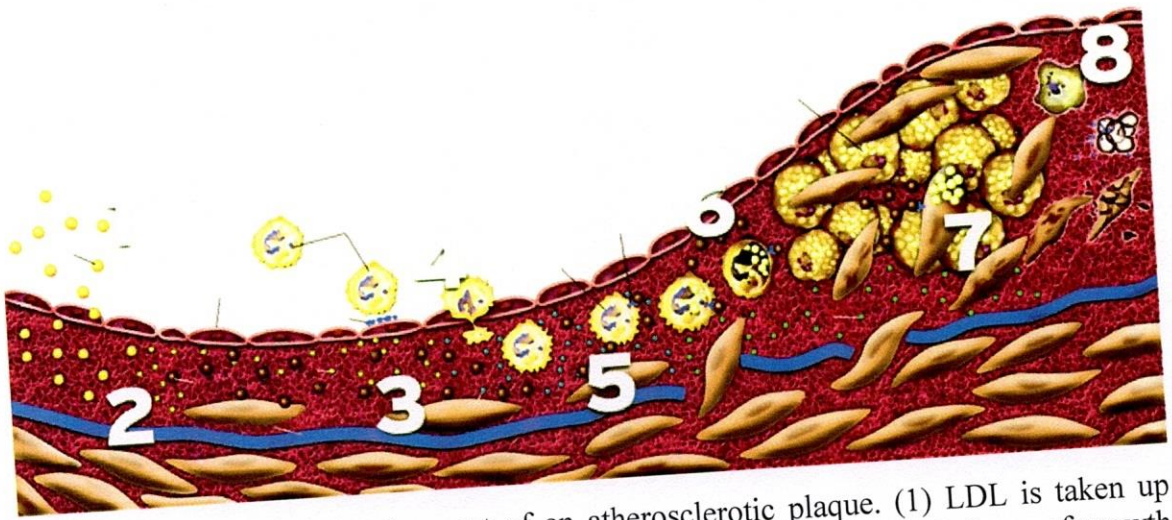


Fig 19: The stages of development of an atherosclerotic plaque. (1) LDL is taken up by the endothelium. (2) Oxidation of LDL by macrophages and VSMCs. (3) Release of growth factors and cytokines. (4) Attraction of additional monocytes. (5) Foam cell accumulation. (6) SMC proliferation. (7, 8) Formation of plaque (Faxon *et al.* 2004)

One of the earliest steps in atherogenesis is the endothelial injury and dysfunction followed by vascular permeability, leukocyte adhesion and thrombosis. Subsequently there is infiltration, retention and modification of atherogenic lipoprotein, mainly low density lipoprotein (LDL) and its oxidized form. (Kinlay and Ganz 1997; Lerman *et al.* 1991).

LDL moves from blood into the vessel wall. It becomes oxidized by action of free radicals or direct activity of leukocytes. Oxidized LDL is chemotactic for monocycle. Monocytes migrate into the intima, take up oxidized LDL particles and

become lipid laden macrophages and foam cells. In response to cytokines and growth factors produced by activated macrophages, smooth muscle cells migrate from the media to intima. The lipid core is covered by smooth muscle cells and matrix forming a stable atherosclerotic plaque (Faxon *et al.* 2004; Newby *et al.* 2010; Crowther 2005).

Various factors that may stimulate endothelial activation are i) elevated LDL, ii) oxidant stress by tobacco smoking, iii) hypertension, iv) diabetes mellitus, v) genetic alternations, vi) infections by microorganisms, vii) estrogen deficiency and viii) advancing age.

2.2.3 Risk factors for developing atherosclerosis

Hyperlipidemic states, diabetes mellitus, smoking, obesity, hypertension, and sedentary life style are of the risk factors for atherosclerosis. However, any one of these alone is not sufficient to produce an atherosclerotic lesion. There is substantial evidence that hyperhomocysteinemia and hyperfibirinogenemia have been linked to atherosclerosis. Hmocysteinemia promotes atherogenesis by causing endothelial damage. Fibrinogen is involved in the formation and growth of atheroma by stimulating smooth muscle cells migration and proliferation and promoting the uptake of lipid by macrophages. (Singh *et al.* 2002).
Genetic alterations adversely promote the development of atherosclerotic disease. Familial predilection is an acknowledged risk factor besides the other predisposing diseases such as polygenic disease and combined hyperlipidemia

2.2.3.1 Tobacco Smoking and atherosclerosis

The exact toxic components of tobacco responsible for producing atherosclerosis have not been clearly identified. Carbon monoxide, nicotine, and many other substances in tobacco are the subjects of discussion but nicotine is probably the most studied component. Although nicotine plays a major role in smoking-related increases in cardiac output, heart rate, and blood pressure, its role in smoking related athero-thrombotic disease remains controversial. Nicotine exposure alone had been reported to cause no change, a decrease, or an increase in atherogenesis. In various models, although high doses of nicotine favor atherogenic changes, the majority of current evidence suggests that nicotine, at concentrations similar to a smoker's blood level, has a minor effect on the initiation or propagation of atherosclerosis. Similarly, the effect of nicotine on thrombo-hemostatic factors such as platelets, fibrinogen, or t-PA, PAI-1 appears to be insignificant in the setting of smoking. (Benowitz 1997 ; Sun *et al.* 2001 ; Mayhan and Sharpe 1999).

The inflammatory response is an essential component in the initiation and evolution of atherosclerosis. Several studies have indicated that smoking causes 20% to 25% increase in the peripheral blood leukocyte count (Smith and Fischer 2001). In vivo, smoking is associated with an increased level of multiple inflammatory markers including C-reactive protein, interleukin-6, and tumor necrosis factor alpha in both male and female smokers (Tappia *et al.* 1995; Tracy *et al.* 1997). Thus there is the evidence that smoking initiates inflammation, the first step in the pathogenesis of atherosclerosis.

The toxins in tobacco smoke lower a person's high density lipoprotein cholesterol (HDL) while raising levels of low-density lipoprotein cholesterol (LDL). Smoking

increases oxidative modification of LDL. Circulating products of lipid peroxidation and autoantibody titers to oxidized LDL are significantly increased in smokers (Heitzer *et al.* 1996). Yokode *et al.* (1988) reported that exposure to cigarette smoking extract caused a modification of LDL, which was actively taken up by the macrophages to form foam-cells in culture. Frei *et al.* (1991) observed that exposure of human plasma to the gas phase of cigarette smoke caused oxidative modification of plasma LDL.

It is suggested that tobacco smoking is related to thrombogenesis, as well as atherogenesis. It has two effects on platelets, an acute effect which causes potentiating of platelet activation occurring shortly after smoking a cigarette, and a chronic desensitization of the cell to activating agents of atherosclerosis (Inoue 2004). Although there are numerous longitudinal investigations, the opinion on platelet aggregation produced as the result of acute effects of smoking have been conflicting. Some have found no such change in platelet aggregability immediately after smoking (Davis *et al.* 1985) and some demonstrated increased platelet aggregatablity immediately after smoking (Renaud *et al.* 1984 ; Schmidt and Rusmussen1984). The relationship between platelet aggregation and cigarette smoking was examined by a large number of studies. Recently, however, platelet aggregability has been assessed by newly developed systems to closely evaluate platelet aggregation. Platelet activation by cigarette smoking is linked to thrombosis formation, including onset of myocardial infarction (Inoue 2004). So smoking increases inflammation, thrombosis, and oxidation of low-density lipoprotein cholesterol, all are related to atherogenesis.

2.2.3.2 Diabetes and atherosclerosis

Diabetes Mellitus predisposes to higher rates of coronary artery disease , cerebral vascular disease, and peripheral arterial disease. Compared to cardiovascular disease in nondiabetics, diabetic patients have a greater overall coronary plaque burden and a higher rate of multivessel disease. The proportion of stenotic segments is directly proportional to the duration of disease (Gao *et al.* 2011). The effects of diabetes on the vasculature are quite extensive as diabetes affects not only the endothelium and smooth muscle cells, but also platelets, lipoproteins, local vasoactive substance production and function, clotting factors, triglycerides, as well as local arterial response to hypoxia and new collateral vessel formation (Beckman *et al.* 2002) . The pathogenesis of diabetic atherosclerosis involves not only the direct effects of chronic hyperglycemia, but also insulin resistance, nonesterified free fatty acid production, dyslipidemia, hypercoagulability, and impaired response to injury (Shrikhande *et al.* 2010) . The development of diabetes-related atherosclerosis follows the same histologic course as atherosclerosis in nondiabetic patients. This includes endothelial injury, smooth muscle cell proliferation, foam cell development and in filtration, platelet activation, and increased inflammation. It is suggested that endothelial injury may be the initial event in the genesis of atherosclerosis, followed by platelet adhesion and aggregation at the site of injury.

2.2.3.3 Role of lipid

Increased triglyceride and LDL and decrease HDL are responsible for the genesis of atherosclerotic lesion. Triglyceride concentration is more predictive than total cholesterol in determining the risk (Chanu 1999).

Reduced HDL is associated with increased atherosclerosis. The protective effects of HDL is mediated by its role in reverse cholesterol transport. It acts as an acceptor, transporter and inactivator of oxidized LDL. In addition, HDL inhibits monocyte adhesion and migration into the intima and also inhibits growth factor induced smooth muscle cell proliferation. It stimulates cell repair and proliferation and preserves endothelium dependant vascular activity. (Acton *et al.* 1999).

2.2.4 Clinical manifestations

Distinct clinical manifestations are seen depending on the type of vascular bed affected. Coronary lesions lead to myocardial ischemia or infarction. Similarly, transient ischemic attacks and stroke are seen in the cerebral circulation, whereas intermittent claudication occurs in the peripheral circulation. Infarction of the gut produces lesions in the splanchnic circulation, while renal artery lesions result in ischemia due to reduced renal perfusion and damage the renal parenchyma, (Singh *et al.* 2002). However the three main territory affected by atherosclerosis are coronary arteries, cerebrovascular arteries and lower limb arteries. Coronary arteries and peripheral arteries are the main two territories of interest in this study.

2.3 : CORONARY ARTERY DISEASE

2.3.1 Definition

Coronary artery disease (CAD), also called coronary heart disease, is the disease involving arteries supplying to heart as a result of narrowing or blockage of the arteries caused by atherosclerosis.

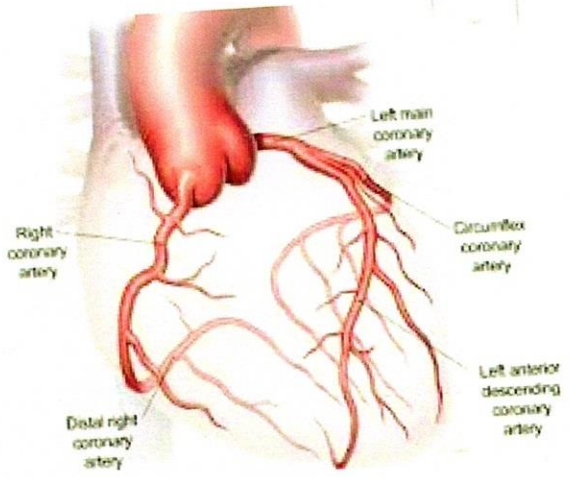


Fig 20: Diagram of Coronary Arteries

2.3.2 Coronary Arteries (Fig-20)

The coronary arteries originate as the right coronary artery (RCA) and left main coronary artery (LMCA) which exit the ascending aorta just above the aortic valve. The left main coronary divides into two major branches: The left anterior descending artery (LAD) and The left circumflex artery (LCX). Left anterior

descending artery supplies blood to the front of the left side of the heart. The circumflex artery encircles the heart muscle and supplies blood to the outer side and back of the heart. These main branches subdivide and course over the surface of the heart (epicardium) as they traverse away from the aorta. These arteries divide into progressively smaller branches that then progress inward to penetrate the epicardium and supply blood to the transmural myocardium (Sinnatamby 2011; Malouf *et al.* 2008). Since coronary arteries deliver blood to the heart muscle, any coronary artery disorder or disease can have serious implications by reducing the flow of oxygen and nutrients to the heart muscle. Atherosclerosis is the most common cause of heart disease leading to impairment of blood flow and coronary ischemia.

2.3.3 Coronary Blood Flow

Coronary blood flow has a direct relationship to myocardial oxygen consumption. There is increase coronary blood flow during exercise to meet the energy requirement of the heart. Normal coronary blood flow at rest is about 200ml/min, which can be up to 1000ml/min on maximal exercise. (Depre *et al.* 2008)

2.3.4 Risk factors of CAD

Risk factors for CAD are those for atherosclerosis. Hypertension, positive family history, continued tobacco smoking, dyslipidemia, diabetes mellitus, obesity, sedentary life style, age and male sex are the major risk factors. The effect of risk factors is multiplicative rather than additive. People with combination of risk factors are at greater risk. Diets deficient of fresh fruits and vegetables is also considered as a risk factor for developing CAD. Platelet activation and high levels

of fibrinogen are associated with increased risk of thrombosis in coronary arteries. (Newby *et al.* 2010)

2.3.4.1 Hypertension and CAD

Several major prospective epidemiologic studies have found that both systolic and diastolic hypertension have a strong, positive, continuous and graded relationship to CAD (Chobenian *et al.* 2003). A widened pulse pressure, an indicator of arterial stiffness, is another blood pressure parameter that predicts CAD (Vaccarino *et al.* 2000). The potential mechanism by which hypertension may cause CAD are impaired endothelia function and increased endothelial permeability to lipoproteins, increased adherence of leukocytes, increased oxidative stress, haemodynamic stress triggering acute plaque rupture and increased myocardial wall stress and oxygen demand.

2.3.4.2 Smoking and CAD

Smoking is a major cause of cardiovascular disease mortality. In the year 2000, an estimated 1.62 million cardiovascular deaths in the world, 11% of total global cardiovascular deaths, were due to smoking (Ezzati *et al.* 2005).

The chemicals in tobacco smoke can promote atherosclerosis which has been discussed in earlier section 2.2.3.1.

In addition to active smoking, passive smoking can also carry a risk of coronary artery disease. In a meta-analysis on 18 epidemiological studies it was found that overall, nonsmokers exposed to environmental smoke had a relative risk of coronary heart disease of 1.25 (95 percent confidence interval, 1.17 to 1.32) as compared with nonsmokers not exposed to smoke. A significant dose-response

relation was identified, with respective relative risks of 1.23 and 1.31 for nonsmokers who were exposed to the smoke of 1 to 19 cigarettes per day and those who were exposed to the smoke of 20 or more cigarettes per day, as compared with nonsmokers not exposed to smoke (He *et al.* 1999).

Smokers who stop smoking reduce their risk of developing and dying from tobacco-related illnesses. The extent of benefit partly depends on the intensity and duration of prior tobacco smoke exposure. Smokers who stop smoking can be expected to live longer and are less likely to develop tobacco-related diseases, including coronary heart disease, cancer, pulmonary disease and malignancy. Smokers also benefit from quitting smoking even after the development of smoking-related diseases, such as coronary heart disease or chronic obstructive pulmonary disease. A study showed that among the men born around 1920, prolonged cigarette smoking from early adult life tripled age specific mortality rates, but cessation at age 50 halved the hazard, and cessation at age 30 avoided almost all of it (Doll *et al.* 2004).

Thus the evidence linking cigarette smoke exposure with cardiovascular disease is clearly present.

2.3.4.3 Diabetes and CAD

Patients with diabetes mellitus have an over ten fold risk for cardiovascular disease in their lifetime (Nathan *et al.* 2005). In the United States, 77% of diabetes related hospital admissions are for cardiovascular complications. A key feature of diabetes contributing to this is the development of an accelerated atherosclerosis (Faxon *et al.* 2004).

2.3.4.4 Age and Sex

The incidence and prevalence of CAD increases sharply with age, so that age might be considered one of the most potent cardiovascular risk factor (Thom *et al.* 2006). CAD is relatively uncommon in premenopausal women. There is a dramatic rise in CAD incidence in women after age 55 years. Early menopause either natural or surgical, is associated with an increased CAD risk. Numerous observational studies show that postmenopausal users of estrogen replacement therapy have a 40 to 50 percent lower risk of CAD events compared with non users (Rossouw *et al.* 2002; Mosca *et al.* 2001).

2.3.4.5 Physical activity.

Physical inactivity is an independent risk factor for CAD and rightly double the risk. Physical activity slows the progression of angiograhically defined coronary altherosclerosis in humans. (Fletcher *et al.* 1996). More than 50 observational studies, primarily on men, have established that physical fitness, on the job physical activity and leisure time physical activity reduce the risk of CAD (Haskell 1994).

2.3.4.6 Obesity

Obesity is over 20% of ideal body weight or a state in which body fat is above the ideal. Normally it can be measured based on Body Mass Index (BMI). A BMI of more than 30 classifies one as being obese. Waist-Hip ratio is also used as a indicator of obesity. Waist-Hip ratio above 0.90 for males and above 0.85 for females is considered as obese. American Heart Association (AHA) mentioned

obesity as a major risk factor for CAD (Poirier *et al.* 2006). Obesity accelerates the progression of coronary atherosclerosis in adolescent and young adult men. (Mcgill *et al.* 2002). In woman obesity contributes independently from physical inactivity to the development of CAD (Wada *et al.* 2006).

2.3.4.7 Atherogenic Diet

An atherogenic diet is considered leading preventable causes of death, second only to tobacco use. (Mokdad *et al.* 2004) Considerable epidemiologic data indicate that populations with diets high in saturated and *trans*-fatty acids have higher rates of CAD (KrisEtherton *et al.* 2001). Conversely, those populations that consume large amounts of calories as vegetables, cereals, and fish have lower rates of CAD (Hu and Willett 2002). Populations that consume larger amounts of sodium in their diet have higher average blood pressures (Intersalt 1988). Caloric imbalance, in part a result of excess calorie consumption, is related to the rising prevalence of obesity and diabetes. On an individual basis, clinical trials of modified diets have demonstrated reductions in angiographic progression (Ornish *et al.* 1990) and significant reductions in recurrence of clinical events (de Lorgeril *et al.* 1999).

2.3.5 Investigations

2.3.5.1 Electrocardiography (ECG)

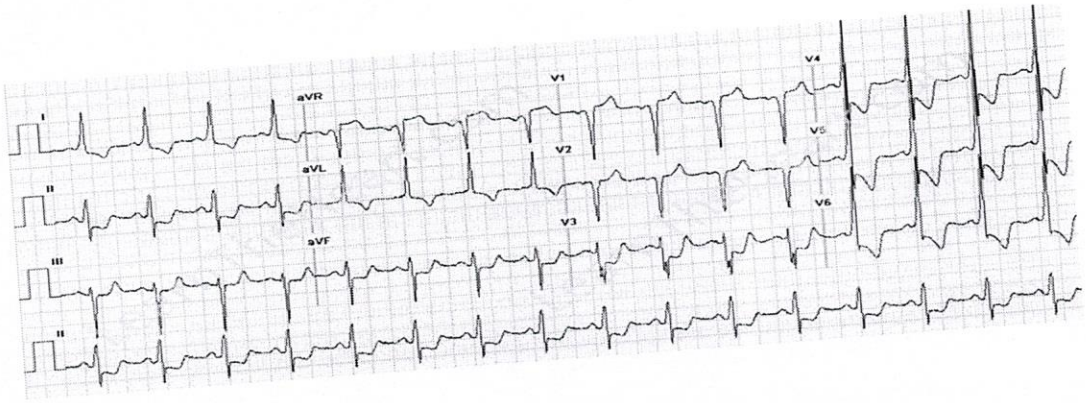


Fig 21 : A 12-lead electrocardiogram

A baseline 12 lead electrocardiogram (ECG) is performed in every patient with suspected CAD (Fig 21). An abnormal resting ECG increases the probability that a patient has ischemia or infarction, but gives no indication as to the severity of any associated obstructive CAD (Daly *et al.* 2003). A normal 12 lead ECG does not exclude a diagnosis of coronary heart disease (Connolly *et al.* 1984).

2.3.5.2 Cardiac Stress Test or Exercise Tolerance Test (ETT)

The cardiac stress test is done to evaluate the cardiovascular system in response to exercise. The test is done with heart stimulation under carefully controlled conditions and careful monitoring. The heart is stimulated either by exercise on a treadmill, or with intravenous pharmacological stimulation. The common practice is walking on a treadmill with the patient connected to an ECG.

Exercise is the body's most common physiologic stress, and it places major demands on the cardiopulmonary system. Thus, exercise can be considered the most practical test of cardiac perfusion and function. The exercise test, alone and in combination with other noninvasive modalities, remains an important testing method because of its high yield of diagnostic, prognostic, and functional information. The adaptations that occur during an exercise test allow the body to increase its resting metabolic rate up to 20 times, during which time cardiac output can increase as much as 6 times. The magnitude of these adjustments is dependent on age, gender, and body size, type of exercise, fitness, and the presence or absence of heart disease.

ETT has been quoted as having a sensitivity of 78% and a specificity of 70% in detecting coronary artery disease (Hill and Timmis 2002). Sensitivity is higher in patients with triple vessel disease and lower in patients with single vessel disease (Gibbons *et al.* 2002).

2.3.5.3 : Echocardiography

Echocardiography, often referred as simply an echo, is actually the ultrasonography of the heart. Rapid assessment of cardiac structure and function can be estimated from the information obtained by placing an ultrasound probe on chest wall. Both B mode images and Doppler signals are studied. However no information regarding patency of coronary artery can be obtained but figuring out the Ejection Fraction (EF) is an important determinant of the severity of systolic heart failure which may follow CAD. EF is the volume of the blood ejected by the heart per beat (stroke volume) divided by the volume of the blood filled heart at the phase of full relaxation. (end-diastolic volume). In a healthy 70-kilogram (150 lb)

man, the stroke volume is approximately 70 mL and the left ventricular end-diastolic volume) is 120 mL, giving an ejection fraction of $^{70}/_{120}$, or 0.58 (58%).

2.3.5.4 : Nuclear Imaging

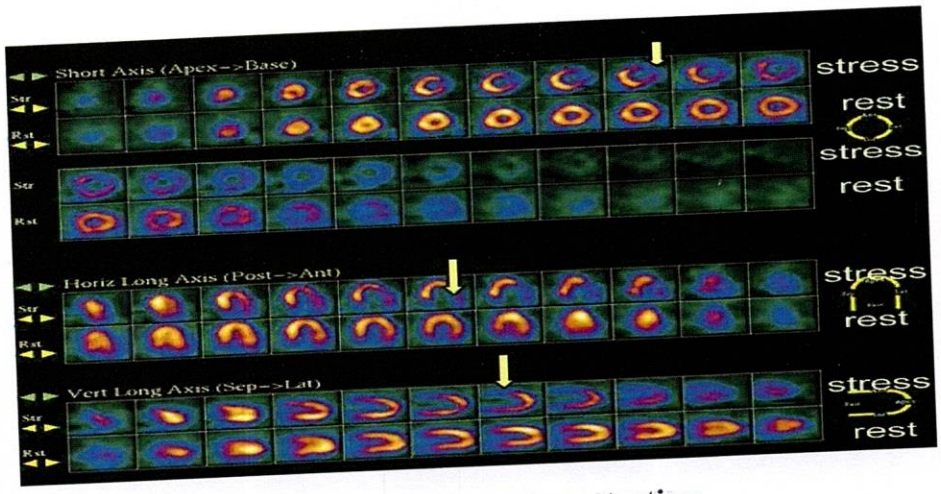


Fig 22 : Nuclear stress testing

Although radionuclide angiography (Fig-22) played a promotional role in noninvasive testing in decades past, by the 1990s, the use of this modality was largely replaced by echocardiography. However some important clinical application of this modality still remains. Gated single-photon emission computed tomography myocardial perfusion imaging (SPECT MPI) procedure is the most commonly used nuclear cardiology procedure. SPECT MPI is performed using a scintillation camera and an intravenously injected radiopharmaceutical that distributes to the heart in proportion to regional myocardial perfusion. ^{99mm} Tc-tetrofosmin is the current radiopharmaceutical agent used now a day. The scintillation camera detectors rotate 180 degrees around the patient in a semicircular or elliptical fashion, collecting a series of planar projection images at regular angular intervals. The three-dimensional (3D) distribution of radioactivity

in the myocardium is then mathematically reconstructed from the two-dimensional projections, and the resulting data is displayed in series of slices in the short axis, vertical long axis, and horizontal long axis orientation. (Berman *et al.* 2008).

The accuracy of diagnostic testing for CAD is defined clinically on the basis of sensitivity and specificity for identification of angiographically significant stenosis, most commonly employing either a 50 percent or a 70 percent diameter-narrowing cut off. ACC/AHA/ASNC guidelines on cardiac radionuclide imaging reported a pooled sensitivity and specificity of 87 and 73 percent of exercise SPECT MPI and 89 and 75 percent for vasodilator SPECT MPI for detection of CAD (ACC/AHA/ASNC guidelines, 2003).

2.3.5.5 Biomarkers of myocardial injury

Some biochemical substances are released in respond to myocardial injury which are considered as marker of myocardial injury. Important markers are Creatinin Kinase, Troponin and Myoglobin which are useful for both diagnosis and for assessment of prognosis (Puleo *et al.* 1994). An ideal marker should be released quickly into the blood and reflect quantitatively the magnitude of necrosis and it should be more specific to cardiac muscle rather than other non myocardial tissue. Creatinin kinase activity has been the most widely used serum cardiac marker but it is elevated in patient with other muscle disease, alcohol intoxication, seizures, pulmonary embolism and in some other disorders.

To improve the specificity it includes measurement of CK-MB levels by specific enzyme immunoassay that use monoclonal antibodies directed against CK-MB and by measuring CK-MB isoforms (Apple *et al.* 1997).

Cardiac troponin has increased sensitivity and specificity relative to CK. (Bertrand *et al.* 2000). Regardless of the etiology of troponin release, an elevated level

implies a worse prognosis (Ohman *et al.* 1996, Antman *et al.* 1996). There is an incremental risk of death or MI in patient with elevated troponin that can be seen in a quantitative fashion, (Aviles *et al.* 2002). An elevated troponin level is an easy and objective method for identifying high risk acute coronary syndrome patient (Hamm *et al.* 1999).

Even patient in whom CK –MB levels are within normal limits, troponin elevation signifies an increased risk of death when compared with those without elevations (Stubbs *et al.* 1996).

2.3.5.6 Coronary angiogram by Cardiac Catheterization

Coronary angiogram by Cardiac Catheterization is an invasive diagnostic procedure to detect atherosclerotic artery disease by acquiring radiographic images of coronary arteries. During the procedure, a small catheter typically ~2.0 mm (6-French) in diameter, is introduced through femoral or radial artery up to the opening of coronary arteries. A large bolus of contrast media is introduced into the left ventricle by a high pressure contrast media injector. The contrast media may cause allergic reaction. There are three types of allergic reaction. i) minor cutaneous manifestations, ii) smooth muscle and minor anaphylactoid response and iii) major cardiovascular and anaphylactoid reaction. Besides that some major complications may occur during cardiac catheterization which are cerebrovascular accident, death, myocardial infarction and serious arrhythmia like ventricular tachycardia and fibrillation. So every Cardiac Catheterization laboratory must be equipped with a defibrillator, suction apparatus, airway tube and emergency drugs (Kern and King III 2008)

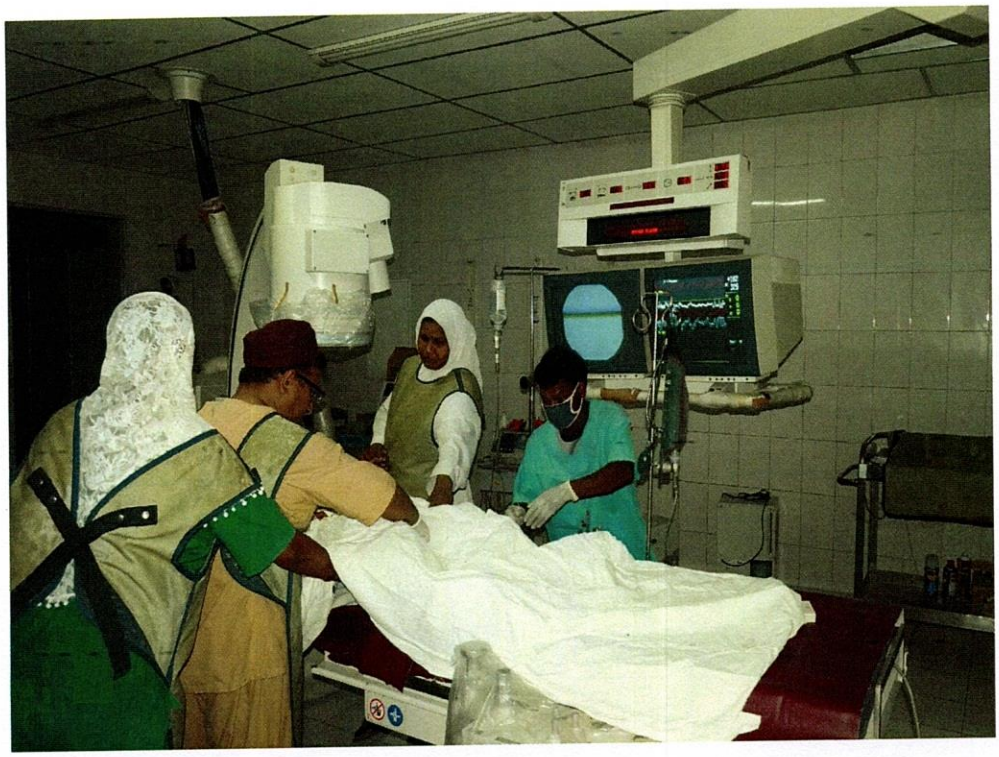


Fig 23 : Preparation of a patient for Coronary Angiogram.

The most widely used technique for vascular access is percutaneous femoral artery catheterization. However, the radial artery approach has many advantages. The radial artery is easily accessible, if there is bleeding it can be easily controlled due to its superficial location, complication after radial artery occlusion is less significant as collateral flow is possible through ulnar artery, patient can sit up and walk easily immediately after the procedure.

An angiographic lumen narrowing is commonly referred as stenosis which is expressed as percentage reduction in the diameter of the narrowed vessel site to the adjacent unobstructed vessel. It is actually a visual estimation and the severity is roughly classified. The exact evaluation is almost impossible. The variation from

one observer to another may range from 15 to 30 %. Even the same observer may render a different interpretation at a time remote from the first reading. This interobserver variability reveals a significant limitation of coronary angiography (Zir *et al.* 1976 ; deRouen *et al.* 1977). Quantitative measurement is possible by intravascular ultrasound assessment.

2.3.5.7 Coronary CT Angiography (CCTA)

Coronary CT angiography (CCTA) is a less invasive alternative to Catheter angiography. Instead of a catheter being inserted into a vein or artery, only a CT-visible dye is injected into the arterial system. Thus the CCTA lowers the risk of cardiac catheterization, arterial perforation and catheter site infection. Still some risks are involved relating to allergic reaction to the dye or contrast media used. It provides 3D images that can be studied on computer, and also allows measurement of heart ventricle size, infarct area and quantifying coronary arterial calcium.

CT scans have been used to look at various static anatomic regions like brain, abdominal cavity, and extremities but have not been useful for the heart because the heart is continuously in motion. Most early CT scanners took 1-8 pictures (slices) a minute, much slower than the rate of the heart. Too much motion artifact are created just as taking a picture of a moving object with a camera results in a blurry picture. A new generation of CT scanners which can take 64 pictures a minute is now available. With the use of a little medication to slow the heart rate to less than 64, CT images of the coronary arteries are now possible (Bryg 2014). Beta-blocker is often administration to lower the heart rate and decrease motion artifact. The level to which the heart rate should be lowered depends on the temporal resolution of the scan. The introduction of multirow spiral CT detector

systems which is known a multislice CT, currently allow acquisition of 4 to 64 simultaneous images, with slice thickness reduced to 0.5 to 0.625 mm. The primary advantage of dual-source CT scanning is greater temporal resolution, which allows CCTA to be performed at higher heart rates without the use of beta blockers. Several contraindications to beta-blocker therapy exist, including a heart rate below 60 bpm, a systolic blood pressure below 100 mm Hg, and decompensate cardiac failure, among others.

2.3.5.8 Electron beam computed tomography (EBCT)

Electron beam tomography (EBCT) is a specific form of CT scan. EBCT is similar to the more frequently used CCTA but has slightly different applications. In CCTA the scanner produce image by rotating an X-Ray tube around a circular gantry through which the patient advances on a moving coach. In EBT the X-ray tube is large and stationary, and partially surrounds the imaging circle. Rather than moving the tube itself, electron-beam focal point (and hence the X-ray source point) is magnetically swept along a tungsten anode in the tube, tracing a large circular arc at a 210 degree on its inner surface. This motion can be very fast. This different design was explicitly developed to better image heart structures, performing a complete cycle of movement with each heart beat. This spiraling approach can create clearer images of the heart even while it is in motion (Wikipedia 2015b, Vann 2009).

Advantage of EBCT

With a 100 msec acquisition time, a freeze frame image of the myocardium and coronary arteries in end diastole can be achieved with little if any motion blur

(Budoff *et al.* 2008). Image acquisition within 50 msec is required to completely avoid cardiac motion artifact (Boyd and Lipton 1983 ; Lu *et al.* 2001). Because EBCT has no moving part, as found in conventional CCTA scanners, imaging time is completed within 50 msec, which is the time required for the electron beam to sweep along the tungsten targets.

Another important advantage of electron beam computed tomography is to get the calcium score. The presence of coronary artery calcification (CAC) is clearly indicative of coronary atherosclerosis (Rumberger *et al.* 1995; Sangiorgi *et al.* 1998). EBCT has been shown to be an accurate noninvasive tool in the detection of coronary artery calcium (Detrano *et al.* 1994). Coronary artery calcium score (CACS) severity, as assessed by EBCT is directly related to the total atherosclerotic plaque burden present in the epicardial coronary arteries (Rumberger *et al.* 1995; Sangiorgi *et al.* 1998). Calcium Score, or Agatston Score, represents the total amount of plaque in coronary arteries. The number can range from 0 to 1,000 or more. The higher the number, the greater the risk. This score can help to find out whether an individual is at risk for a heart disease event in the next 10 years. A review of research shows that the higher the calcium score, the more likely one is to have atherosclerosis that threatens one's long-term health. (Agatstone 2008). Calcium Score is an excellent way of predicting the likelihood of it happening but not absolute way to predict who is going to have a heart attack. Other variable like smoking habit, hypertension, diabetes must be considered beyond one's calcium score. If someone continues to smoke, reluctant to control his blood pressure, a low Calcium Score will not protect him. On the other hand, someone with a moderately high Calcium Score, if he begins an aggressive prevention program immediately, his level of risk can sharply decline within months.

Coronary calcification is thought to begin early in life, but the progress is more rapid in older individuals who have further advanced atherosclerotic lesions (Janowitz *et al.* 1993). Recent studies have suggested that coronary calcification is an active as opposed to a degenerative process. It is an active, organized, and regulated process occurring during atherosclerotic plaque development where calcium phosphate in the form of hydroxyapatite precipitates in atherosclerotic coronary arteries in a similar fashion as observed in bone mineralization (Fitzpatrick *et al.* 1994, Ikeda *et al.* 1993). A comparative study was done by Rumberger *et al.* (1995) on Thirty-eight coronary arteries from 13 autopsy hearts. Coronary arteries were dissected, straightened, and scanned with EBCT. A strong linear correlation exists between total coronary artery plaque area and the extent of CAC as found in individual hearts ($r= 0.93$, $p< 0.001$) and in individual coronary arteries ($r=0.90$, $p< 0.001$) However, the total calcium area underestimates total plaque area, with approximately five times as many noncalcified as calcified plaques (Rumberger *et al.* 1995).

Significant (> 50 percent) coronary artery stenosis by angiography is almost universally associated with the presence of CAC as assessed by EBCT. However, the severity stenosis is not directly related to the total CACS. A study done by Sangiorgi *et al.* (1998), compared calcium extent to coronary artery luminal diameter stenosis. Although coronary stenosis severity increased with increasing CAC, this relationship was not significant. It might be due to remodeling of coronary arteries that occur with increasing plaque burden so as to maintain luminal diameter and arterial patency (Glagov *et al.* 1987). Although the extent of coronary calcification does not precisely predict stenosis severity, noncalcified plaques are almost universally associated with <50 percent diameter stenosis (Sangiorgi *et al.* 1998). These data indicate that lack of coronary calcification predicts a very low likelihood of obstructive CAD.

However, the Absence of Coronary Calcification Does Not Exclude Obstructive Coronary Artery Disease or the Need for Revascularization in Patients Referred for Conventional Coronary Angiography (Truong *et al.* 2010). Total coronary occlusion frequently occurs in the absence of any detectable calcification. In the trial by Gottlieb *et al.* (2010) done between November 2005 and January 2007 on 291 patients, they found that 19% with CS 0 had ≥ 50% stenosis and 13% underwent revascularization.

However EBCT is used less often because the equipment is expensive and not widely available.

2.3.5.9 Intravascular Ultrasound of Coronary Arteries

Intravascular Ultrasound (IVUS) of Coronary Arteries can provide quantitative evaluation of coronary stenosis which minimizes the chance of significant observer variability in visual interpretation of angiogram (Nissen *et al.* 1997). IVUS also provides information regarding the deeper intramural structures within the vessel wall which is not amenable to coronary angiography. Characterization of atheroma size, plaque distribution, and lesion composition are possible by IVUS. lipid-laden lesions appear hypoechoic, fibromuscular lesions generate low-intensity echoes, and fibrous or calcified tissues are echogenic. (Nissen and Yock 2001; Takahashi *et al.* 2002). It is used as an important complimentary method for examination of the coronary arteries during diagnostic and interventional catheterization. (Topol and Nissen1995).

IVUS equipment consists of a dedicated catheter with a miniaturized ultrasound transducer capable of generating very high frequency sound (20-50 MHz). The catheter is carefully introduced through a preplaced guiding catheter and guide

wire. The vessels are examined in real time and recorded digitally for subsequent quantitative analysis. Normally two strong acoustic interfaces are

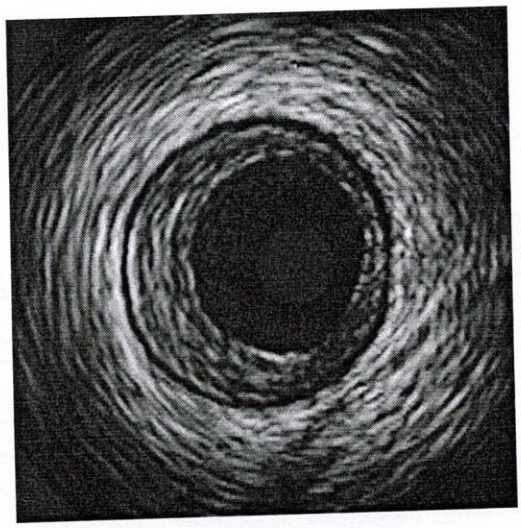


Fig 24 : Intravascular ultrasound image demonstrating the classic three-layered appearance of intima, media, and adventitia.

visualized by ultrasound (Fig 24), i) the interface between the lumen and the endothelium which indicates the leading edge of the media and ii) at the junction of media and external elastic membrane which indicates the outer border of media These two interfaces are echodense. Between these two echodense lines, a sonolucent layer is evident which is the tunica media. So a trilaminar appearance is seen, a middle hypoechoic layer sandwiched by two echogenic layers.

However this finding is not universal finding. Thin intimal layers reflect poorly in 30 to 50% of normal segment which results in a monolayer appearance specially in young individual. A three-layered appearance suggests the presence of at least 178 microns of intimal thickening and is seen more frequently with advancing age. Histological analysis at autopsy revealed a significantly greater degree of intimal

thickening in segments with three layers (243 +/- 105 microns) than in nonlayered segments (112 +/- 55 microns). Discriminate analysis of these data predicted the threshold between the two groups to be 178 microns. Measurements of medial thickness were not different between these two groups (235 +/- 61 versus 210 +/- 76 microns). In the nonlayered group, the average patient age was 27.1 +/- 8.5 years, whereas in the three-layered groups, the average age was 42.8 +/- 9.8 years. (Fitzgerald *et al.* 1992).

The threshold for abnormal intimal thickness by IVUS is controversial. The actual histological measurement does not correlate well to measurements obtained by IVUS. In a comparative study, ultrasound measurements of the intima media thickness averaged 20 percent greater than histologic measurements (Wong *et al.* 1993). In a necropsy study, normal intimal thickness not including media was age-dependent, averaging 0.21 mm in 21- to 25 year-olds, 0.22 mm in 26-to30- year-olds, and 0.25 mm in 36- to 40 year- olds (Velican and Velican1981). In various histological and ultrasound studies, normal intimal thickness ranges between 0.10 and 0.35 mm, and the normal medial thickness ranges from 0.15 to 0.25 mm.
In a study of 262 recently transplanted patients, normal intimal thickness by IVUS was shown to be <0.3 mm in younger hearts whose age is below 40yrs and in person whose age is more than 40 yrs old, it is less than 0.5 mm (Tuzcu *et al.* 2001). Considering the histologic and ultrasound data, most clinical studies have defined threshold for coronary disease by ultrasound as a measured intimal thickness >0.5mm. (Tuzcu *et al.* 1995)
IVUS is also not free from serious risk. Few serious untoward effects were noted during the procedure of IVUS. Focal coronary spasm is common. There is potential risk of intimal injury or vessel dissection like any other intracoronay instrumentation. Data from a multicentre European registry shows a 1.1%

complication rate in 718 patients who underwent IVUS (Batkoff and Linker 1996). Another report from 28 centres documents spasm in 2.9 % and major complications , such as occlusion and dissection. (Hausmann *et al.* 1995).

IVUS is not routinely performed during coronary interventions even in developed countries like United States, Europe and Japan. In USA aproximately 5 to 10 percent of interventional procedures are currently performed with ultrasound guidance. Use in Europe is considerably less reflecting differing practice patterns and reimbursement rates (Tuzcu *et al.* 2008). IVUS is more likely to be incorporated into practice algorithms in countries where its use is deemed to be cost effective. In Bangladesh the scope is still now very limited.

2.4. PERIPHERAL ARETERIAL DISEASE

2.4.1 Definition

Peripheral Arterial Disease (PAD) is diseases of the arteries located outside the heart and brain. Most often, atherosclerosis is thought in terms of its affect on arteries of the heart and of the brain. But atherosclerosis can affect any other blood vessel throughout the body. Blood vessels in the legs are the ones most often affected. Other arteries frequently affected include those that supply blood to the kidneys and those in the arms. (Stoppler 2014).

2.4.2 Epidemiology

PAD is the leading cause of disability among people older than 60 as well as those with diabetes. In 2010 about 202 million people had PAD worldwide. In the developed world it affects about 5.3% of 45 to 50 years olds and 18.6% of 85 to 90 year olds. In the developing world it affects 4.6% of people between the ages of 45 to 50 and 15% of people between the ages of 85 to 90. In the developed world PAD is equally common among men and women while in the developing world women are more commonly affected. In 2013 PAD resulted in about 41,000 deaths up from 16,000 deaths in 1990 (Fowkes *et al.* 2013 ; GBD 2013).

About 8 million people in the United States have PVD. It occurs mostly in people older than 60, affecting about 12% to 20% of people in that age group. It is also common among people with diabetes. Men are slightly more than women to have PAD. The disease is more common in smokers. The combination of diabetes and smoking almost always results in more severe disease. (Bhimji 2014)

2.4.3 Causes and Risk Factors

Almost all peripheral arterial disease is due to atherosclerosis and so CAD and PAD shares common risk factors but tobacco use and diabetes have greater effect. Other risk factors include hypertension, hyperlipidemia and hyperhomocysteinemia (Violi *et al.* 2012; Fowkes *et al.* 2013).

Tobacco Smoking – Tobacco use in any form is the single most important modifiable cause of PAD internationally. Smokers have up to a tenfold increase in relative risk for PAD in a dose-related effect. (Joosten *et al.* 2012). Exposure to second-hand smoke from environmental exposure has also been shown to promote changes in blood vessel endothelium which is a precursor to atherosclerosis. (Price *et al.* 1999) Smokers are 2 to 3 times more likely to have lower extremity peripheral arterial disease than coronary artery disease. More than 80%-90% of patients with lower extremity peripheral arterial disease are current or former smokers (Smith *et al.* 1990). The risk of PAD increases with the number of cigarettes smoked per day and the number of years smoked. (Cole *et al.* 1993)

Diabetes mellitus – Numerous studies have demonstrated an association between diabetis mellitus and the development of PAD (Gordon and Kannel 1972; Widmer *et al.* 1985; Stout 1990). There is increased risk of PVD in Diabetes Mellitus due to endothelial and smooth muscle cell dysfunction in peripheral arteries. The risk of developing lower extremity peripheral arterial disease is proportional to the severity and duration of diabetes (Beks *et al.* 1995). The distribution of lower extremity lesions in DM shows a higher propensity of atherosclerotic disease in the deep femoral artery, as well as in all vessels below the knee. (Beckman *et al.* 2002;

Jude *et al.* 2001). Persons with diabetes have a sevenfold higher rate of lower extremity amputation than persons without diabetes (Jonason and Ringqvist 1985; Hughson 1978).

Dyslipidemia – Almost 50% of patients with lower extremity arterial disease have hyperlipidemia. In the Framingham Study a fasting cholesterol level >270 mg/dL (7 mmol/L) was associated with a doubling of the incidence of intermittent claudication (Kannel *et al.* 1970). An association between lower extremity arterial disease and hypertriglyceridemia has also been reported, but the strength of this association is unclear (Gofin *et al.* 1987, Kannel *et al.* 1970).

Hypertension – Elevated blood pressure is correlated with an increase in the risk of developing PAD. The risk is increased 2.5- to 4-fold in men and women, respectively (Kannel and Mc Gee 1985).

Risk of PAD also increases in individuals who are over the age of 50, male, obese, heart attack, or stroke or with a family history of vascular disease (Hooi *et al.* 2001).

Other risk factors include levels of various inflammatory mediators such as C-reactive protein, fibrinogen' hyperviscosity, hypercoagulable state. (Ridker *et al.* 2001)

2.4.4 Clinical features

The symptoms of peripheral artery disease depend upon the location and extent of the blocked arteries. Up to 50% of people PAD may have no symptoms. Symptoms range from pain, cold feet, and bluish discoloration to stroke or gangrene. If the

condition is not reversed, the affected body part is injured and eventually starts to die. It's important to find narrowed arteries before damage occurs.

Symptoms of PAD in the legs and feet are generally divided into 2 categories:

A) Claudication— The most common symptom of peripheral artery disease is intermittent claudication, manifested by pain (usually in the calf) that occurs while walking and dissipates at rest. Pain in muscles when walking or using the affected muscles that is relieved by resting those muscles. This is due to the unmet oxygen demand in muscles with use in the setting of inadequate blood flow.

B) Critical limb ischemia, consisting of:

- Rest pain, a pain in the soles of the feet, particularly when the feet are elevated, such as when in bed.
- Tissue loss, consisting of arterial insufficiency ulcers, which are sores or wounds that heal slowly or not at all, and Gangrene.

2.4.5 Diagnosis

2.4.5.1 Edinburgh Claudication Questionnaire for PAD involving lower extremity

This is a test used by many medical professionals to diagnose peripheral artery disease. It is a series of 6 questions and a pain diagram. It is accurate at diagnosing PAD in people with symptoms up to about 90% of the time (Leng and Fowkes 1992).

2.4.5.2 Ankle/brachial index (ABI) for PAD involving lower extremity

Upon suspicion of PAD in lower limb, the first-line study is the ankle brachial pressure index (ABPI/ABI). This is one of the most widely used tests for a person who has symptoms suggesting intermittent claudication. This test compares the blood pressure in the arm (brachial) with the blood pressure in the legs. In a person with healthy blood vessels, the pressure should be higher in the legs than in the arms. When the blood pressure readings in the ankles are lower than that in the arms, blockages in the arteries which provide blood from the heart to the ankle are suspected. An ABI above 0.90 is normal; 0.71-0.90 indicates mild PVD; 0.41-0.70 indicates moderate disease; and less than 0.40 indicates severe PAD. (Rooke *et al.* 2013). In people with suspected PAD but normal resting ABIs, exercise testing of ABI can be done.

It is possible for conditions which stiffen the vessel walls (such as calcifications that occur in the setting of long term diabetes) to produce false negatives usually, but not always, indicated by abnormally high ABIs (> 1.40). Such results and suspicions merit further investigation and higher level studies.

2.4.5.3 Treadmill exercise test

If necessary, the ABI will be followed by a treadmill exercise test. A base line ABI is obtained prior to exercise. The patient is then asked to exercise (usually patients are made to walk on a treadmill at a constant speed) until claudication pain occurs (or a maximum of 5 minutes), following which the ankle pressure is again measured. A decrease in ABI of 15%-20% would be diagnostic of PAD. (Rooke *et al.* 2013)

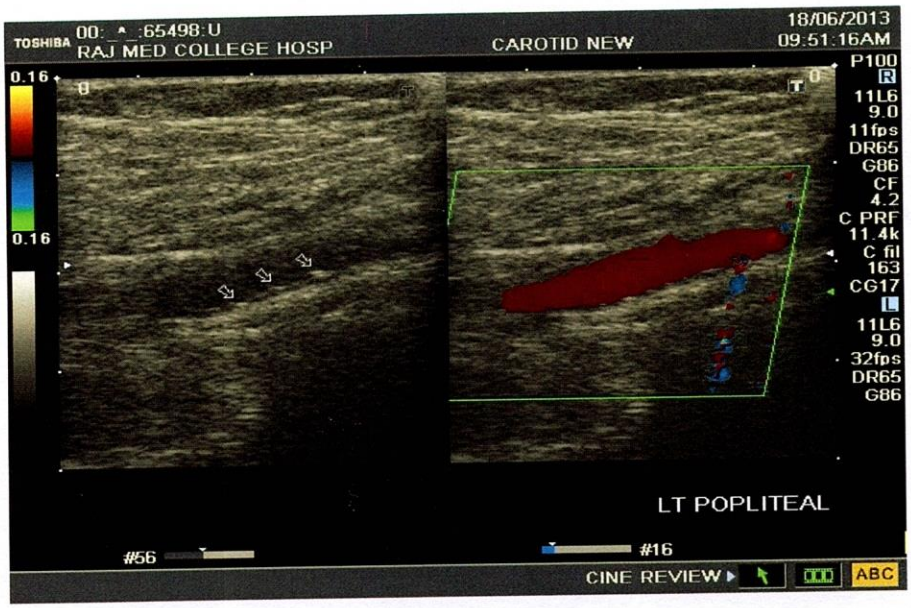


Fig 25: Small plaques deposited in wall of left popliteal artery (arrow marks)

2.4.5.4 Duplex ultrasound

Duplex US provides safe and reliable information regarding arterial anatomy and also the haemodynamic effects of stenosis. B-mode images are obtained initially allowing a clear evaluation of anatomic structures and atheromatous plaques (Fig 25). Use of colour Doppler helps to proper identification of vessels and to see the direction of vessels. Then spectral Doppler is used to gather information regarding hemodynamic status of blood. Velocity information, turbulence in flow can be determined from spectral analysis. Details procedure of carotid and lower limb examination was described in earlier section 2.1.6 and 2.1.7 respectively.

2.4.5.5 Limitations of Duplex US for lower extremity

Distal arteries are frequently difficult to be imaged due to their small size. So sensitivity of Doppler ultrasonography in the leg arteries is relatively lower.

Bergamini *et al.* (1995) examined 404 arterial segments in 44 patients. They found sensitivity and specificity values for common femoral artery were 86% and 96 % respectively. For popliteal artery above knee it was 84% and 90% respectively. But below knee the sensitivity came down to 47% though specificity was high which was 98%. There is limited number of studies conducted on below-knee arteries and the sensitivity and the specificity were found to be 75-83% and 77-95%, respectively. (Hatsukami *et al.* 1992)

Doppler US in the entire lower extremities is operator dependent. Interobserver variability was reported especially where the vessel diameter is smaller below popliteal artery. However in larger vessels the inter-observer variability of the technique is less pronounced. When a stenosis of ≥50% is considered, operator-

dependent differences were found to be quite low, except for the pedal arteries (Winter-Warnars *et al.* 1996, Koelemay *et al.* 2001). In addition, the presence of diabetes mellitus, which is known to cause early calcifications in the vascular wall, does not cause inter-observer variations in Doppler ultrasonography readings.

In several studies, Doppler ultrasonography was reported to have difficulty in differentiating a 99% stenosis from complete occlusion (Legemate *et al.* 1991a, Hatsukami *et al.* 1992, Linke *et al.* 1994).

Obesity and presence of intestinal gas cause difficulty in imaging of the pelvic arteries. Age related or accelerated vessel wall calcifications easily impair the conduct of the Doppler signals (Hingorani *et al.* 2007). In addition, successful application of the method may not be possible in the areas with ulcers or marked scars.

Although Doppler US is a non-invasive technique, approximately one fifth of the patients report mild pain or discomfort during or immediately after the procedure which might be further difficult in individuals with poor cooperation or mobility. However, in contrast with CA and MRA, the occurrence of a life-threatening adverse event is unlikely (Collins *et al.* 2007).

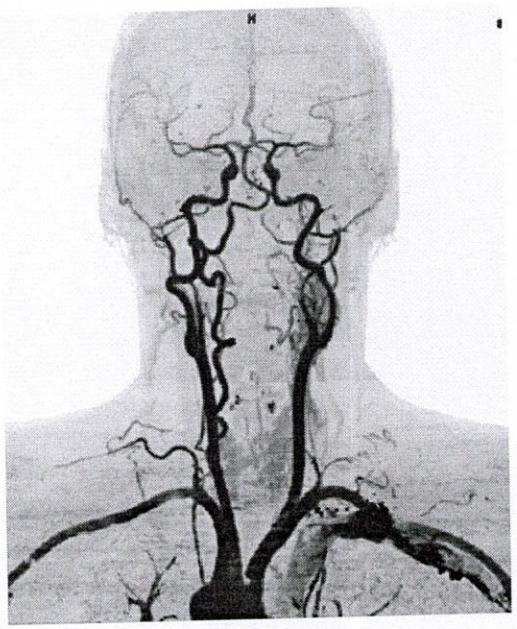


Fig 26.A

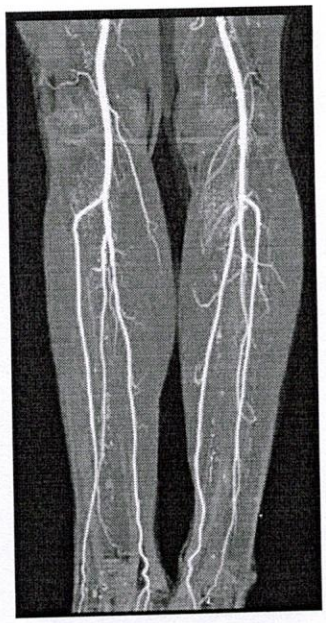


Fig 26.B

Fig 26 : A) Angiography of carotid vessels, B) MRA of lower limb vessels

2.4.5.6 Imaging techniques of Arteries

Conventional angiography or arteriography is the standard imaging technique to judge the anatomy of peripheral arteries (Hirsch 2006). A catheter is inserted into the common femoral artery and selectively guided to the artery in question. While injecting a radiodense contrast agent an X-ray is taken (Fig 26A)

Drawbacks of catheter angiography include risk of distal embolization and arterial damage at the puncture site. Anaphylactoid reaction and contrast nephropathy may occur due the iodinated contrast used.

CT angiography and MRA (Fig 26B) are alternative imaging techniques. Iodinated contrast is still required in CTA but MRA provides information similar to CTA without the need of iodinated contrast. MRA is a safe and accurate alternative to CTA and conventional angiography (Sommerville *et al.* 2005).

2.4.5.7 Comparison between Duplex US and Catheter Angiography

Technology of Doppler ultrasonography equipment is rapidly advancing which increasing the sensitivity and specificity of the method. For the success of Doppler ultrasonography in the demonstration of stenosis, sensitivity and specificity reached to 100% in aortoiliac arteries. In femoro-popliteal arteries the sensitivity and specificity are 90% and 100% respectively (Legemate *et al.* 1991b, Hatsukami *et al.* 1992). Linke *et al.* (1994) performed Doppler US evaluation in 25 patients with intermittent claudication. 134 arterial segments were evaluated. CA was also performed in same patients. Sensitivity and specificity of Doppler ultrasonography were found 89% and 95% respectively and the combination of both methods was well-correlated.

A relatively large study was done in by Sensier *et al.* (1996) on 79 patients. They evaluated 1658 segments from the aortoiliac segment to tibial arteries. A significant overall consistency was found between Doppler ultrasonography and arteriography. For femoropopliteal segment, both sensitivity and specificity were 88%. A similar retrospective trial was performed two years later in 1998 by the

same investigator group and it was concluded that Doppler US could also be recommended to evaluate branches below popliteal artery (Sensier *et al.* 1998).

Aly *et al.* (1998) performed both CA and Doppler US of 90 patients. 3108 arterial were examined. In this work, sensitivity and specificity of femoral artery stenosis were found to be 100% and 99%, respectively.

So, presented data by many authors suggest that CA could successfully be replaced by Doppler US.

2.4.5.8 Comparisons between Duplex US and Magnetic Resonance Angiography(MRA)

Contrast enhanced MRA has the highest diagnostic value for the diagnosis of stenosis, with a sensitivity range of 92%-99.5%, average 95% and a specificity range of 64%-99%, average 97% (Collins *et al.* 2007). In one of the earliest works, Doppler ultrasonography was reported to be even more sensitive than MRA for the detection of infrainguinal stenosis (Mulligan *et al.* 1991). For the stenosis in the iliac arteries, other researchers found similar sensitivity and specificity values for MRA, CA and Doppler US (Wikstrom *et al.* 2000). During the same period, Visser and Hunink (2000) performed a meta-analysis by reviewing the already published studies collectively in an attempt to delineate the diagnostic value of Doppler ultrasonography and/or MRA in PAD. They calculated the sensitivity value as 97.5% for MRA and 87.6% for Doppler US and the difference was statistically significant. Specificity values, however, were found to be similar (96.2% for magnetic resonance and 94.7% for Doppler ultrasonography). The authors recommended that MRA could replace CA as the preferred method with high diagnostic accuracy.

Subsequent study that evaluated 668 segments in a total of 249 patients, sensitivity and specificity values were found to be statistically different for Doppler US (76% and 93%) and MRA (84% and 97%) when compared to each other (Leiner *et al.* 2005). A prospective study was performed by Bueno *et al.* (2010) who examined 1720 segments on 40 patients) where the utility of Doppler US and MRA was evaluated by using CA as reference point. When the detection of stenosis ≥50% was taken as the sole criterion, sensitivity and specificity values were calculated to be 81.4% and 99% for Doppler US, and 91 and 99% for MRA. In the same study, the detection of total occlusion sensitivity and specificity values were calculated as 90% and 97% for Doppler US, and 95.4% and 98% for MRA. The latter study demonstrated a relatively low sensitivity value for Doppler US in the detection of significant stenosis in the lower limb arteries whereas, the specificity value was quite acceptable.

To formulate preoperative plan for lower extremity revascularization, Doppler US is used. MRA appeared less accurate than Doppler US to determine the preoperative period in subjects with lower extremity PAD. (Hingorani *et al.* 2004). High-quality Doppler US has been proposed as a reasonable alternative to CA in subjects with lower limb ischemia. In addition to diagnostic purposes, Doppler ultrasonography is recommended to be used simultaneously during balloon angioplasty and stent placement for infrainguinal arterial occlusive disease due to its ease of use, safety and reliability.(Ascher *et al.* 2003).

2.4.5.9 Guidelines for Doppler US in lower extremity

The American College of Cardiology (ACC) and the American Heart Association (AHA) jointly published PAD management guideline in 2006 with the title ''ACC/AHA 2005 Practice Guidelines for the management of patients with PAD''

(Hirsch *et al.* 2006). The guideline committee recommended that Doppler US should be the first modality of choice for screening subjects with intermittent claudication. It should be used for the determination of the anatomic localization, grading and post-operative follow-up of the stenosis of femoropopliteal and femorotibial-pedal vein grafts. Doppler ultrasonography was also recommended for the selection of subjects that could benefit from endovascular intervention. ACC/AHA guideline committee also addressed Doppler US as a quality tool to select those individuals that could benefit from the revascularization surgery by identifying the level of arterial segments that require surgical anastomosis. It was noted that the utility of Doppler ultrasonography in identification of long-term success of the percutaneous transluminal angioplasty was not clear. However, it could be an option for the evaluation of patency of the synthetic femoro-popliteal bypass grafts in the routine follow-up.

Trans-Atlantic Inter-Society Consensus Document on Management of Peripheral Arterial Disease (TASC) published ''Inter-Society Consensus for the Management of PAD'' (TASC II) guideline in 2007 who gave same emphasis on Doppler US, MRA and CTA for detection and localization of in the lower limb arterial stenosis.(Norgren *et al.* 2007). However, TASC II guideline noted that, while some patients might be operated based only on the ultrasonography results, angiography-based imaging methods were used in the majority of the cases in the clinical practice.

ACC/AHA 2005 guideline was updated in 2011 (Creager *et al.* 2012) with an attempt to establish a harmony with the TASC II guideline. Following this update, Doppler ultrasonography still maintained its diagnostic value by itself or with other tools for the diagnosis of the PAD of the lower extremities. For European

countries, the first guideline of PAD was published by the European Society of Cardiology (ESC) in 2012 (Tendera *et al.* 2011). Like other guidelines, ESC guidelines recommended Doppler US first diagnostic tests to confirm and localize stenotic lesions. To localize stenotic lesions and consider revascularization options, this latest guideline also indicated the need for Doppler US and did not mentioned that CTA or MRA was superior. Finally, the ECS guideline recommended that any patient suggested for surgery based on any of the imaging tools should also be tested hemodynamically, which can be achieved only by Doppler arteriography.

The most recent guideline recommendations on the management of PAD were published by the ACC foundation in 2013 (Anderson *et al.* 2013). In this update, Doppler US measurements were demonstrated among the top diagnostic tests to provide an accurate assessment of lower extremity PAD location and severity, and to provide accurate follow-up after revascularization. Doppler arteriography was also addressed as a useful tool to select patients as candidates for endovascular intervention and surgical bypass.

CHAPTER 3

METHODOLOGY

CHAPTER 3

METHODOLOGY

3.1: Study type: This study was a cross sectional descriptive study

3.2: Study time: Duration of the study was 03 years (From September 2009 to September 2012). It included article reviews, development of thesis protocol, patient examination and data collection, data analyses and paper writing.

3.3: Study place: The study was done in Department of Cardiology, Rajshahi Medical College Hospital, Rajshahi, Bangladesh.

3.4: Sample size: Total 210 patients were included in the study.

3.5: Sample size calculation: Sample size was figured out by the following formula

$$n = \frac{z^2pq}{d^2}$$

Where n = Sample size, p = The prevalence of occurrence, q = 1- p, z = Area under normal curve corresponding to the desired confidence level (CI) and it is the amount of uncertainty that one can tolerate. d = Margin of error is the amount of error that one would tolerate

Now, for the present study, prevalence of IHD = 11.6 % (Mandal *et al.* 2009)

So p = 0.116 ≅ 0.12

q= 1- 0.12 = 0.88

z= 1.96

d = standard error 5% = .05

So n = $\frac{1.96 \text{ X } 1.96 \text{ X } 0.12 \text{ X } 0.88}{0.05 \text{ X } 0.05}$ = 162.26 ≅ 163

To make the sample size authentic extra 20% of the sample was added.

163 + 33 = 196 ≅ 210

3.6: Sampling technique:

Purposive sampling technique was followed. After proper diagnosis of the patient of coronary artery blockage by coronary angiogram they were given a serial number who justified the criteria to be selected as sample every day. Then one patient was selected for the study by simple random sampling. If the patient was not available on particular that day he or she was dropped from the study.

3.7: Inclusion criteria:

1. Patients having coronary artery disease confirmed by coronary artery angiogram.
2. Age between 30 to 80 years.
3. Both male and female were enrolled

3.8: Exclusion criteria:

1. Patients who could not give the history properly or no responsible attendant/relative to give the patient's history.
2. Age below 30 and over 80 years.
3. Clinically unstable patients requiring respiratory or intensive care or could not be moved for relevant investigations.
4. Patient who did not give consent to participate in the study.

3.9: Procedure of data collection:

This study was carried out on 210 admitted patients presented with symptoms of CAD and subsequently proved of having coronary artery blockade by coronary angiogram. The patients were interviewed by the researcher himself by using a questionnaire (Appendix -i) and by face to face interview. The study objectives were explained and written consent was obtained for physical examination and investigations. (Appendix –ii & iii) Basic demographic information including age, residence (Rural or Urban), educational attainment, occupation, family income were noted. Detailed history was taken about dietary habit (Vegetarian or non vegetarian, amount of red meat consumption), smoking and alcohol consumption, physical activity, family history of cardiac disease, past history of hypertension, heart disease, diabetes mellitus and hyperlipidemia. Symptoms regarding CAD and PAD like chest pain, breathlessness, sweating, vertigo, intermittent claudication were noted. Thorough physical examination, including examination of cardiovascular system was done. Emphasis was given on risk

factors for atherosclerosis and sign of PAD like blood pressure, obesity, carotid bruit, peripheral arterial pulsation and leg ulcer.

3.10: Investigations

Fasting blood glucose, fasting serum lipid profile, ECG, echocardiography and coronary angiogram were done in all cases. Echocardiography and coronary angiogram were done by expert cardiologists. **Duplex ultrasound of carotid vessels and vessels of both lower limbs were examined by the investigator himself.**

3.11: Blood Sample collection:

Fasting (at least for 12 hours) blood sample was taken from each patient for lipid profile, blood sugar and other relevant investigations. Blood samples were obtained from an anticubital vein. Investigator himself collected the samples.

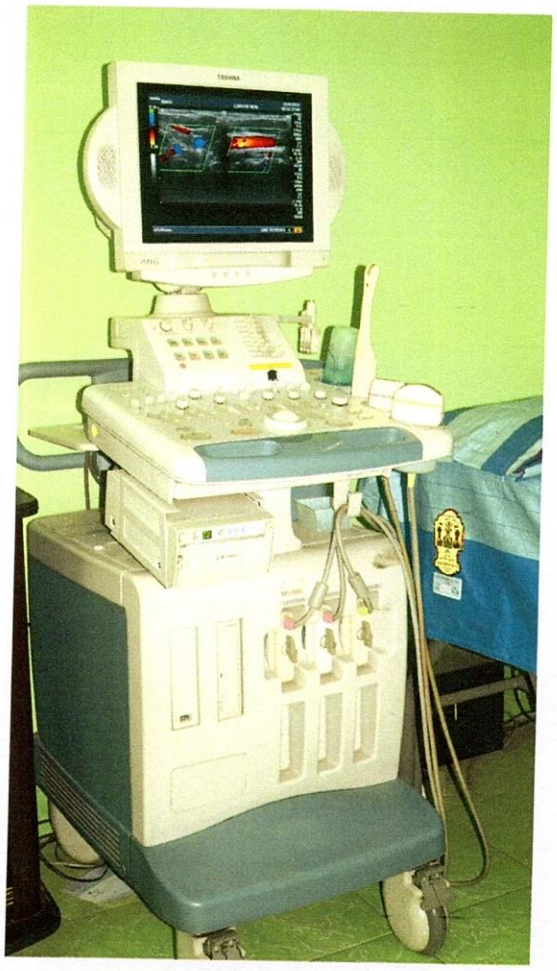


Fig 27 : Toshiba NemioXG Ultrasonogram Scanner

3.12: Duplex US Study

Duplex Ultrasound of blood vessels was carried out using a Toshiba Neimo ultrasound scanner (Fig 27) with a 7.5 MHz transducer with the use of a standard examination technique. Sample volume was set at about half of the total diameter of target vessel and placed in the centre of the vessel. The Doppler angle was kept in between 55 to 60 degree.

3.12.1 Duplex US of Carotid arteries

Cervical carotid arteries on both sides were scanned for carotid duplex study. Patients were examined in the supine position with slight head tilt. Neck was little extended by placing a pillow under their shoulders. Excessive extension of the neck was avoided. The carotid arteries were scanned from as low as possible to as high as possible behind the angle of mandible.

Initially common carotid arteries were assed in B mode image and presence of any plaque was noted. It there was plaque, the reduction in lumen area was calculated at the narrowest point of the artery with carotid plaque and expressed as percent reduction compared with the area of the original arterial cross section at the site of the disease by the fallowing formula:

Degree of stenosis = $(1- A_s / A_n) \times 100$,

where A_s is cross sectional area of the intrastenotic colour filled residual lumen and A_n is the area of the original vascular cross section including the plaque.

Intimia-medial thickness was measured on an image of distal common carotid wall in longitudinal section and the vessel scanned perpendicular to the US beam. The measurement was taken from far wall and from both the right and left arteries. Focal thickening was considered as plaques and excluded in IMT measurement.

Vessels were then examined with colour and spectral Doppler. The external and internal carotid arteries were identified by their course, branching

pattern and characteristic waveform on spectral Doppler with relatively high systolic and low diastolic velocities in external carotid artery. The spectral

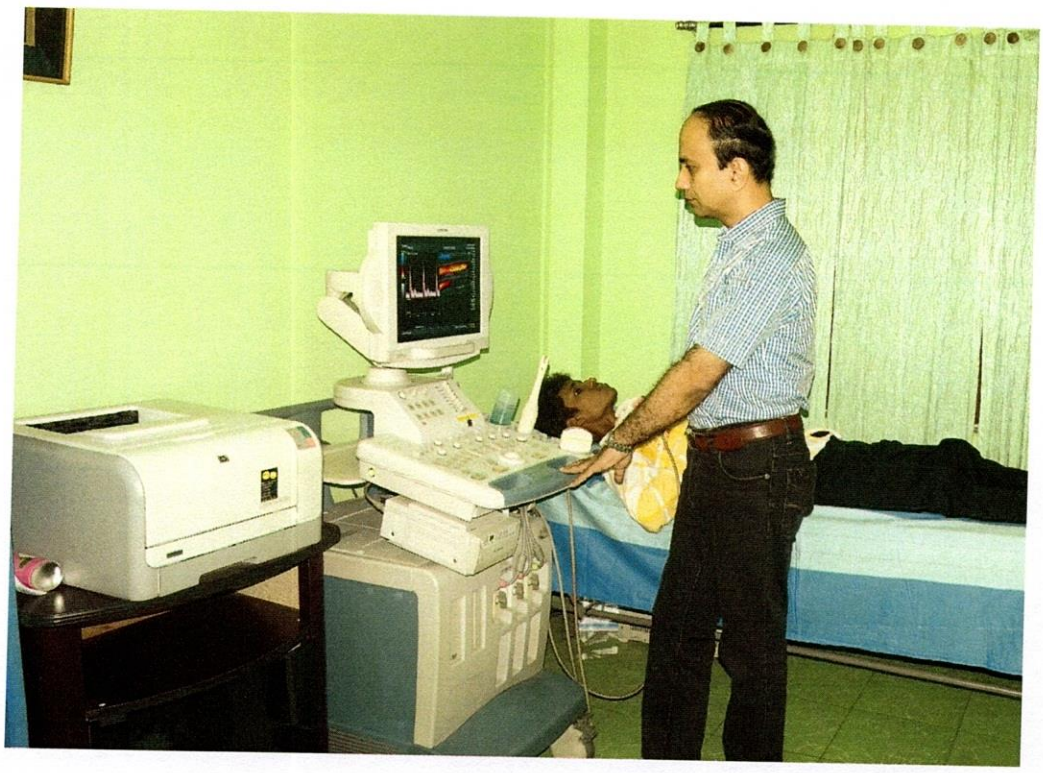


Fig 28: Procedure of Duplex ultrasound of Carotid arteries by the investigator.

waveform was recorded from common carotid artery 2-3 cm below the bifurcation, the internal carotid artery from 1-2 cm above the bulb or as high as possible and if there was plaque, at proximal to and distal to any plaque. Peak-systolic velocity and end-diastolic velocity were recorded. All Doppler waveform were obtained in longitudinal plane. Almost all the recordings were made at a reasonably standard angle of 55 to 60 degree. Diagnostic

criteria for internal carotid artery stenosis were considered as following chart based on Robinson *et al.* (1988).

Diameter Stenosis	PSV of ICA	EDV of ICA	ICA/CCA systolic ratio
50%	> 1.5 m/sec	> 0.5 m/sec	>2
70%	> 2.3 m/sec	> 0.75 m/sec	>3

3.12.2 Duplex US of Lower limb arteries:

The major arteries of both lower limbs were examined by the same machine mentioned earlier. The common femoral, the superficial femoral, the popliteal, anterior tibial and posterior tibial arteries were examined. Same tranducer with same frequency set up was used which was used for carotid vessels. Both colour Doppler and spectral Doppler were used. The examination was done with the patient lying supine on bed. The distal external iliac artery and common femoral arteries were located using colour Doppler as it leaves the pelvis under the inguinal ligament lateral to the femoral vein. The bifurcation of common femoral artery into the superficial femoral profunda femories arteries was then examined. The superficial femoral artery was examined as far as down as it can be followed on the medial aspect of the thigh. The popliteal artery was then located in popliteal fossa and followed superiorly. The popliteal artery was then examined and followed down to the point of division into the tibioperoneal trunk and anterior tibial artery. The anterior tibial artery was examined from anterior approach through the extensor muscle lying between the tibia and fibula.

The posterior tibial artery was examined on the medial aspect of the mid calf area behind the tibia, and as it passes behind the medial malleolus.
Peak systolic velocity , RI value and PI value were noted for each artery. Pattern of waveform was observed. Arterial disease in the lower extremities were classified into 4 categories on the basis of ultrasound findings, including 1) 0 to 49% stenosis, 2) 50-75% stenosis, 3) >75% stenosis, and 4) 100% stenosis or total occlusion (Cossman *et al.* 1989).

Velocity Criteria for diagnosis of lower limb arteries were done based on Cossman *et al.* (1989) as follow :

Stenosis(%)	**PSV (cm/sec)**	**Velocity Ratio**
0-49	150-200	1.5-2:1
50-75	200-400	2-4:1
> 75	>400	>4:1
Occlusion	No flow	No flow

Velocity ratio means ratio of PSV at the stenosis compared with the PSV 1-2 cm upstream.

3.13 Data recording and analysis:

All relevant information from history, clinical findings and investigations were recorded in a predesigned questionnaire which was approved by the supervisor. After proper verification, data were coded and entered into the computer and were analyzed according to the objectives of the study by using SPSS16. Descriptive variables were explained with mean and standard deviation. Statistical significance was found by applying relevant statistical

tests at appropriate probability level. Probability value of $P<0.05$ was considered to indicate significance.

3.14 Ethical consideration:

The research protocol was approved by the Ethical committee of Rajshahi Medical College, Bangladesh. The aim and objectives of the study along with its procedure and benefits of the study were explained to the respondents in easily understandable native language and then written consent was taken from each participant. It was assured that all information and records would be kept confidential and the procedure would be used only for research purpose and the findings might be helpful for developing awareness regarding prevention of coronary artery disease.

3.15 Operational Definition:

Hypertension: Hypertension was considered to be present if the patients were taking antihypertensive drugs or if the systolic blood pressure ≥140 mm of Hg and diastolic blood pressure ≥90 mm of Hg on three separate reading, according to JNC-8 guideline (Paul and James 2014).

Diabetes : A patient was confirmed as having Diabetes Mellitus if patient was oral hypoglycemic agents or insulin or if the fasting blood glucose was greater than 7.0 mmol/L (WHO diagnostic criteria).

Dyslipidaemia was assessed by raised fasting serum cholesterol >200 mg/dl, LDL>130 mg/d, TG >150 mg/dl and HDL <40 mg/dl (National Cholesterol Education Program 2002, Iqbal *et al.* 2003).

Smoking : A smoker was considered who smoked at least 1 cigarette or biri per day and continued for at least 6 months within the last 10 years from the time of study. Smoking status was noted as pack year. A non smoker was considered who never smoked or smoked occasionally, not more than 1 cigarette or biri per day, less than six month or quite smoking for last 10 years. (Agarwal *et al.* 2012). Smoking was assessed as a part of questionnaire. Smoking habit was recorded as packed year which is essentially the magnitude of primary smoke exposure. Pack year was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. One pack contains 20 cigarettes. So, to calculate smoking pack-years, the number of cigarettes smoked per day is divided by 20 and then multiplied by the number of years smoked.

Waist Hip ratio: It was measured according to the World Health Organization's data gathering protocol (WHO 2008). The waist circumference was measured at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Hip circumference was measured around the widest portion of the buttocks, with the tape parallel to the floor (Fig 29). Measurements were taken at the end of a normal expiration. Value of waist circumference was divided by hip circumference. Each measurement was repeated twice; if the measurements were within 1 cm of one another, the average was calculated. If the difference between the two measurements exceeded 1 cm, the two measurements were repeated.

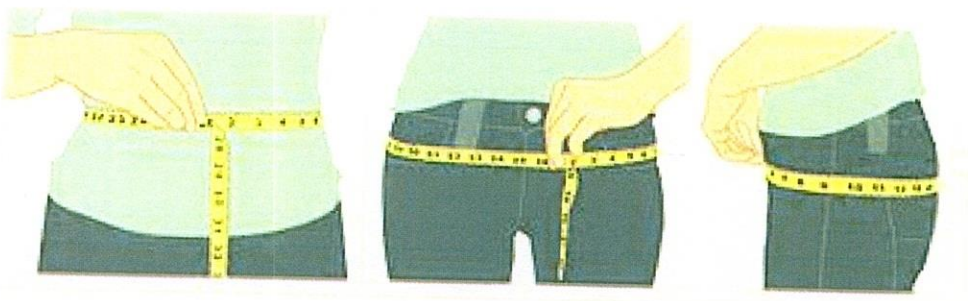


Fig 29 : Measurement of waist and hip circumference.

Abdominal obesity is defined as a waist–hip ratio above 0.90 for males and above 0.85 for females (WHO 2008)

Economic status categorization: Economical status was categorized according to per month income of each patient. Considering the international poverty line US$ 37.5 (i.e. 3000tk approx.) as reported by UNICEF in 2013, patient was considered as very poor who earned less than 3000 Bangladeshi taka per month. Poor: who earned near about 3,000 taka to 10,000 taka,

middle class: 10,000-20,000 taka and was considered as rich who earned more than 20,000 taka per month.

Physical Activities categorization: Physical activities or working life style was categorized into sedentary worker, light worker and heavy worker. Those were categorized as sedentary worker who had lifestyle with almost no or irregular physical activity. Lifting and carrying was limited to 20kg or less, standing and walking was limited to less than two hours out of an eight hour working day, the majority of the time was spent sitting. A job could allow him to alternate between sitting and standing, and still be considered a sedentary job. (Stasiuk 2009).

In light work, sitting was limited to 2 hours out of an eight-hour day, and standing or walking, off and on, for a total of approximately 6 hours in an 8 hour workday in order to meet the requirements of frequent lifting or carrying of light objects.

Hard work was considered who works almost constant standing or walking, or kneeling, squatting, bending, climbing along with lifting heavy weight.

Consumption of Red meat: Consumption of red meat included beef, pork, lamb or mutton as main dish, and 85 gm per dish (Pan *et al.* 2012).

Residence: Resident was considered where patients were dwelling for last 5 years or where he or she spent maximum time of his or her last 10 years. Area under jurisdiction of any city corporation or municipality corporation was considered as urban area.

Body mass index (BMI): BMI was figured out by the formula weight in Kg/ square of Height in meter ($BMI = \frac{Weight}{Height^2}$).

Normal weight was defined as BMI of 18.5 to 24.9. Below 18.5 was considered underweight. Overweight was considered who had BMI within 25. to 29.9 and obesity was defined as a BMI > 30. (WHO)

Low Ejection Fraction: Lower limit of normal left ventricular ejection fraction was considered as 50%. (Pfisterer *et al.* 1985)

CHAPTER 4

RESULT

CHAPTER 4

RESULT

The study population comprised of total 210 patients who had definite evidence of CAD, proved by coronary catheter angiography.

For purpose of analysis entire study was subdivided into three major groups on the basis of number of coronary artery involvement

One vessel disease = 114 (54.3%) patients.

Double vessel disease =40 (19.0%) patients.

Triple vessel disease = 56 (26.7%) patients.

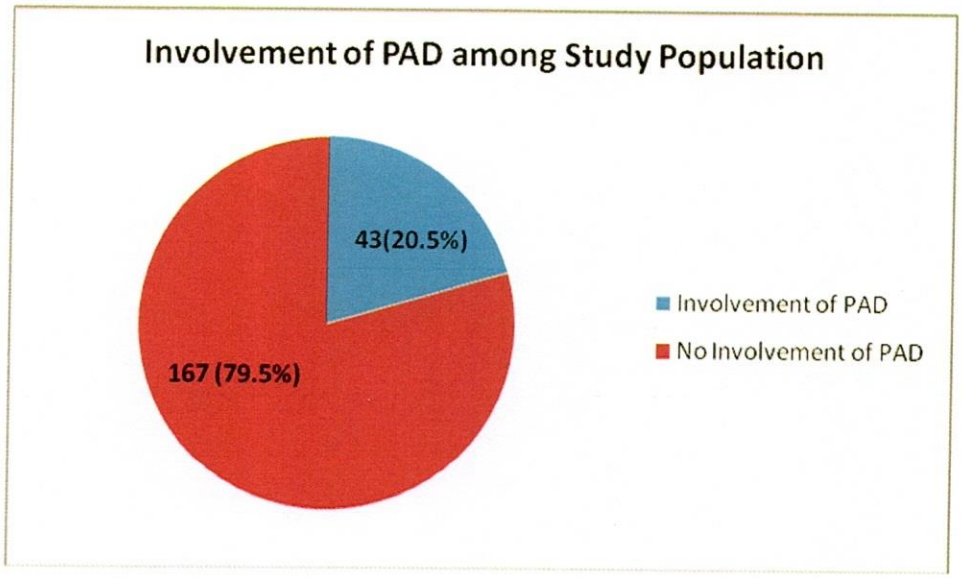


Fig-30 : Percentage of PAD involvement in total study population

Among 210 study population we found 43 (20.5%) patients had peripheral arterial disease (Fig-30). Demographic profile of total respondent and PAD patient are given in Table 1 and 2 respectively. Risk factors among respondent and PAD patients are shown in Table 3 and Table 4 respectively.

Table 1 : Demographic profile of Respondents. (n=210)

	Number	Percentage
Mean Age	51.3±10.4 yrs	
Sex		
Male	196	93.3%
Female	14	6.7%
Residence		
Urban	84	40.0%
Rural	126	60.0%
Marital Status		
Married	208	99.0%
Unmarried	2	1.0%
Profession		
Service	50	23.8%
Retired	32	15.2%
Unemployed	2	1.0%
House Wife	14	6.7%
Laborer	6	2.9%
Farmer	56	26.7%
Business	40	19.0%
Professional	6	2.9%
Others	4	1.9%
Food Habit		
Vegetarian	1	0.5%
Regular Red meat	68	32.4%
Occasional Red meat	141	67.1%
BMI		
<18.5 (Underweight)	2	1.0%
18.5 to 25 (Normal)	108	51.4%
25 to 30 (Overweight)	92	43.8%
>30 (Obese)	8	3.8%
Hip/Waist Ratio		
High	208	99.0%
Normal	2	1%
Physical Activity:		
Sedentary	152	72.4%
Moderate	24	11.4%
Hard Worker	34	16.2%
Economic Status:		
Below Poverty	5	2.4%
Poor	98	46.7%
Middle Class	101	48.0%
Rich	6	2.9%
Literacy Level:		
Complete illiterate	6	2.9%
Below Primary	68	32.4%
Primary to High School	62	29.5%
Above High School	74	35.2%

Table 2: Demographic profile of PAD patient (n=43)

	Number	Percentage
Mean Age	54.67±10.7 yrs	
Sex		
Male	39	90.7%
Female	4	9.3%
Residence		
Urban	18	41.9%
Rural	25	58.1%
Marital Status		
Married	43	100%
Unmarried	0	0
Profession		
Service	8	18.6%
Retired	8	18.6%
Unemployed	0	0
House Wife	4	9.3%
Laborer	2	4.7%
Farmer	10	23.3%
Business	7	16.3%
Professional	2	4.7%
Others	2	4.7%
Food Habit		
Vegetarian	0	0
Regular Red meat	20	46.5%
Occasional Red meat	23	53.5%
BMI		
<18.5 (Underweight)	0	0
18.5 to 25 (Normal)	18	41.9%
25 to 30 (Overweight)	21	48.8%
>30 (Obese)	4	9.3%
High Hip/Waist Ratio		
High	43	100%
Normal	0	0
Physical Activity:		
Sedentary	32	74.4%
Moderate	7	16.3%
Hard Worker	4	9.3%
Economic Status:		
Below Poverty	1	2.3%
Poor	21	48.8%
Middle Class	20	46.5%
Rich	1	2.3%
Literacy Level:		
Complete Illiterate	0	0
Below Primary	10	23.3%
Primary to High School	9	20.9%
Above High School	24	55.8%

Table 3: Major Risk factors among CAD patients (n=210).

Risk factor	YES	No
Smoking	154 (73.3%)	56 (26.7%)
Mean pack year	14.43±16.4	
Family History of CAD	65 (31.0%)	145 (69.0%)
Hypertensive	101(48.1 %)	109 (51.9 %)
Diabetic	83 (39.5%)	127 (60.5%)
Dyslipidemia	114(54.3)	96(45.7%)

Table 4 : Major Risk factors among PAD patients (n=43).

Risk factor	YES	No
Smoking	35(81.4%)	8 (18.6%)
Mean pack year	15.8±19.9	
Family History of CAD	16 (37.2%)	27 (62.8%)
Hypertensive	29 (67.4%)	14 (32.6%)%)
Diabetic	27(62.8%)	16 (37.2%)
Dyslipidemia	34(79.0%)	9(21.0%)

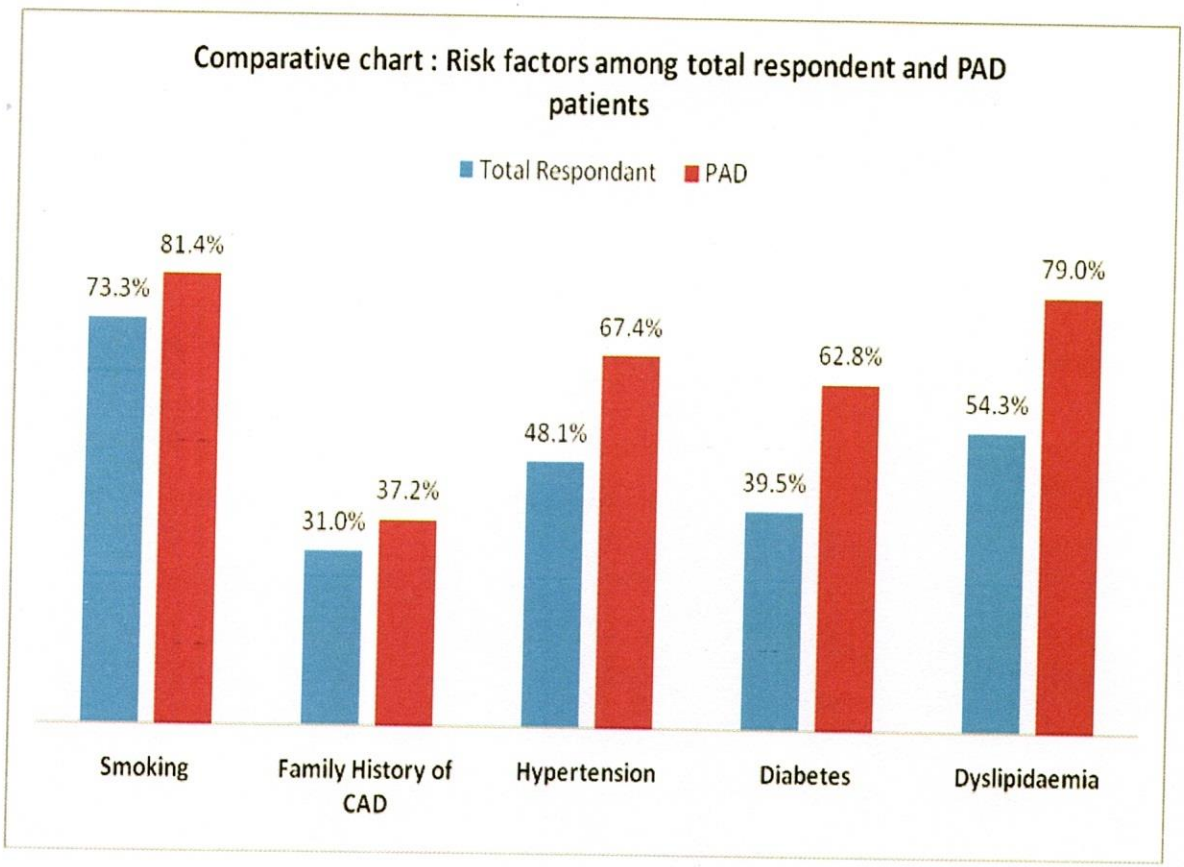


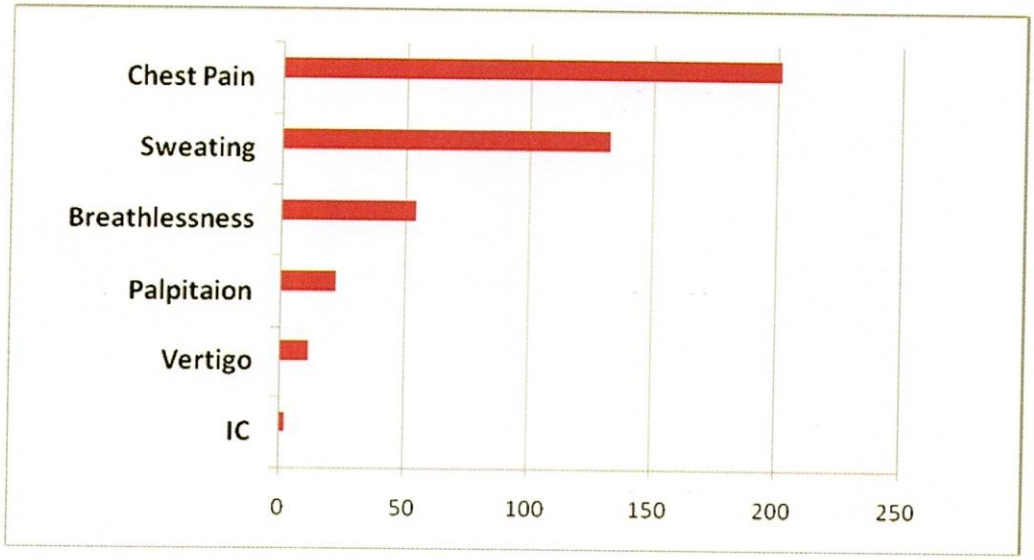
Fig 31: A comparative graph showing major risk factors among total study population and among patients with PAD. Percentages of incidence of all the risk factors are showing higher values in case of PAD patients.

Clinical pictures

In most of the patients (201, 95.7%) the presenting complaint was precordial chest pain. Only in two patients (1%), the presenting complaint was sudden collapse, one presented with sudden profuse sweating with restlessness, and in three cases breathlessness was the only presenting symptoms. Those who presented with chest pain, 131 also had associated profuse sweating, 54 had associated breathlessness and 22 had associated palpitation and 11 complained vertigo.

Only two (1%) patients complained intermittent claudication. None of the patient had any complaint of leg pain at rest.

On examination in 4 (1.9%) patients carotid bruit was found. Peripheral arterial pulsation could be felt in all patients from Femoral to Arteria Dorsalis Pedis. No leg ulcer was found in any patient.



** IC = Intermittent claudication*

***Multiple involvements were present.*

Fig 32: Graph showing clinical presentation of respondents.

Table 5: Segmental Distribution of Coronary Artery Involvement (>50% lumen blockage)

Coronary Arteries	Number of involvement (%)*
Left Main Artery Involvement	16 (7.6%)
Left Anterior Descending	143 (68%)
Left Circumflex Artery	87 (41.4%)
Right Coronary Artery	128 (60.9%)

**Multiple involvements were present.*

Table 5 shows that among 210 CAD patients, 143 (68%) had stenosis in LAD, with 16(7.6%) in LMCA. LCX was involved in 87(41.4%) and RCA was involved in 128(60.9%) patients. Some of the patients had multiple involvements, in multiple arteries and also in multiple segments of same arteries. Only 18 (8.5%) patients had distal segment involvement but all of them had proximal involvement also. So no patient was found who had got isolated distal involvement.

Gender distribution:

Among the cases, gender distribution shows very high male propensity and only fourteen cases (6.7%) were female. Male outnumbered the female by 196 (93.3%). Male female ratio was 14:1.

Total number of male patients was also higher in PAD. Thirty nine were male and only four were female. Male female ratio of PAD was 9.7:1.

Table 6: Gender Distribution of PAD involvement

Sex	PAD Involvement		
	Yes	No	Total
Male	39 (19.9%)	157 (80%)	196
Female	4 (28.6%)	10 (71.4%)	14
Total	43	167	210

$X^2 = 0.604$ $df = 1$ $p = 0.315$

But percentage calculation among male and female group who suffered PAD showed different picture. Table 6 shows that among CAD patient comparatively more patients from female group developed PAD. Out of 14 female patients 4 i.e. 28.5% had peripheral artery involvement and 19.9% from male group (39 out of 196 male) had peripheral artery involvement. But the association was not statistically significant.

Age distribution:

Age distribution of the study population has been shown graphically in Fig-33. Mean age was 51.3±10.4 yrs. Study population was divided into five different age groups; viz. group I: 30-39 years, group II: 40-49 years, group III: 50-59 years, group IV : 60-69 years, and group V: 70-80 years. In this study, it was found that the highest (76, 37.2%) belongs to group III or 5^{th} decade. The next to highest is group II or 4th decade, (58,27.6%). The lowest number of patients was from group V or the 7^{th} decade. (12, 5.7%). It was found that 24 (11.4%) from group I (3^{rd} decade) and 40 (19%) from group IV (6^{th} decade.)

Mean age of PAD involvement was 54.67±10.7yrs. The highest group who had PAD involvement was group III or 5^{th} decade, same as CAD sufferer. Fifteen (34.8%) patients were from this group. Next to the highest is the group IV or 6^{th} decade (13,30%). The two lowest number of patients having PAD involvement represented group I and group V i.e. 3^{rd} and 7^{th} decade, three and four patients from each group respectively which is also as same pattern as seen in total CAD sufferer patients. (Fig-34)

But if the percentage of PAD involvement among each age groups is noticed, it is seen that the highest (33.3%) involvement was from 7^{th} decade and the lowest (12.5%) involvement was from 3^{rd} decade. There was a graded increase in percentage of PAD patinets among the individual age group from group I to group VI (Table 7 and Fig 35) though the trend was not found to be statistically significant.

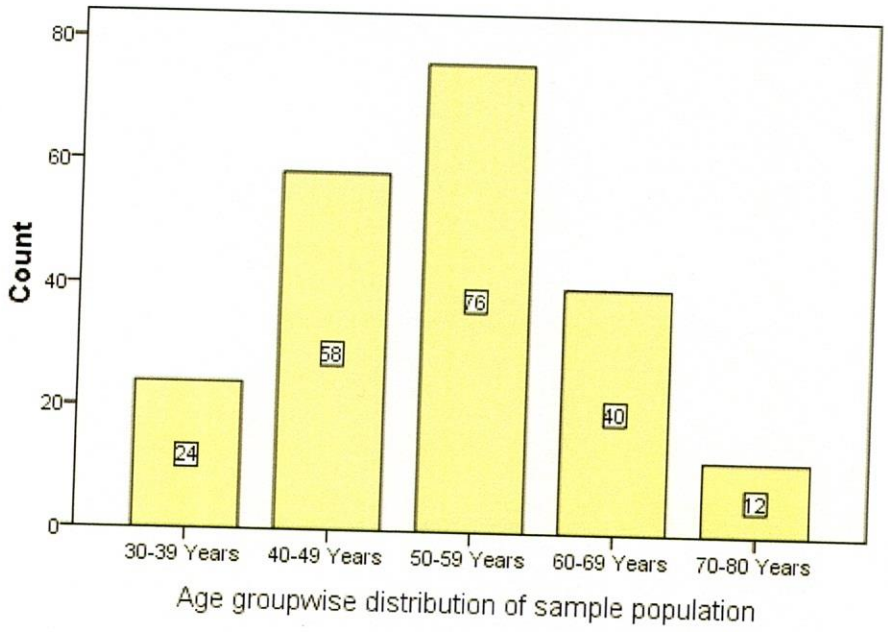


Fig 33 : Age Incidence of CAD

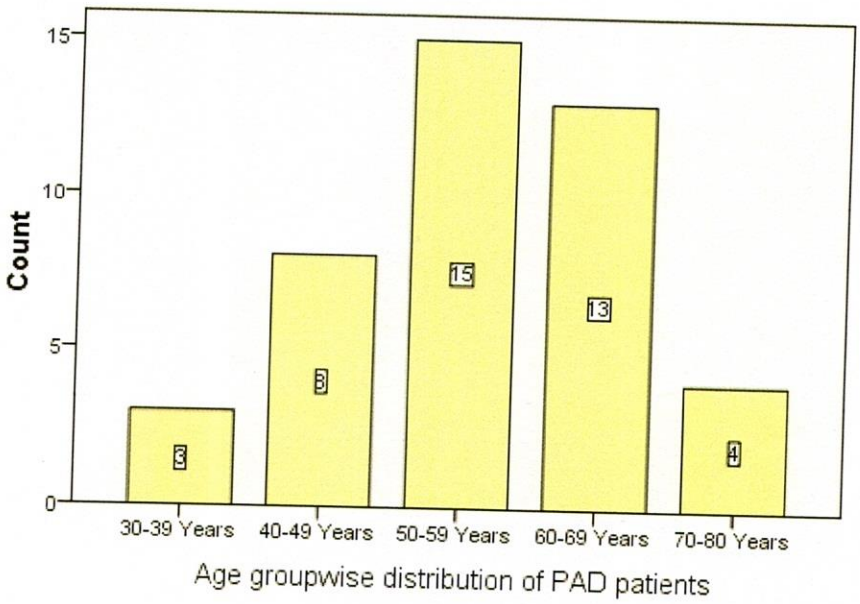


Fig 34: Age Incidence of PAD

Table 7: Age distribution of PAD involvement among each age group

	PAD involvement		
Age Group	**Yes**	**No**	**Total**
30-39 Years	3(12.5%)	21(87.5%)	24
40-49 Years	8(13.8%)	50(86.2%)	58
50-59 Years	15(19.7%)	61(80.3%)	76
60-69 Years	13(32.5%)	27(67.5%)	40
70-80 Years	4(33.3%)	8(66.7%)	12
Total	43(20.5%)	167(79.5%)	210

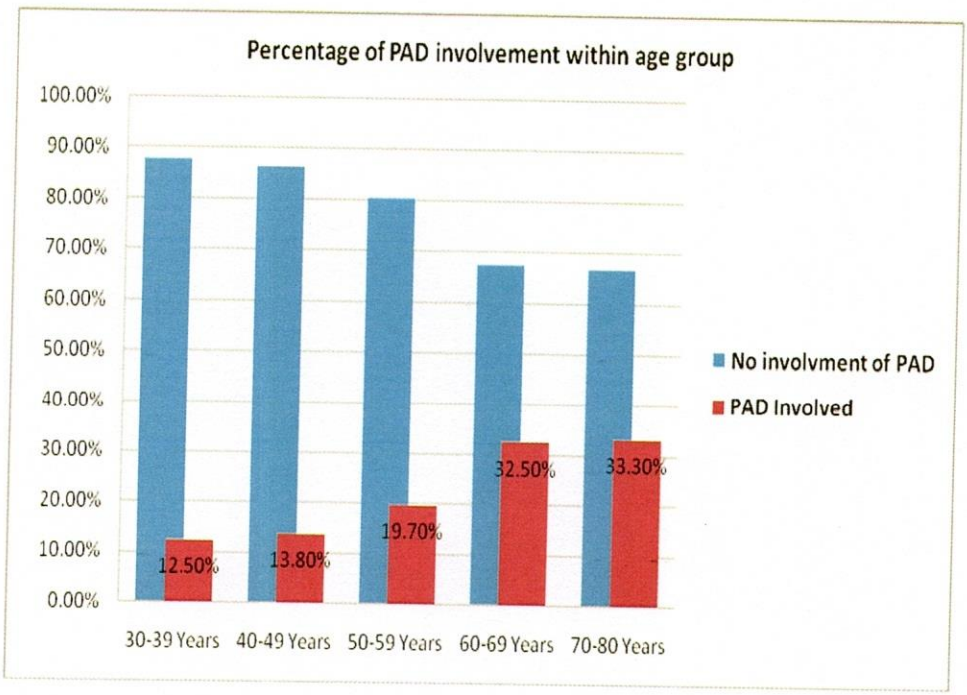


Fig 35 : Comparative graph showing age of PAD patients among the each age group of study population. There is a slow increase in the number of Pad involvement from 3rd to 7th decade of respondents.

Residence

Among 210 patients, 84 (40%) patients came from urban area and 126 patients (60%) came from rural area. Among 43 PAD patient 18 (41.9%) were from urban area and 25 (58.1%) were from rural area.

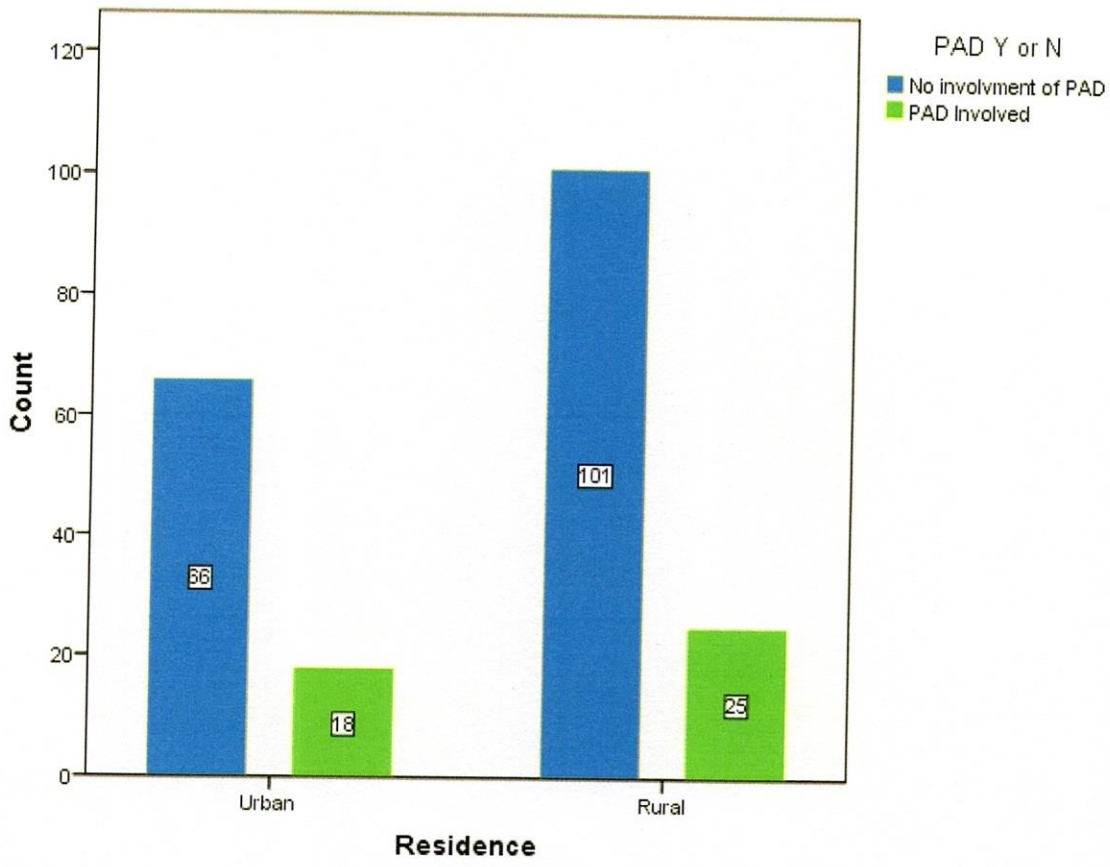


Fig 36 : Graph showing Residence of PAD and non PAD patients.

Of 84 urban people 18 (21.4%) developed PAD, 66 (78.6%) did not develop PAD and 25 of 126 (19.8%) rural people developed PAD, 101(80.2%) did not. So percentage of urban people suffering from PAD was slightly higher than that was from rural people.

Occupation

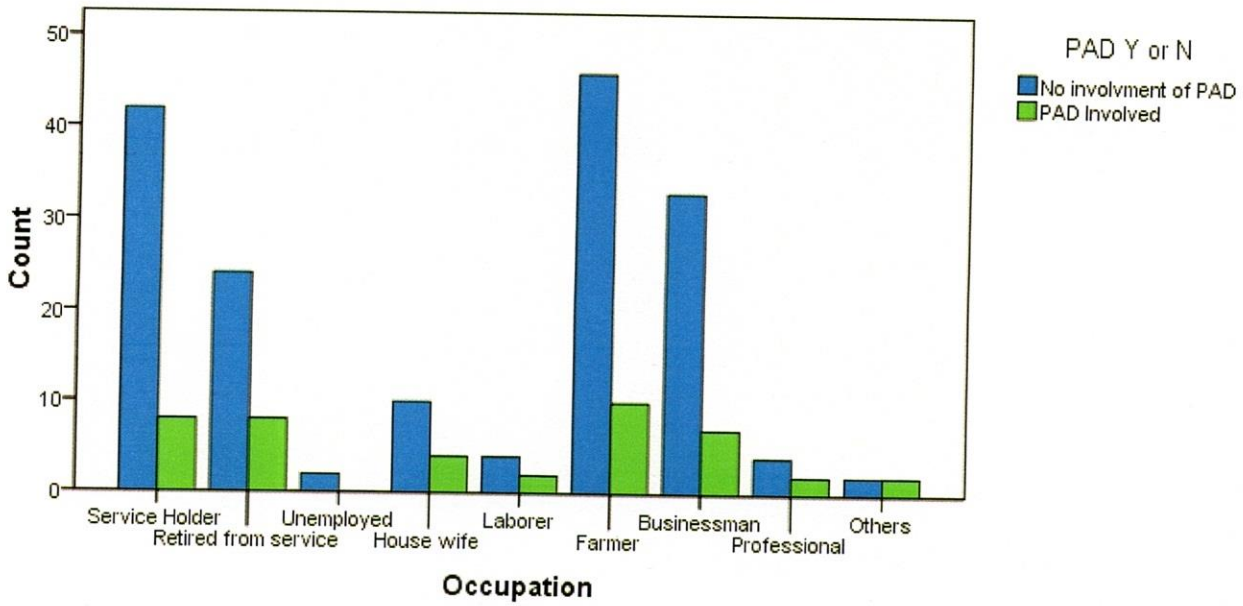


Fig 37 : Occupational distribution of respondents and PAD sufferer.

Most of the patients in study population were service holder, either acting or retired. Fifty (23.8%) were government or non government acting service holder and thirty two (15.2%) were retired from service. The group next to the highest group of occupation was farmer. Fifty six (26.7%) patients were farmer, Forty (19%) were businessman. All the female patients (14 patients, 6.7%) were housewives. Six (2.9%) were day labor and another 10 (4.7%) were from other professional. Only two patients (1%) were found unemployed.

Same trend of occupational status is seen in patients who suffered from PAD.

Figure 37 shows comparative bar graph of CAD patients and CAD with PAD patients.

Economic status:

Economic status was assessed. Among study population five patients (2.4%) were below poverty line and only 6 patients (2.9%) could be considered as rich. One from each of these two extreme groups suffered from PAD i.e. 20% from below poverty line (1 out of 5) and 16.6% from reach people (1 out of 6).

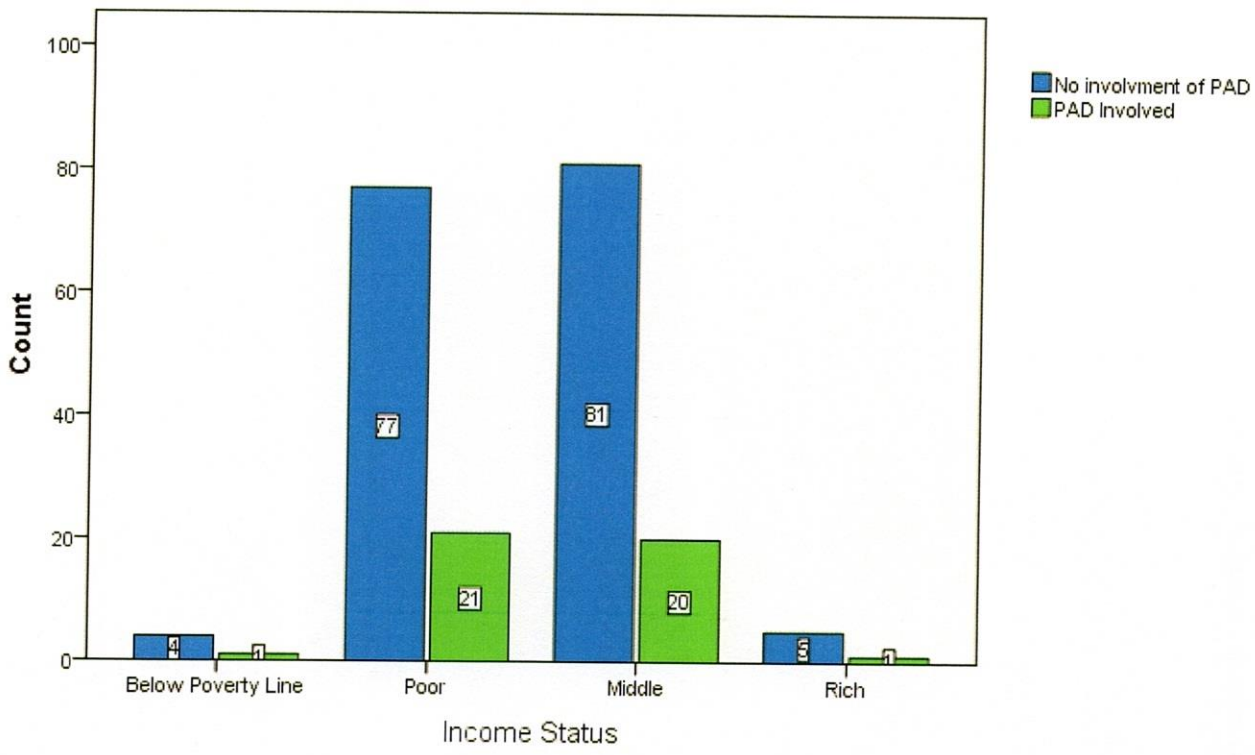


Fig 38 : Graph showing Economic Status distribution among PAD and non PAD group

Most of the patients of total respondents (101, 48.1%) belonged to middle class and next to them were from poor group (98,46.7%).

Out of 101 middle class patients 20 (19.8%) had PAD, 81(80.2%) did not. Out of 98 poor patients, 21(21.4%) had PAD involvement and 77(78.6%) did not. So the highest group of PAD involvement was from the poor group.

Literacy level:

Literacy level of each case was documented. This was categorized in four types. Completely illiterate: who cannot read or write anything, literate having education level below primary level education, those who have more than primary level education but did not pass secondary school certificate and the fourth group who had education level above the secondary school certificate.

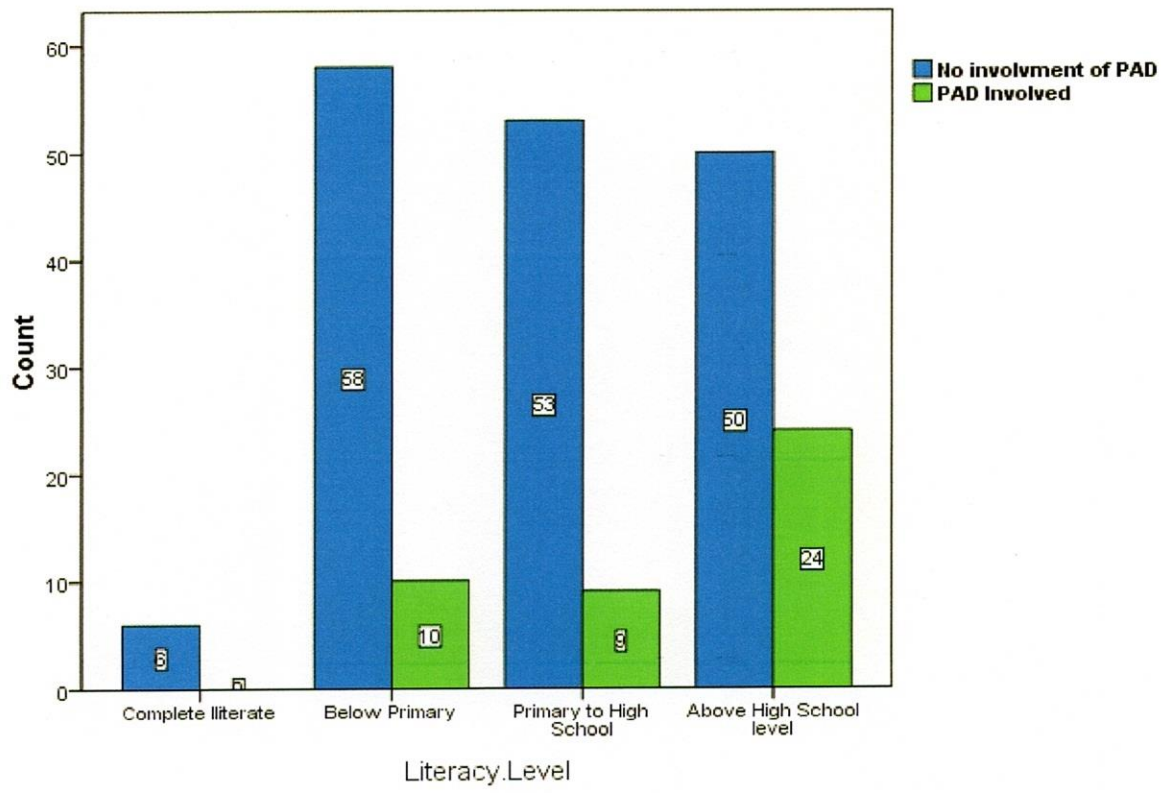


Fig 39: Graph showing Literacy level of PAD and Non PAD patients

Most of the cases, i.e 74(35.2%) had above high school level education. Out of 74, 24 patients (32.4%) developed PAD, 50(67.6%) did not have PAD. The next to highest group was the 2nd group i.e. below primary level education (68, 32.4%). In this group only 10 (14.7%) developed PAD and 58(85.3%) did not get PAD.

Those who had education level in between primary to high school level, 9(14.5%) of them suffered from PAD, 53(85.5%) did not. Six patients were completely illiterate, none of them showed any evidence of PAD.

Dietary Habit

Only one patient was found vegetarian. Among the non vegetarian group 68 person (32.4%) took regular red meat, more than three days per week. and 141 person (67.1%) took red meat less than 3 days in a week. Out of 68 persons who took regular red meat, 20(29.4%) of them suffered from PAD. On the other hand out of 141 persons who took occasional red meat, 23(16%) of them suffered from PAD. (Table-8)

Table 8: Relation of Diet in PAD involvement:

Dietary Habit	**PAD involvement**		
	Yes	**No**	**Total**
Taken regular red meat	20 (29.4%)	49 (71.0%)	68
Taken occasional red meat	23 (16.3%)	118(83.7%)	141
Vegetarian	0	1(100%)	1
Total	43	167	210

*X2=4.5 d=1 p=**0.033***

Table 8 shows that incidence of PAD is higher in patients who took regular red meat which was statistically significant.

Family History of CAD in first degree relatives :

A familial incidence of CAD was found in 65 (31%) subjects. In 145(69%) subjects no family history of heart disease was found. Among PAD patients, 16 (37.2%) had the family history of cardiac disease i.e. 24.6% (16 of 65) of patients among positive family history of CAD, developed PAD. Forty nine patients who had previous family history of heart disease did not develop PAD (75.4% of patients with positive family history).

Out of 145 patients, who had no family history of CAD, 27(18.6%) developed PAD and 118 (81.4%) did not develop PAD.

The association was not statistically significant (p=0.32).

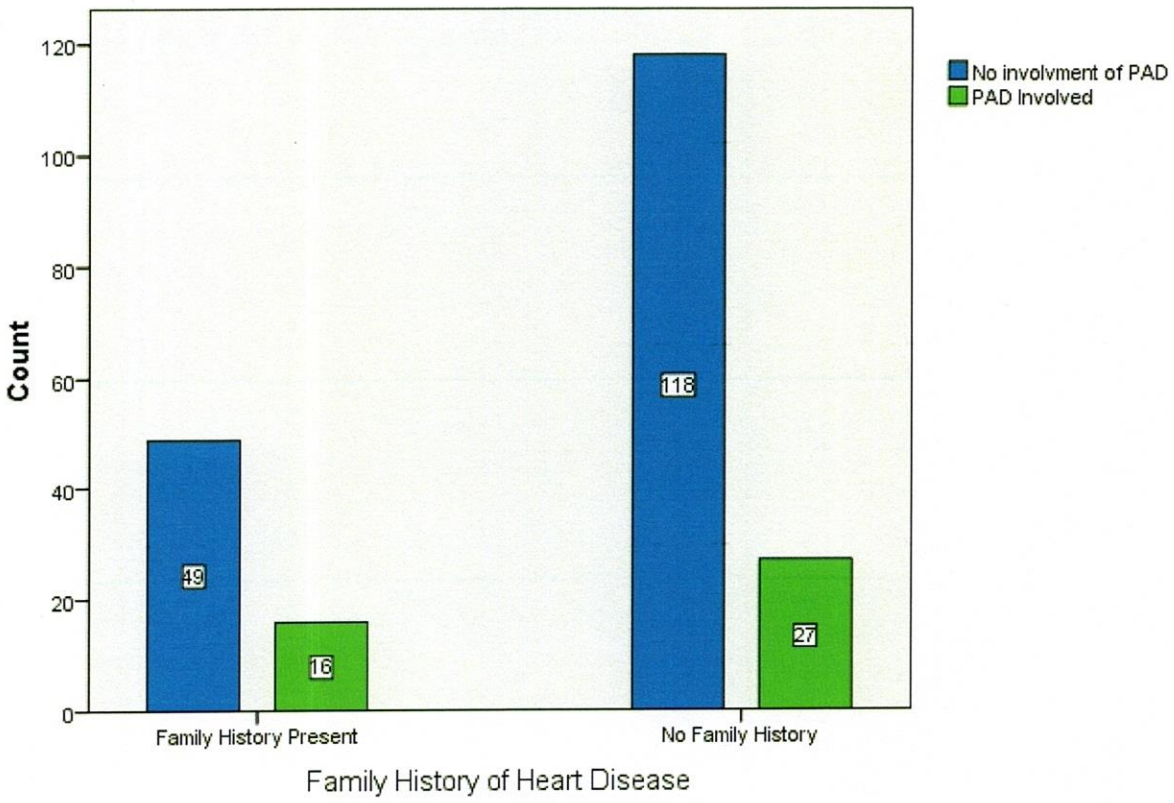


Fig 40 : Family History of Heart disease among PAD and non PAD group

Smoking

All the patients fulfilled the criteria of recent smoker. None could be considered as past smoker. Total number of smoker in study group was 154 (73.3%) and 56 (26.7%) person were non smoker. Mean pack year of the smokers was 14.43± 16.4 years.

Out of 154 smoker 35 (22.7%) developed PAD and out of 56 non smoker 8 (14.3%) developed PAD (Table 9).

Out of 43 patients of PAD, percentage of smoker was 81.4% (35 out of 43) and non smoker was 18.6% (8 out of 43). Mean pack year of smoker having PAD patients was 15.8 ± 19.9 years.

Table : 9 : Distribution of smoker in PAD patients.

	PAD involvement		
	Yes	**No**	**Total**
Smoker	35 (22.7%)	119(77.3%)	154
Non smoker	8(14.3%)	48(85.7%)	56
Total	43	167	210

$X^2 = 1.7$ $df = 1$ $p = 0.180$

Alcohol Consumption

Only 2 (1%) patients gave the history of regular alcohol consumption. Sixteen patients (7.6%) gave the history of occasional alcohol consumption which was not very remarkable. Rest 192(91.4 %) told they never drank any form of alcohol in their life. None of the patient with PAD had the history of alcohol consumption.

Physical Activity

Most of the patients were sedentary worker (152,72 %). Among the rest ,24 person (11.4%) might be considered as having medium level of physical activity and 34 (16.2 %) patient had to do hard manual labor for their profession. 156(74%) patients had no history of regular exercise. 50(23.8%) had the habit of regular walking. Only 2 (1%) patients were found who went to gymnasium regularly and only one patient had the habit of swimming.

Among PAD patents sedentary, moderate and hard labor were 32 (74.4%), 7(16.3%) and 4 (9.3%) respectively.

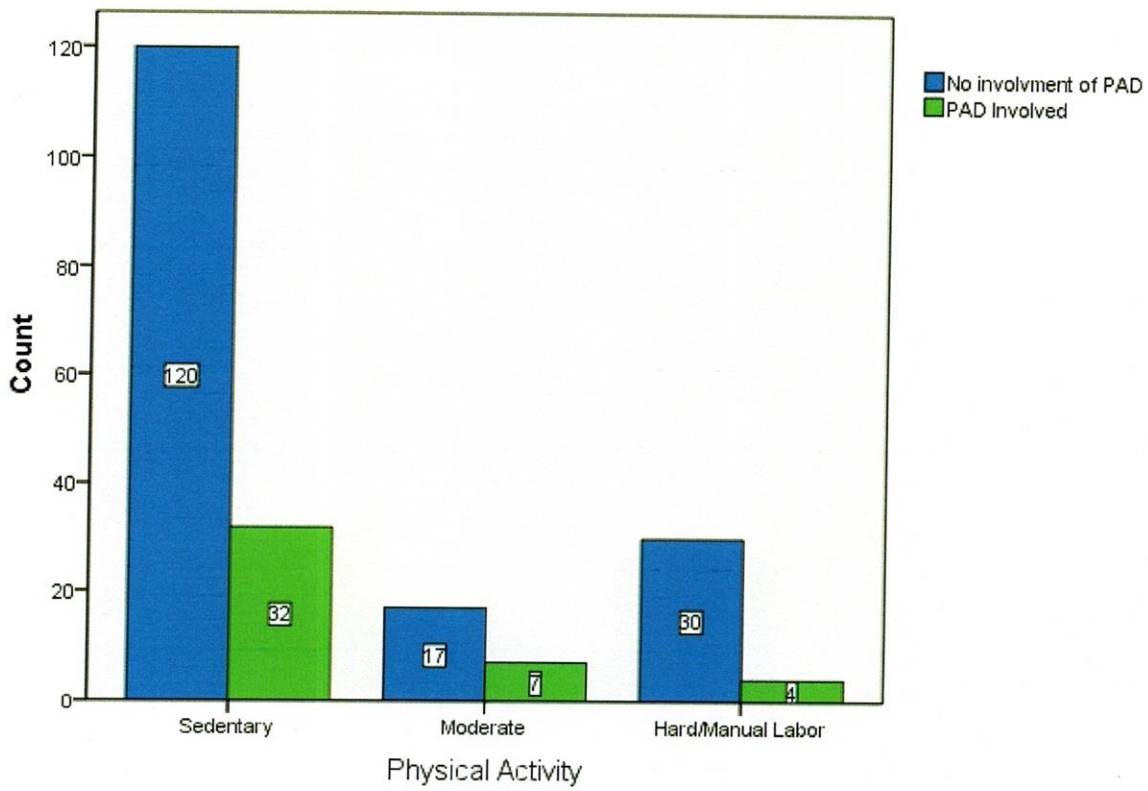


Fig 41: Graph showing physical activity distribution among PAD affected patients. It shows that the patients with moderate physical activities mostly suffered from PAD (29.2% of moderate worker, 21.1% of sedentary worker and 11.8% of hard manual worker developed PAD).

Hypertension

Among 210, ninety (42.9%) patients were taking anti hypertensive drugs. One hundred and four (49.5%) patients said that they had no history of previous hypertension but six of them were found hypertensive when blood pressure was checked. Rest 16 (7.6%) patients said that they never checked their blood pressure in their life and five of them were found hypertensive when blood pressure was checked. So total 90+6+5=101 patients were considered hypertensive.

Twenty nine patients out of 101 hypertensive patients i.e. 28.7% of hypertensive patient had PAD which was statistically significant (Table-10).

67.4% of total PAD (29 of 43) was hypertensive.

Table-10 : Distribution of hypertension among PAD patients.

Hypertension	**PAD involvement**		
	Yes	**No**	**Total**
Hypertensive	29(28.7%)	72 (78.3%)	101
Normotensive	14 (12.8%)	95(87.2%)	109
Total	43	167	210

*x2=8.1 d=1 p=0.**004***

Presence of Diabetes Mellitus

Among 210, 83 (39.5%) cases were diabetic. Sixty two of them were known case of diabetic taking antidiabetic agents or were in diet control. Twenty one were discovered as new cases on laboratory findings. 127 (60.5%) cases were non diabetic.

Table-11 : Distribution of Diabetic patients among PAD patients

Diabetic	**PAD involvement**		
	Yes	**No**	**Total**
Diabetic	27(32.5%)	56 (67.5%)	83
Non Diabetic	16 (12.6%)	111(87.4%)	141
Total	43	167	210

*x2=12.2 d=1 p=**0.000***

Twenty seven (44%) of total 43 patients with PAD had the history of diabetes. Table 11 shows that of 83 diabetic cases, the percentage of PAD involvement was 32.5 % and of 141 nondiabetic cases, the percentage of PAD involvement was 12.6%. The involvement was statistically highly significant.

Dyslipidemia

Among 210 cases 41(10.5%) patients were taking lipid lowering agent for their dyslipidemia. Seventy four (35.2%) person said they had no history of any dyislipidemia . A large number, 95(45.2%) persons said that they never checked it in their life. So emphasis was given on laboratory findings. Total cases of dyslipidemia on the laboratory finding were 132(62.8%). One hundred and two (48.6%) cases showed hypercholesterolemia, 122 (58.1%) cases showed abnormal TG-cholesterol, 74 (35.2%) cases showed high LDL-cholesterol and 84 (40%) cases showed low HDL-cholesterol.

Total cases of dyslipidemia in PAD patients were 34 which were the 79% of total PAD patients and 25.7% of total dyslipidemic patients.

Table 12- Dyslipidemia in patient with PAD

Abnormal lipid profile	PAD Involved	PAD not involved	Total	p value by chi square test
High serum total cholesterol	26 (25.5%)	76 (74.5%)	102	0.080
High serum TG	33 (29.5%)	89 (70.5%)	122	**0.005**
High serum LDL	12(16.2%)	62 (83.8%)	74	0.259
Low serum HDL	18(21.4%)	66 (78.6%)	84	0.780

Abnormal lipid profile among PAD patients are shown in table-12. The table shows that association of PAD and high TG level was statistically significant.

Body mass index (BMI):

Mean BMI was 24.70±2.9. Two persons (1.0%) had BMI below normal level, was considered underweight. One hundred and eight (51.4%) had normal BMI, 92 (43.8%) had the level of above average but below obesity level. Eight (3.8%) was considered obese, had high BMI level.

Mean BMI of PAD patient was. 25.7±2.9. Twenty one (48.8%) of them were overweight, four (9.3%) of them were obese and 18 (41.9%) had BMI within normal range. So 50% of obese (4 out of 8) patient had PAD. (Table-13)

Table 13: BMI of PAD patient

	PAD Involvement		
BMI	**Yes**	**No**	**Total**
Underweight	0 (0%)	2 (100%)	2
Normal	18 (16.7%)	90 (83.3%)	108
Overweight	21 (22.8%)	71 (77.2%)	92
Obese	4 (50%)	4 (50%)	8
Total	43	167	210

$X^2 = 6.0 \quad df = 3 \quad p = 0.108$

Table 13 shows a gradual increase in percentage of PAD patients as the BMI is increased, though statistically was not significant.

Hip Waist Ratio:

It was found that 200 (99.0%) patient had higher Hip Waist Ratio, considering 0.9 as normal for male and 0.85 as normal for female. All 43 patients of PAD had high H/W Ratio.

Duplex Study

In 210 study population both sided CCA, ICA, Common Femoral, Popliteal, Anterior Tibial and Posterior tibial, total 210X12=2520 segments of arteries were examined. Involvement of PAD was found in 43(20.6%) patients in one or multiple segments of arteries. Twenty six (12.3%) of them had only carotid involvement, 15(7.4%) had only lower limb arteries involvement and two (0.95%) had both carotid and lower limb involvement (Fig-42). Total carotid involvement was in 28(13.3%) patients and total lower limb involvement was in 17(8.0%) patients. Among the carotid stenosis, 18(8.5%) were critical and 10(4.7%) were non critical stenosis and among the lower limb arteries stenosis, 11(5.2%) were critical and 6 (2.8%) were non critical stenosis. Total Twenty seven (12.8%) patients had critical stenosis with turbulent flow and 16(7.6%) had non critical stenosis with hemodynamically stable condition in one or multiple arteries of any of the carotid or lower limb territories.

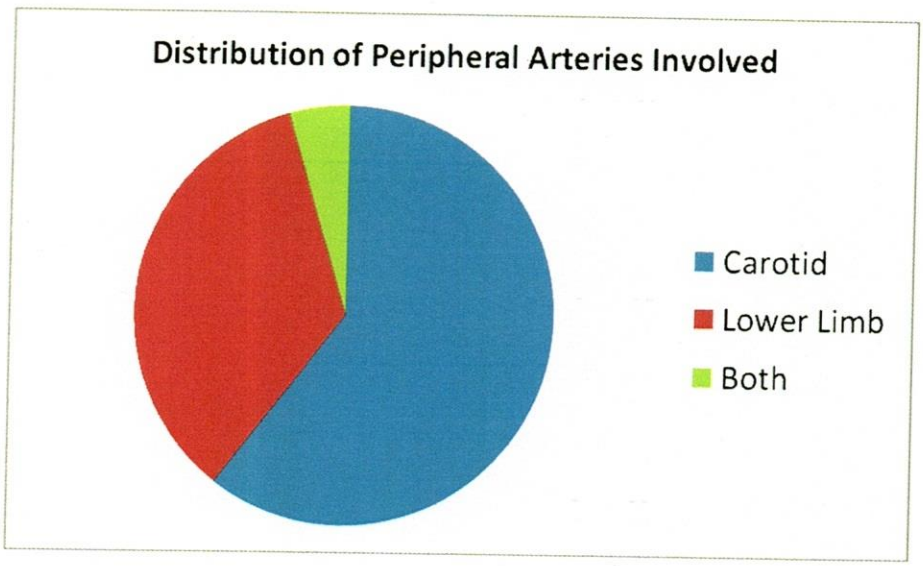


Fig-42 : Distribution of peripheral arteries involvement.

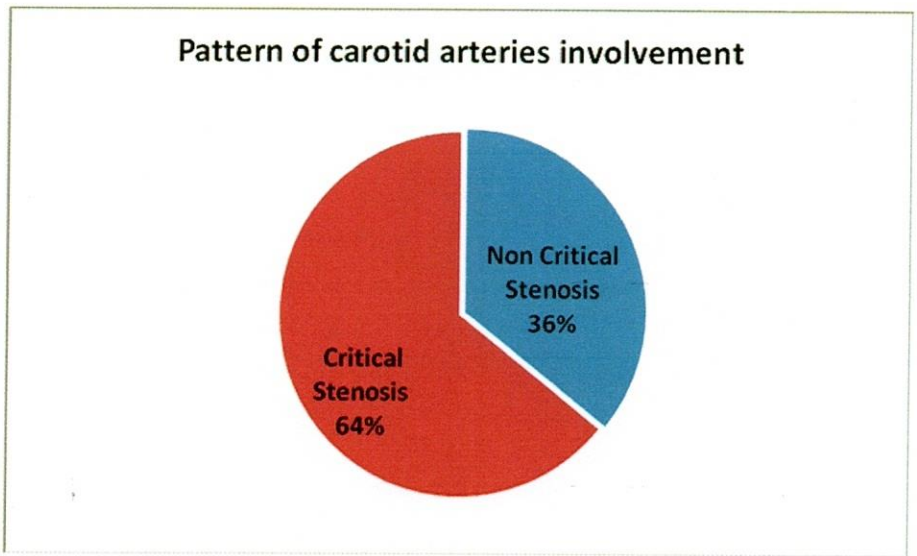


Fig-43 : Distribution of carotid arteries involvement (n 43)

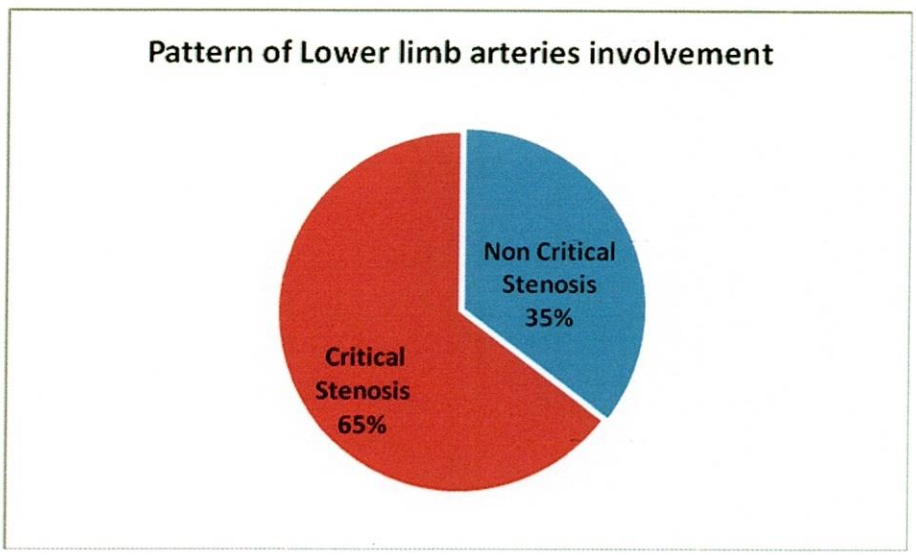


Fig-44 : Distribution of lower limb arteries involvement (n 43)

Carotid Arteries:

B mode image showed plaques in wall of RCC in 20 cases which causes lumen area stenosis > 50% in 12 cases and < 50% in 08 cases.

In LCC 12 cases show plaques in wall among which 06 had > 50% stenosis and 06 had < 50% stenosis.

RICA showed Stenosis in 02 cases, both >50% stenosis. LICA showed stenosis in 02 cases and both had < 50% stenosis. (Table 14)

Some patients had multiple involvements. Total number of patients having either one or more carotid system involved was 28 (65.12%). Among them 18 had more than > 50% stenosis and 10 had < 50% stenosis determined on the basis of B mode image and hemodynamic study by Duplex US.

Table 14: Patterns of Carotid system Involved

	> 50% Stenosis	< 50% Stenosis	Total*
Right CCA	**12**	**8**	**20**
Left CCA	**06**	**06**	**12**
Right ICA	**02**	**00**	**02**
Left ICA	**00**	**02**	**02**

**Some patients had multiple involvements.*

Carotid Intima-media thickness (CIMT):

Mean intima-media thickness of right common carotid artery was 0.85±0.23 mm and that of left common carotid artery was 0.87±0.23 mm. Mean intima-media thickness of right and left internal carotid artery were 0.44±0.48 mm and 0.43±0.44 mm respectively.

Table 15 : Correlation of Age and Right Common Carotid IMT

Correlations

		Age	RCCA Intima Media Thickness
Age	Pearson Correlation	1	.254**
	Sig. (2-tailed)		.000
	N	210	210
Intima Media Thickness	Pearson Correlation	.254**	1
	Sig. (2-tailed)	.000	
	N	210	210

**. Correlation is significant at the 0.01 level (2-tailed).

Table 16 : Correlation of Age and Left Common Carotid IMT

Correlations

		Age	LCCA Intima Media Thickness
Age	Pearson Correlation	1	.223**
	Sig. (2-tailed)		.001
	N	210	210
Intima Media Thickness	Pearson Correlation	.223**	1
	Sig. (2-tailed)	.001	
	N	210	210

**. Correlation is significant at the 0.01 level (2-tailed).

Table - 17 : Result of independent-samples t-test comparing mean of both common carotid IMT on presence of PAD.

Group Statistics

	PAD total	N	Mean	Std. Deviation	Std. Error Mean
Intima Media Thickness	No involvement of PAD	167	.823	.2296	.0178
	PAD Involved	43	.981	.2333	.0356

P value = **.000**

Table 17 shows Mean Carotid IMT of total PAD patients was higher than that of patients without PAD and statistically significant.

Table - 18 : Result of independent-samples t-test comparing mean of both common carotid IMT on presence of Carotid stenosis.

Group Statistics

	Carotid Involvement	N	Mean	Std. Deviation	Std. Error Mean
Intima Media Thickness	Not involved	182	.829	.2357	.0175
	Involved	28	1.029	.1802	.0341

P value : **.000**

Table 18 shows Mean Carotid IMT was significantly higher in patients who have got Carotid arteries involvement.

Table - 19 : Result of independent-samples t-test comparing mean of both common carotid IMT on presence of lower limb artery stenosis.

Group Statistics

	Lower limb involvement	N	Mean	Std. Deviation	Std. Error Mean
Intima Media Thickness	Not involved	193	.849	.2328	.0168
	Involved	17	.929	.2953	.0716

P value = .182

Table 19 shows Mean Carotid IMT was higher in patients who has got lower limbs arteries involvement but not statistically significant.

Table -20: Result of independent-samples t-test comparing mean of both common carotid IMT on common risk factors:

	Mean IMT±SD	p value by independent-samples t-test
BMI: Normal High	0.861±0.22mm 0.848±0.21mm	0.702
EF: Normal High	0.902±0.22mm 0.842±0.22mm	0.131
Family History of Heart disease : Present Absent	0.840±0.22mm 0.862±0.24mm	0.537
Smoking : Yes No	0.866±0.23mm 0.823±0.23mm	0.622
Hypertension: Yes No	0.890±0.22mm 0.820±0.25mm	**0.035**
Diabetes Yes No	0.872±0.22mm 0.844±0.24mm	0.404
Dyslipidemia Yes No	1.107±0.26mm 0.831±0.22mm	**0.000**

From table 20 it is seen than mean carotid IMT is significantly higher in patients who are hypertensive patients and patients with dyslipidemia. In diabetic patients it also shows higher value though statistically not significant. Mean IMT also shows higher value in patients with higher BMI, patients with positive family history of cardiac disease and who had history of more tobacco consumption.

Lower Limb Arteries

Lower limb arterial stenosis were evaluated by seeing the heamodynamic status. (Velocity, Doppler indices and shape of waveform). Total 17 patients were found having some degree of stenosis in single or multiple segments. They are showed in table 21. Among them 15 had involvement only in leg arteries, two had both in leg and carotid system. Among 17 patents, 11 had critical stenosis, 06 had non critical stenosis. Segmental distributions were, 9 in femoral segment, 06 in popliteal segment, and 09 in tibioperoneal segment (Table 21).

Table 21 : Segmental Distributions of PAD involvement.

	Non Critical <50% stenosis	Critical >50% stenosis	Total*
Stenosis in Rt Common Femoral Artery	3	2	5
Stenosis in Lt Common Femoral Artery	3	1	4
Stenosis in Rt popliteal Artery	2	1	3
Stenosis in Lt popliteal Artery	1	2	3
Stenosis in Rt Anterior Tibial Artery	1	0	1
Stenosis in Lt Anterior Tibial Artery	1	3	4
Stenosis in Rt Posterior Tibial Artery	0	0	0
Stenosis in Lt Posterior Tibial Artery	1	3	4

**Some patients had multiple involvements.*

Relation of PAD and CAD

Relation of PAD and CAD in different aspect was analyzed. Involvement of PAD in relation to severity of CAD and risk factors were studied.

Occurrence of PAD among CAD patient was found 20.4%. However if only critical stenosis was considered the prevalence was 12.9%. We found 7.6% patient who had plaques in their wall and narrowing of lumen but hemodynamically stable condition.

Table 22: Relationship of PAD involvement with number of vessels involved in CAD

CAD	PAD involvement	Without PAD involvement	Total
Single vessel Disease	21(18.4%)	93(81.6%)	114
Double vessel Disease	8(20%)	32(80%)	40
Triple vessel Disease	14(25%)	42(75%)	56
Total	43	167	210

X^2=1.0 df=2 p=0.605

Relationship of PAD and number of vessels affected in CAD is shown in Table-22. An upward trend of PAD involvement is seen with the severity of coronary artery involvement. In single vessels disease the occurrence of PAD was 18.4%. Mild increase in occurrence of PAD was seen in double vessel disease which was 20%. Then a sharp increase of PAD involvement was found in triple vessels disease which was 25%.

So there was gradual increase in percentage of PAD involvement with number of coronary vessels affected, though it was not significant statistically.

Table 23: Relationship of number of vessels involved in CAD with degree of involvement of PAD.

	CAD			
PAD	**Single vessel Disease**	**Double vessel Disease**	**Triple vessel Disease Total**	**Total**
Critical Stenosis of PAD	13(48.1%)	6(22.2%)	8(29.6%)	27
Non Critical Stenosis of PAD	8(50%)	2(12.5%)	6(37.5%)	16
Total	21	8	14	43

$X^2=0.709,\ df=2,\ p=0.702$

Table 23 shows that out of 27 patients having critical stenosis , 48.1%, 22.2% and 29.6% patients had single, double and triple coronary vessel involvement. Out of 16 patients having non critical stenosis, single, double and triple coronary vessel involvement were 50%, 12.5% and 37.5% respectively. Statistically this association was not significant.

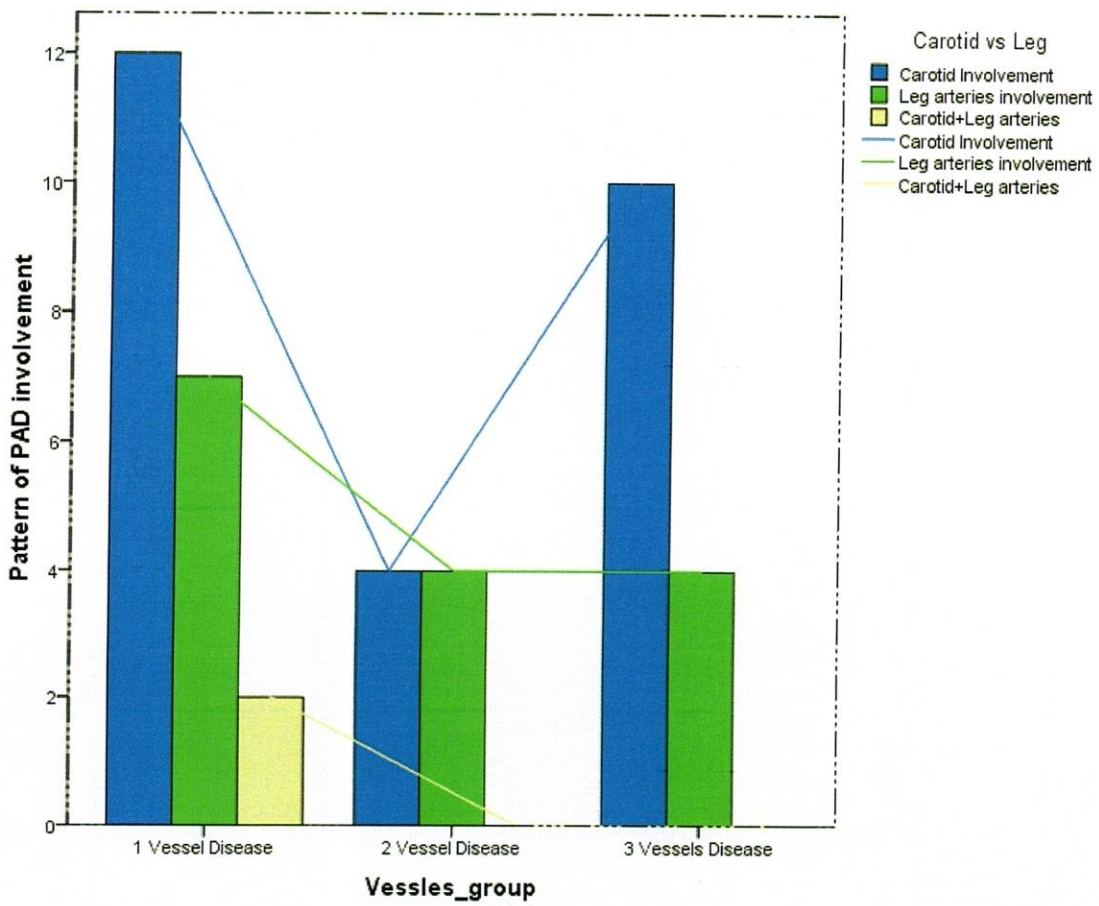


Fig 45 : Graph showing relation of segment of PAD involvement with number of coronary vessels involved.

Table 24. Relationship between CAD and carotid arteries stenosis:

CAD	Carotid arteries stenosis		Total
	Yes	**No**	
Single Vessel	14 (12.3)	100 (87.7)	114
Double Vessel	04 (10.0)	36 (90.0)	40
Triple Vessel	10 (17.9)	46 (82.1)	56
Total	28 (13.3)	182 (86.7)	210 (100)

$X^2 = 1.48$, $df = 02$, $p = .476$

Table 24 shows individual carotid arteries involvement in single, double and triple vessels disease. Carotid stenosis was present in 12.3%, , 10%, 17.9% cases of 1-, 2-, and 3- vessels disease respectively. The association was not significant. Involvement in triple vessel disease was much higher than single and double vessel disease but involvement in double vessels disease was lesser that that of single vessel disease. However, the increase of percentage of PAD involvement was sharp from double to triple vessel disease.

Table 25. Relationship between CAD and Lower limb arteries stenosis:

CAD	Lower limb arteries stenosis		Total
	Yes	No	
Single Vessel	7 (6.1)	107 (93.9)	114
Double Vessel	04 (10.0)	36(90.0)	40
Triple Vessel	6 (10.7)	50 (82.1)	56
Total	17	193	210

$X^2 = 1.29, df = 02, p = .523$

Table 25 shows individual lower limb artery stenosis in the category of single, double and triple vessel disease. The involvement was 6.1%, 10.0% and 10.7% respectively. The association was not significant. Involvement in double vessel disease was higher than single vessel disease but no remarkable increase was seen in triple vessel disease in comparison to double vessel disease.

Table 26. Relationship between LMCA and PAD involvement:

LMCA	PAD Involvement		
	Yes	**No**	**Total**
Involved	4 (25%) (9.3%)	12 (75%) (7.2%)	16 (100%)
Not involved	39 (20.1%) (90.7%)	155 (79.9%) (92.8%)	194 (100%)
Total	43 (100%)	167(100%)	210 (100%)

$X^2 = 0.218, df=1, p=0.422$

Sixteen patients had left main coronary involvement. Table 26 shows that among them only 4(25%) developed PAD, 12(75%) did not get PAD. Conversely 9.3% of PAD patients had LMCA involvement and 90.7% of PAD patients had no involvement of LMCA. The association was not statistically significant.

Table 27. Relationship between LMCA and Carotid arteries involvement:

LMCA	Carotid arteries stenosis		
	Yes	**No**	**Total**
Involved	2 (12.5%) (7.1%)	14 (87.5%) (7.7%)	16
Not involved	26 (13.4%) (92.9%)	168 (86.6%) (92.3%)	194
Total	28	182	210

$X^2 = 0.010, df=1, p=0.919$

Table 27 shows that among 16 patients of LMCA involvement only 2 patients (12.5%) had associated carotid artery involvement and rest 14(87.5%) did not have any involvement in carotid arteries. Conversely only 7.1% of patients with carotid artery stenosis had stenosis in LMCA and 92.9% of patients with carotid artery stenosis did not have stenosis in LMCA. The association was not statistically significant.

Table 28. Relationship between LMCA and Lower limb artery involvement:

LMCA	Lower limb arteries stenosis		
	Yes	**No**	**Total**
Involved	2 (12.5%) (11.8%)	14 (87.5%) (7.3%)	16
Not involved	15 (7.7%) (88.2%)	179 (92.3%) (92.7%)	194
Total	17	193	210

$X^2 = 0.452, df=1, p=0.502$

Table 28 shows the association of LMCA involvement with lower limb arteries stenosis. Only 2 patients of 16 (12.5%) had lower limb artery stenosis. Rest 14(87.5%) of patients with LMCA involvement did not get lower limb arteries stenosis. Conversely only 11.8% of patients with lower limb artery stenosis had LMCA involvement and 88.2% of lower limb artery stenosed patient did not have stenosis in LMCA. The association was not statistically significant.

Table 29: Relation of Intima Media Thickness of Carotid Arteries to Different pattern of Coronary Artery Involvement

CAD	Mean Intima Media Thickness in mm			
	Right CCA	Left CCA	Right ICA	Left ICA
Single Vessels disease	0.783±0.21	0.813±0.22	0.425±.064	0.432±.059
Double Vessels disease	0.932±0.22	0.942±.021	0.422±0.11	0.412±0.12
Triple Vessels disease	0.946±0.24	0.968±0.23	0.436±0.13	0.427±0.16
LMCA Involvement	0.941±0.23	0.942±0.27	0.431±0.13	0.424±0.14

Intima media thickness of carotid arteries was figured out in different groups of coronary involvement. Mean IMT of both CCA in 1-, 2- and 3- vessel disease and LMCA involvement are shown in Table -29. It is observed that there was an upward trend of mean carotid IMT with increase severity of CAD, categorized as single, double and triple vessels disease.

Mean CIMT was higher in LMCA involvement than that of single vessel and double vessel disease but no remarkable difference of CIMT was seen in-between patients with triple vessel disease and patients with LMCA involvement.

Mean IMT of each internal carotid artery does not show any remarkable correlation with number of coronary vessels involvement and LMCA involvement.

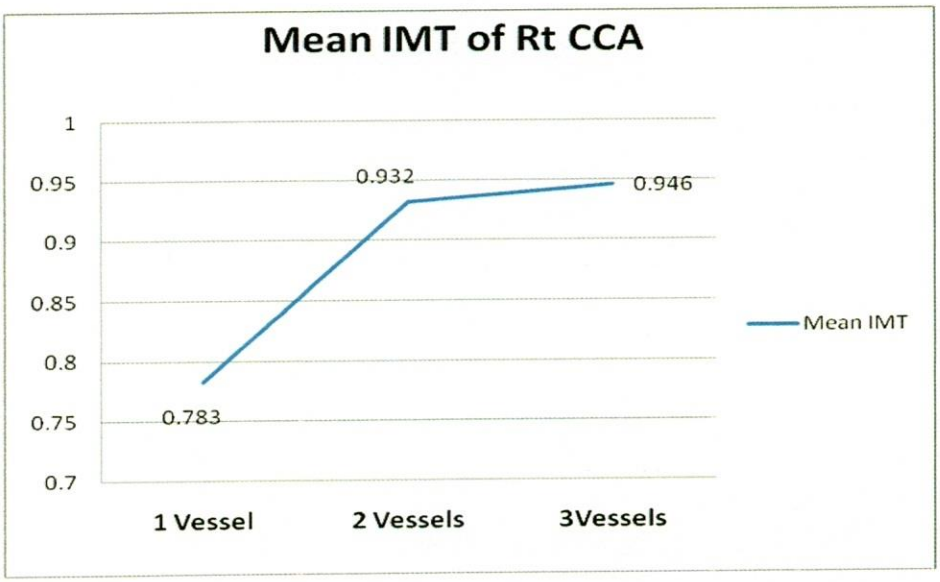


Fig 46: Relation of IMT of right CCA with number of vessels involved in CAD patients

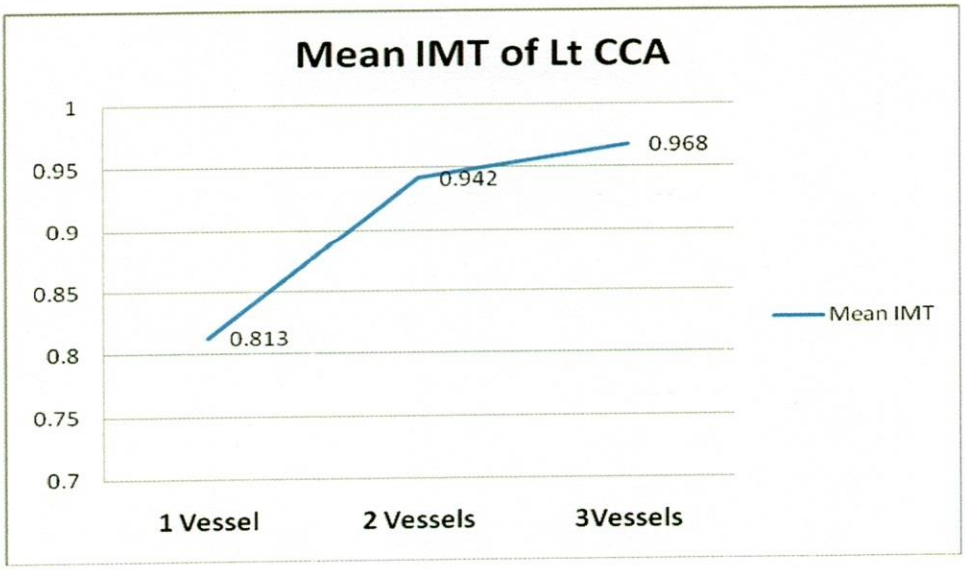


Fig 47: Relation of IMT of Left CCA with number of vessels involved in CAD patients

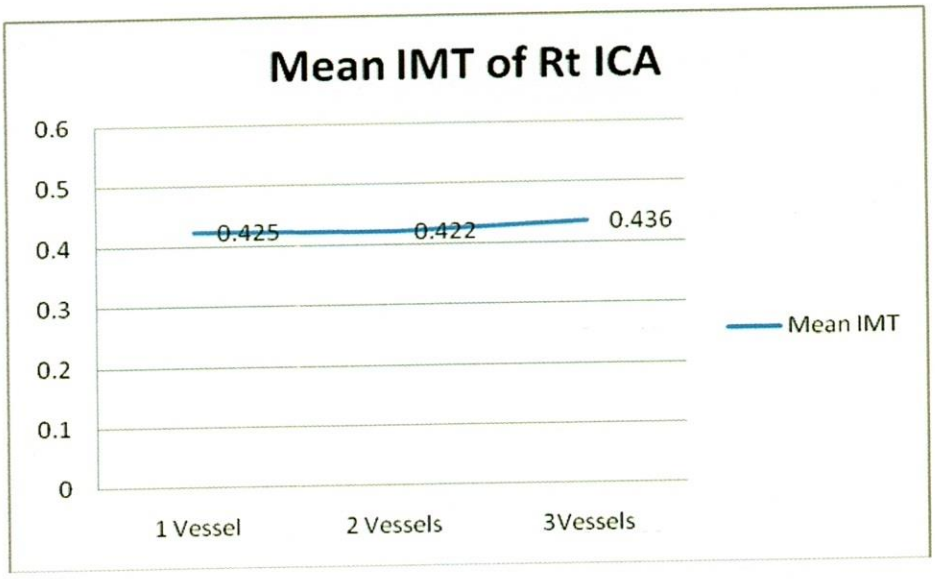


Fig 48: Relation of IMT of right ICA with number of vessels involved in CAD patients

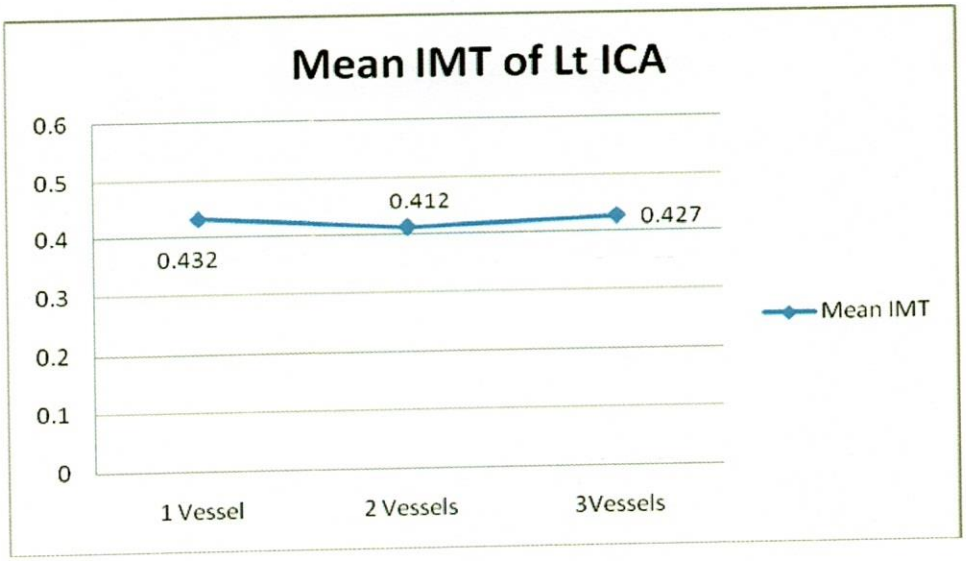


Fig 49: Relation of IMT of Left ICA with number of vessels involved in CAD patients

Relation of PAD with Ejection Fraction of left ventricle:

The mean ejection fraction of total study population was 52.7±6.6.

The mean ejection fraction of PAD patients was 52.98±6.5

21.9% of total respondent had EF lower than normal. In PAD 23.2% patient had EF lower than normal.

Table 30: Relationship between PAD involvement and Ejection Fraction of Left Ventricle

	PAD Involvement		
Ejection Fraction	**Yes**	**No**	**Total**
Low	10(21.7%)	36 (78.3%)	46
Normal	33(20.1%)	131 (79.9%)	164
Total	43	167	210

$X^2 = 0.058$ $df = 1$ $p = 0.810$

Table 30 shows that slightly higher trend to develop PAD who had low EF. Twenty one percent patients developed PAD among patients who had low EF and 20.1% developed PAD among patients who had normal EF.

Table 31: Relationship between Carotid artery stenosis and Ejection Fraction of Left Ventricle

	Carotid Involvement		
Ejection Fraction	**Yes**	**No**	**Total**
Low	6(13.0%)	40 (87.0%)	46
Normal	22(13.4%)	142 (86.6%)	164
Total	43	167	210

$X^2 = 0.004$ $df = 1$ $p = 0.948$

Table 31 shows that percentage of involvement of PAD in patients with normal left ventricular EF and those with normal LVEF are almost same.

Table 32: Relationship between lower limb stenosis and Ejection Fraction of Left Ventricle

	PAD Involvement		
Ejection Fraction	**Yes**	**No**	**Total**
Low	4(8.7%)	42 (91.3%)	46
Normal	13(7.9%)	151 (92.1%)	164
Total	43	167	210

$X^2 = 0.029$ $df = 1$ $p = 0.866$

Table 32 shows that more patients (percentage) of low LVEF developed PAD in comparison to patients with normal LVEF but the association was not significant statistically.

Table 33: Risk factors in PAD and non PAD group:

	CAD having PAD (n=43)	CAD without PAD (n=167)	p value
Mean Age	54.6±10.7	50.48±10.2	0.955
Smoking Number of smoker Mean Pack Year	 35(81.4) 15.8±19.9	 119(71.3) 14.0±15.4	 0.180 0.722
Physical Activity Sedentary Moderate Hard	 32(74.4) 7(16.3) 4(9.3)	 120 (71.8) 17 10.1) 3017.9)	0.256
Family History	16(37.2)	49(29.3)	0.320
Hypertension	29(67.4)	72(43.1)	**0.004**
Diabetic	27(62.8)	56(33.5)	**0.000**
Total Cholesterol	205±44.9	192±62.3	**0.017**
High LDL	111±40.5	116±52.0	0.511
Low HDL	41.7±4.0	40.9±6.3	0.334
High TG	230±137	169±79.3	**0.007**
High H/W Ratio	43(100%)	157(94%)	**0.005**
Mean BMI	25.7±2.9	24.4±2.9	**0.009**

Table 33 shows the difference between PAD and non PAD group in term of risk factors. Continuous data are presented as means and standard deviations. Categorical data are presented proportion. Figures in parenthesis are percentage. An independent sample t test was run for continuous variable and chi square test was done for discrete variable.

It was found that PAD patients had statistically significant association in case of more number of hypertensive and diabetic persons, higher total cholesterol concentration, higher triglyceride level, and obesity compared to patients without PAD.

Table 34 : Relationship of risk factors with degree of stenosis of PAD

Risk Factors	PAD Stenosis >50% (n=27)	PAD Stenosis <50% (n=16)	P value
Smoking	20(74.1%)	15(93.8%)	0.109
Family History	9(33.3%)	7(43.8%)	0.495
Hypertension	20(74.1%)	9(56.2%)	0.228
Diabetes	20(74.1%)	7(43.8%)	**0.047**
High Total Cholesterol	13(48.1)	13(81.2)	**0.032**
High LDL	7(25.9%)	5(31.2%)	0.707
Low HDL	10(37.0%)	8(50.0%)	0.405
High TG	20(74.1%)	13(81.2%)	0.590

Table 34 shows that only percentage of diabetes mellitus and hypertension were more in critical stenosis in comparison to non critical stenosis of which the association with diabetes mellitus was only statistically significant. Though a significant p value is seen in case of High Total Cholesterol level but it was seen high in non critical stenosis. Habit of tobacco smoking, history of positive family history, low HDL and high S. triglyceride also show higher percentage in case of non critical stenosis.

Table 35: Relationship of Risk Factor of CAD with younger age.

Risk Factor	**Age <40**(n=24)	**Age >40**(n=186)	**P value**
Smoking			
Yes	20(83.3%)	134 (72.0%)	0.239
No	4(16.7%)	52(28.0%)	
Family History			
Yes	14 (58.3%)	51 (27.4%)	**0.002**
No	10 (41.7%)	135(72.6%)	
Hypertension			
Yes	15 (62.8.3%)	86 (46.2%)	0.133
No	9(37.5%)	100(53.8%)	
Diabetes			
Yes	11 (45.8%)	72 (38.7%)	0.502
No	13(54.2%)	114(61.3%)	
Sedentary			
Yes	14 (58.3%)	138 (74.2%)	0.078
No			
Cholesterol			
Yes	12 (50.0%)	90 (48.4%)	0.882
No	12(50%)	96(51.6%)	
LDL			
Yes	10 (41.7%)	64 (34.4%)	0.484
No	14(58.3%)	122(65.6%)	
Low HDL			
Yes	10 (41.7%)	74 (39.8%)	0.859
No	14(58.3%)	112(60.2%)	
TG			
Yes	14(58.3%)	108(58.1%)	0.980
No	10 (41.7%)	78 (41.9%)	

Common risk factors were statistically analyzed among young patients who were of age below 40 yrs. Table 35 shows only positive family history was significantly associated with the younger age group (below 40 yrs) involvement.

Table 36: Binary Logistic regression to assess independent predictors of PAD

Variable	Regression Coefficient	P value
Age	-0.016	0.618
Smoking	0.493	0.180
Red meat Diet	0.870	**0.033**
Physical Activity	-0.249	0.385
Family History	-0.240	0.320
Hypertension	-0.542	**0.005**
Diabetes	-0.122	**0.048**
High BMI	0.814	**0.034**
Total Cholesterol	0.989	0.080
LDL	-1.078	0.259
HDL	0.227	0.780
Triglyceride	0.709	**0.005**

Table-36 shows result of binary logistic regression to assess significant independent predictor of PAD. Diet reach with red meat, hypertension, diabetes, BMI and S. triglyceride level were found to be significant predictors of PAD.

CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

Coronary artery disease, peripheral arterial disease, and cerebrovascular disease are the three major manifestations of atherosclerosis and share same risk factors to develop. So the question may arise whether the occurrence of atherosclerosis plaques in one vascular territory can predict that an individual is likely to prone to the development of atheromatous plaques in other territories. Coexistence of PAD with other atherosclerotic disorder was reported by many authors. In a study among 468 persons with PAD, 270 (58%) and 159(34%) had coexistent CAD and ischemic cerebrovascular disease respectively (Aronow and Ahn, 1994). Another study showed 68% and 42% persons with PAD had coexistent CAD and ischemic cerebrovascular disease respectively (Ness and Aronow 1999). Sheehan (2004) stated peripheral arterial disease of lower extremities as a marker of atherothrombotic disease in other vascular bed. But most of the studies were done on PAD patients. Study with CAD patients was limited. Dieter *et al.* (2003) found 40% patients of CAD patients had coexistent PAD.

This study was a cross sectional descriptive study to see the status of peripheral arteries in the patients who suffered from CAD. Peripheral arteries of 210 patients were examined by Duplex ultrasound who were diagnosed as having CAD, based on presence of more than 50% stenosis in a major coronary arteries by coronary angiography.

The study was conducted at Cardiology Department of Rajshahi Medical College Hospital, Rajshahi, Bangladesh. Medically stable patients with CAD

were requested to participate in the study prior to discharge. After informed written consent, general history and history regarding risk factors for atherosclerosis were noted. Duplex ultrasonography on both sided major carotid arteries and major lower limbs arteries were examined.

Total 2520 segments of both sided carotid and major lower limb peripheral arteries were examined.

For purpose of analysis, the entire respondent group was subdivided into single vessel disease, double vessels disease and triple vessels disease on the basis of number of coronary arteries involved. This classification of CAD was described by Kallikazaros *et al.* (1999), Ziembicka *et al.* (2004) and Ogata *et al.* (2005). The data concerning patients with LMCA involvement were analyzed separately.

Occurrence of PAD among CAD patients in this study was 20.4% among which 93.3% was male and 6.7% was female. The incidence of independent carotid and lower limb arteries involved in the entire study population was 13.3% and 08%. These figures are much lesser than those were found by Dieter *et al.* (2003) but a small number of patients, only one hundred patients were recruited for their study.

High discrepancies in prevalence of PAD in different studies were found. Discrepancies in relation to gender and age were also eminent. Selvin and Erlinger (2004) found the prevalence of PAD in the United States among adults aged 40 years and over was 4.3%, and among those aged 70 years or over, the prevalence was 14.5%. Ramos *et al.* (2009) reported 4.5% prevalence of PAD. Mahameed (2009) reported prevalence of PAD with adjusted age was 12%. Prevalence of PAD in china was shown 14.7% in men and 23.2% in women (He *et al.* 2006). Another study showed the

prevalence of PAD was estimated to be 10%–25% in people aged ≥55 years and increased to approximately 40% in people aged >80 years (Norman *et al.* 2004).

Table 37 shows the PAD prevalence in different studies in respect of age and sex found from a review study by Vavra and Kibbe,2009.

Table 37 : Prevalence of PAD in different studies.

Study (Year)	Patients (n)	Age (Years)	PAD Prevalence	
			Men	Women
Moussa *et al.* (2009)	788	>70	11.6	23
Sigvant *et al.* (2007)	5080	60-90	9.4	12.6
Ostchega *et al.* (NHNES) (2007)	3947	>60	12.5	12.0
Collins *et al.* (2006)	403	>50	17.4	15.9
Kroger *et al.* (2006)	4814	45-75	8.2	5.5
He *et al.*(2006)	2334	>60	14.7	23.2
Diehm (2004)	6880	>65	19.8	16.8
Mean prevalence			13.4	15.6

Though no data was available regarding prevalence of PAD in Bangladesh, if it is compared with the prevalence rate of other countries, occurrence of PAD patients in this study was higher than the prevalence rate among entire population. This study was a hospital based cross sectional study and was among the CAD patient. Other studies mentioned above were on entire population. So, incidence of PAD among CAD is higher if compared to the prevalence rate in entire population.

Incidence of PAD among diabetic patients was figured out by Premalath *et al.* (2000) showing 3.2% prevalence of PAD whereas Janka *et al.* (1980) showed 14.9% prevalence in a similar study design. The former one was done among south Indian people and the later one was done in western

country. Many people in south India are vegetarian (Wikipedia c). Average meat consumption in some European countries was estimated 75 to 136 kg per anum whereas in India, Bangladesh, Nepal it was only 3.6 to 6.7 kg per anum (UN FAO 2012). Relation to diet developing PAD was tried to find out in this study. Only one patient was found vegetarian and he did not get PAD. However, the study people were divided into two groups, who took regular red meat and who took occasional red meat. A statistically significant difference of PAD occurrence among these two groups was found. The percentage of people having the habit of taking regular red meat was higher in PAD in comparison to total CAD patient. Among the group of patients having habit of taking regular red meat, incidence of PAD was also much higher. 29.4% of patient having habit of regular red meat suffered from PAD in comparison to 16.3% of patient who did not have the habit of taking regular red meat ($p=0.03$). Substantial evidence from epidemiological studies shows that meat intake, particularly red meat, is associated with increased risks of cardiovascular disease. (Micha *et al.* 2010). Saturated fat and cholesterol from red meat may partially explain this association. (Hu *et al.* 1999)

Geographical variation or ethnic variation may also be present. A disproportionably higher PAD prevalence was found among African American compared with non Hispanic whites. This ethnic variation was independent of known risk factors such as diabetes, hypertension and obesity (Selvin and Erlinger 2004). Bangladeshis in New York, USA had more extensive and severe heart disease with 53% having triple vessel disease compared to 26% among whites (Silbiger *et al.* 2011). Bangladeshis appear to share with other South Asian populations the same susceptibility to CAD but studies involving the immigrants in abroad have found that,

Bangladeshis are even more prone to develop CAD among the south Asians and are associated with higher morbidity and mortality related to CAD. The probability of existence of an even more prone 'Bangladeshi ethnicity' is possible. Complex interaction between genetic make-up and environmental factors may underlie this 'Bangladeshi ethnicity'. A genetically predisposed population may explain the high prevalence of CAD in Bangladesh. Recently, 6 novel genetic loci have been identified in South Asians, which are associated with type 2 diabetes mellitus (DM), a major risk factor for CAD (Kooner *et al.* 2011). The association between ACE gene polymorphism and blood pressure in Bangladeshi population has been studied by Chowdhury *et al.* (1998) and Morshed *et al.* (2002). A positive association was found between ACE insertion/deletion (I/D) polymorphism and hypertension in Bangladeshi population which is the major risk factor for atherosclerosis. Some novel gene polymorphisms have been found in PAD such as SLCZA10, PAOD1, Lsq-1 and CHRNA3 (Katwal and Dokun 2011). SLCZA 10 was found as an independent polymorphism gene that could lead diabetic patients to PAD (Jiang *et al.* 2010). Significant rules of genetic polymorphisms have been reported in recent studies. Therefore PAD is a genetics and environmental interaction disease with same risk factors of CAD (Ghasemi *et al.* 2015).

Reports of PAD prevalence according to gender are also conflicting. (Table 32). The prevalance was 16.9% and 20.5% in men and women respectively, reported by Vavra and Kibbe (2009) but in a study by Diehm *et al.*(2004), the overall prevalence among male and female was just opposite. They found PAD 19.8% in man and 16.8% in women. Mahameed (2009) reported that PAD affects men and woman equally. In this study high male propensity

was revealed. The explanation might be that this study was occurrence of PAD among CAD patients and CAD has definite cause of higher male involvement. However, Vavra and Kibbe (2009) suggested that despite some conflicting reports, overall PAD prevalence appeared to be higher in men. Findings in present study also agree with this suggestion. Sex hormones play a major role in the pathogenesis of atherosclerosis. Testosterone increases atherosclerosis in males and on the other hand estrogens are protective and prevent or delay atherosclerosis (Vitale *et al.* 2009, Kaushik *et al.* 2010). Between 70% and 89% of sudden cardiac events occur in men. (Roger *et al.* 2012).

The prevalence of PAD reported by many authors show that the prevalence is also highly age dependant. It increases with age. Even after adjusting for traditional risk factor in a multi-variable cardio-vascular disease prediction model, age remains a fundamental predictor of cardio-vascular disease risk. (Dhingra and Vasan 2012). It was also thought that the contribution of age in the multi-variable models may be due to increased intensity and the duration of exposure to others traditional risk factors of cardio-vascular disease. In present study mean age of the entire study people was 51.3 ± 10.4 years and mean age of PAD was 54.6 ± 10.7 years ie. mean age of PAD was higher. A gradual increase in percentage among age group was noticed in patients having PAD. Of the patients who are from third decade, 12.5% suffered from PAD, on the other hand 33.3% patents of 7^{th} decade suffered from PAD. Some previous studies also found similar trend. In patient younger than 60 years of age, the prevalence was approximately 03% and 15% to 20% in patients over 70 years of age (Vavra and kibbe 2009). In a survey conducted by Criqui (2001) the prevalence of PAD was 18.8% in people >

70 years of age. Dormandy *et al.* (1989) found prevalence of PAD 1%-2% for man age below 50 years and above 50 years the prevalence was 5%. Lower extremities peripheral arterial disease (PAD) is a highly prevalent condition in the elderly (Lloyd *et al.* 2010).

The increase in prevalence of PAD with age is more noticeable in woman. In patients younger than 70 years of age, the prevalence of PAD was 11.5% in woman but over 85 years of age, PAD prevalence increased to 39% in woman compared with only 27% in men (Diehm *et al.* 2004). The incidence of cardiovascular disease increases in women as estrogen declines with age and menopause. An elevated free testosterone level may be a risk factor for coronary atherosclerosis. (Phillips *et al.* 1997)

But the alarming finding was the involvement of younger age group. 11.4% of total study population had age below 40 years and 39% below 50 years. This premature nature of the disease was quite unheard of in this country 25 years ago (Munwar 2012). It is increasingly affecting younger groups in their 40s. In this study it was found that family history of CAD was statistically significantly associated with CAD patients below age of 40. Presence of diabetes mellitus, hypertension, smoking habit and dyslipidemia ware also noticed in higher percentage in patients younger than 40years though statistically not significant.

Although it is general saying that cardiovascular disease is a disease of rich people or people from upper socio economical status (Munwar 2012), in this study it was found that 46% of patient coming from poor group and 2.4% of them had the income below the poverty line. So it can not be said that it only

affects rich people. Number of rich people in the present study was only 3.8%. However one reason of so low population from rich may be that this study was done in public hospital run by the government. Rich people have the tendency to avoid this type of hospital and they usually go to corporate hospital for their treatment. So the economic status of the study population done in a public hospital does not always reflect the exact situation. But it can be said that poor people are not risk free.

Economic status of people having PAD reflects more or less similar picture that was seen in total study population.

Life style change, control of blood pressure, diabetes mellitus, and dyslipidemia are important controllable factor for controlling the disease. Literacy level of the patient plays an important role in awareness of this life style change program. About one third of study group and one fourth of PAD patient had very low level of education, below the primary level. Surprisingly when history about risk factors like past history of hypertension, diabetes and hyperlipidemia, were asked, 7.6% patient told that they never checked their blood pressure in their life and a large number, 45.2% never checked their lipid profile in their life. After checking lipid profile it was found 45% of them had got dyslipidemia though they were not at all aware of this. Among this group who did not check their lipid profile ever in their life, 61.4% had got education below high school level. This finding reflects the impact of education level in controlling risk factor for cardiovascular disease. Higher risk associated with lower levels of education was also described by Winkleby *et al.* (1992). They said that among three factors of socioeconomic status: education, income and occupation, the relationship between education and cardiovascular risk factors were

strongest and most consistent. During the past 30 years, the United States experienced large reductions in the prevalence of several cardiovascular disease risk factors, including hyperlipidemia, blood pressure, and smoking. An education and income related disparity in smoking was noticed. There were large declines in smoking prevalence among people with high incomes and education but only marginal reductions among those with low incomes and education. Diabetes prevalence increased most among persons with low incomes and education (Kanjilal *et al.* 2006).

Zaman *et al.* (2007) found out the prevalence of IHD in a rural population of Bangladesh. They found the prevalence is 3.4% in rural population in 2007. This study revealed 60% patients of CAD were from rural population and 58.1 % of PAD were from rural population. So it appears that atherosclerotic coronary and other arterial disease is an important problem in rural population of Bangladesh and seems to be much higher from that was in 2007. Higher prevalence of CAD in rural populations was also observed in USA. 38.8% higher among respondents living in rural areas compared with urban areas was observed. (O'Connor and Wellenius, 2012)

A study by Sayed *et al.* (2010) in ten villages of Mymensing district was done to find out the risk factors related CAD and PAD. They showed that adjusted for age, social class and obesity, the subject with higher age, higher blood sugar level and family history of heart disease had significant risk of CAD. In addition life style change in village people may be a cause. Over the years the consumption of rice and wheat has decreased among village people in Bangladesh, resulting in overall decrease in cereal consumption from 59% to 41.33% as a percentage of monthly expenditure on major food

items (Hossain MM, cited in Monwarul and majumder 2013). This may contribute to hypertriglyceridemia. On the other hand liberal amount of cooking oil is used during preparation of traditional Bangladesh cuisine. People are working outside and staying away from home. They are adopting habit of taking traditional fast foods like Singara, Samucha, Puri, Paratha, containing high amount of cholesterol and saturated fatty acids, mainly derived from animal fats and palm oil (Nessa *et al* 2002). Like city dwellers they are also now more likely to be exposed to marketing schemes and advertisements for unhealthy processed foods and tobacco. Soft drinks, artificial fruit juice, western type of fast food have become more accessible and they are gaining sugar and fat from those.

The association between PAD and smoking has been shown in number of studies. Heavy smokers have a four-fold higher risk of developing PAD compared to non-smokers. In the study by Norman *et al.* (2006) smoking was found to be more prevalent in the PAD group than in the non PAD group (24% Vs 12.6%). Norgen *et al.* (2007) stated that PAD shares risk factors with those for other cardiovascular disease but smoking is more powerfully associated with PAD than other cardiovascular diseases. He *et al.* (2006) mentioned cigarette smoking as a major risk factor for PAD in china. Present study showed percentage of smoker was higher in PAD if compared to non PAD group but statistically was not very significant.

Relative risk of PAD was several folds higher in ex smoker than in those who had never smoked and the risk increased to 16 fold in current smoker compared with those who had never smoked (Cole *et al.* 1993). A dose response relation between the number of cigarettes smoked and increasing

risk of PAD was reported by the same author. Quitting for ≥ 10 years nearly eliminated excess risk associated with smoking. This study could not compare this finding as all the patients in present study were recent smoker. None of them had the history of quitting for 10 years or more than 10 years.

From the history it was found that only two patients had intermittent claudication. But 10 patients were found having critical stenosis involving one or more segments of lower limbs arteries. Criqui (2001) stated that prevalence of IC in people more than 50 years were 2% to 7% in men and 1.2% in woman, which underestimates the presence of PAD. PAD is two or five times more common than is suggested by a history (Hilleman 1998; Fowkes 1988; Criqui *et al.* 1985). This study also revealed that 8 out of 10 patients (80%) did not complain any leg symptoms though they had stenosis in leg arteries. This result is in good agreement with those reports. PAD prevalence among asymptomatic adults 40 years and older significantly increased from 3.7 percent in the 1999-2000 survey to 4.2 percent in the 2001-02 survey and 4.6 percent in the 2003-04 survey. (American Heart Association 2007). The prevalence of IC was 2.2% in men and 1.2% in women. (Meijer 1998). It is estimated that as many as ten million people have PAD in USA but only four million presents with IC. (Golomb *et al.* 2001)

Relation of PAD and CAD severity

Number of coronary vessels involvement is a prognostic factor of CAD. Burggraf and Parker (1975) subdivided the CAD into three major categories viz: single vessels, two vessels and three vessels disease. Later on this type of classification was described by many authors to indicate the severity of

the disease (Geroulakos *et al.* 1994, Kallikazaros *et al.* 1999, Ziembicka *et al.* 2004,Ogata *et al.* 2005). Involvement of PAD in different aspect with number of coronary vessels involvement was checked in present study. This study showed a gradual increase in number of PAD patient with number of coronary arteries involvement. Percentage of PAD sufferer among triple vessel disease was higher than that single vessels disease. But when individual carotid and lower limb arteries were considered the picture was different. No association of independent carotid and lower limb arteries stenosis was seen with number of stenosis in coronary arteries.

The report from Kallikazaros *et al.* (1999) and Ziembicka *et al.* (2004) showed a linear progressive increase in carotid involvement with number of vessels involved. In this study number of carotid involvement was higher in three vessels disease than one and two vessel disease but two vessel diseases did not show higher involvement of carotid vessels than one vessel disease (Table 38).

Table 38: Carotid Artery involvement found in different studies

	Kallikazanes *et al.*	Ziembicka *et al.*	This Study
One vessel disease	5.3%	3.8%	12.3%
Two vessels disease	13.5%	8.6%	10.0%
Three vessels disease	24.5%	16.6%	17.9%
LMCA Involved	40%	N.A	25%

Involvement of left coronary artery was also compared to the involvement of PAD. The study showed involvement of PAD was not significantly

associated with involvement of left main coronary artery involvement but the percentage of PAD involvement was higher in patients with LMCA involvement if compared to percentage of involvement among total study population. Twenty five percent of patients who had LMCA involvement had got PAD. Distribution of carotid and leg involvement among LMCA involved patients was equal. No significant relation was found between LMCA involvement and isolated carotid arteries involvement, LMCA involvement and isolated lower limb arteries involvement.

Kallikazaros *et al.* (1999) also compared triple vessel disease with left ventricular ejection fraction and found incidence of triple vessel disease was 24% and 63% in the normal and impaired ejection fraction subgroups respectively. Thatipelli *et al.* (2007) observed no relation between PAD and LV wall motion index. Maldonado *et al.* (2008) reported inverse relation between PAD involvement and left ventricular mass. No association of EF with total PAD involvement or isolated carotid or lower limb artery stenosis was found in present study. Present study also failed to find any significant association between low EF and carotid IMT.

Carotid Intima Media Thickness

There is a growing belief that carotid IMT can be regarded as an indicator of generalized atherosclerosis (Grobbee and Bots 1994). Several cross-sectional studies have shown that increased common carotid intima-media thickness may be used as a marker of atherosclerosis elsewhere in the arterial system (Bots *et al.* 1994, Polak *et al.* 1993).

A significant increase in IMT was observed among patients with one, two and three vessels CAD by Ziembicka et al. (2004) and Geroulakos *et al.* (1994). In the present study a linear trend between IMT and severity of CAD was also found but mean IMT for each vessel group was lesser than that was found by other authors. Table 39 shows the comparative result of this two studies and present study. In this study mean IMT values was lesser but mostly nearer to the findings of Geroulakos *et al.*(1994) and far different from the values given by Ziembic *et al.*(2004).

Table 39 : Mean IMT with number of coronary arteries stenosis in different studies including the present study.

	Geroulakos *et al.*	Ziembic *et al.*	This Study
Single vessel	0.9±0.17	1.15±0.2	0.79±0.22
Double vessel	0.96±0.17	1.26±0.2	0.93±0.21
Triple vessel	0.99± 0.21	1.4±0.3	0.95±0.22

Ziembicka *et al.* (2004) stated that if mean IMT was over 1.15 mm, patient had 94% probability of having CHD. This study figured out number of patients having IMT 1.15mm or greater and it was found that only 15% of CAD patients had IMT > 1.15 mm.

The study by Geroulakos *et al.* (1994) was a case control study and the mean carotid IMT for the controls was 0.71 ± 0.16 mm and for the CAD patients was 0.91 ± 0.18 mm. Ziembicka *et al.* (2004) mentioned mean carotid IMT for patients with normal coronary arteries 1.01±0.19 mm. But normal carotid artery IMT thickness was considered 0.8 mm by many authors (Pignoli *et al.* 1986; de Groot 2008; Naqvi and Lee 2014) the value above which indicates

the process of atherosclerosis. This study found 60.2% patient had IMT < 0.8 mm but had significant coronary artery blockage. All the previous studies were done on western people, which may not be feasible for population in Bangladesh as the average body built differs from that of western people. But no such study has been done in Bangladesh as far as it is known. A further large case control study is needed to determine the normal value of IMT in the population of this country.

Jarauta *et al.* (2010) did a study on potentially healthy individuals who had no cardiovascular risk factors. The carotid IMT in subjects without traditional risk factors was strongly dependent on age. The upper limit of normal was 0.59 mm in those under 25 years to 0.95 in those aged over 65 years. In this study coronary IMT was correlated with increase of age (person's correlation coefficient= 0.254, $P < 0.001$).

Matsuchima *et al.* (2004) did a study on 124 patients and found out the correlation between CAD and carotid IMT, pulse wave velocity and the ankle brachial index (ABI). They found relatively good correlation between carotid IMT and Genesini score but no correlation with the ABI. Bots *et al.* (1997) also provided evidence that an increased common carotid IMT is associated with future cardiovascular and cerebrovascular events but the level of IMT does not reflect the presence of arterial stenosis in the CCA and the arterial blood flow in these subjects was virtually normal. This study also did not find any statistically significant association between carotid IMT and carotid artery stenosis though the mean of carotid IMT was higher in the group with carotid artery stenosis in comparison to those without carotid stenosis. (1.0±0.18 vs 0.82±0.23).

Many authors stated that an increased cross-sectional carotid intima-media thickness was associated with established cardiovascular risk factors,(Heiss

et al. 1991; Salonen and Salonen 1991; Walter *et al.* 1992). In the present study significant association with hypertension and dyslipidemia was found but not with diabetes and smoking though in both cases mean IMT were higher than in non diabetic and non smoker patients.

Ziembicka *et al.* (2004) determined four independent predictors of CAD. Age, dyslpidemia, smoking and IMT. Thus IMT may be considered another risk factor for CAD. Measurement of IMT of carotid arteries corresponds to the histological intima and media (Jadhav 2001; Homma *et al.* 2001).
Treadmill testing and echocardiography have limited specificity in diagnosing CAD.(Hill and Timmis 2002, Iliceto *et al.* 1994). The development of Doppler US machine facilitates comprehensive analysis of IMT. IMT is commonly recognized as the initial stage in the development of atherosclerosis.

However, whether increased IMT itself reflects local atherosclerosis is still a subject of debate. (Bots *et al.* 1997). Atherosclerosis is a disease of intima. Ultrasound image can not differentiate intima and medical layers. Hence it is called intima-media complex. (Stary *et al.* 1992). The thickening of this complex reflects an adaptive response of the vessel wall to changes in shear stress, tensile stress and blood flow and subsequent change in lumen diameter (Glagov *et al.* 1987).
Several cross sectional studies have shown that increased carotid IMT may be use as a marker of atherosclerosis elsewhere in the arterial system. (Bots *et al.* 1993; Polak 1993). In the present study a significant association between increased IMT thickness and PAD involvement was recorded.

Risk Factor

Risk factors for atherosclerosis have been well defined. Increasing age, tobacco smoking, hypertension, diabetes mellitus, dyslipidemia, sedentary life style and obesity are well recognized risk factors for atherosclerosis and responsible for developing CAD, PAD and cerebrovascular diseases. This study tried to find out whether certain risk factors are responsible to develop synchronous PAD and CAD.

All the PAD patients of this study had one or multiple above mentioned risk factors. If it is compared to patients having synchronous CAD and PAD with patients only CAD without PAD, it was seen that red meat in diet, obesity, high serum level of total cholesterol and triglyceride, presence of hypertension and diabetes mellitus were statistically significantly associated with PAD. There was no significant difference of serum LDL and HDL level between PAD and the non PAD group.

Some previous studies, Walter *et al.* (1992) and Mohan *et al.* (1995) also found association between serum total cholesterol and PAD. On the other hand Agarwal *et al.* (2012) reported no significant differences between total cholesterol, LDL, HDL or triglyceride levels between PAD and Non PAD group.

Numerous studies have demonstrated an association between diabetis mellitus and the development of PAD (Stout 1990, Widmer *et al.* 1985, Gordon and Kannel 1972). Agerwal *et al.* (2012) stated that PAD is one of the macrovascular complication of type 2 diabetes mellitus. They found

duration of diabetes mellitus, an HbA1c greater than 7% was a significant predictor of PAD. Dieter *et al.* (2003) and Eagle *et al.* (1994) also mentioned association between diabetes and PAD. Persons with diabetes have a sevenfold higher rate of lower extremity amputation than persons without diabetes (Jonason and Ringqvist 1985; Hughson *et al.*1978)

Dieter *et al.* (2003) found older age, greater smoking history, diabetes mellitus, hypertension and dyslipidemia were risk factors of developing PAD. Eagle *et al.* (1994) mentioned PAD patients were more likely to have hypertension, diabetes, family history of CAD, previous history of CAD and smoking.

Luo *et al.* (2007) reported older age, female gender, elevated triglyceride level, low HDL, diabetes mellitus and smoking were associated with PAD. Present study result agrees in most points of finding to those result. Although advanced age and smoking was not statistically significant for development of PAD in present study, but percentage of smoking was much higher in PAD group and increased incidence of occurrence in old age was also noticed in this study.

CHAPTER 6

CONCLUSION, LIMITATION AND RECOMMENDATIONS

CHAPTER 6

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5.1 Conclusion:

This study provided a wide range of information on the occurrence of PAD among CAD patients, pattern of PAD involvement in CAD patients, relation of PAD involvement with the severity of CAD, role of risk factors in development of CAD and PAD patients. Though this was a cross-sectional study, yet attempt was taken to find out the real picture of the CAD sufferer in the regarding factors mentioned above. Duplex ultrasound was used to determine the extent of involvement of PAD.

The major arteries which are commonly involved in PAD were examined. On the basis of findings on 2550 segments of peripheral arteries of 210 CAD patients, the occurrence of PAD was 20.4%. The study uncovered that most of the patients of PAD were asymptomatic and were diagnosed for the first time after enrollment in study. So Duplex US can be considered as a useful method to diagnose PAD before they come into a critical stage to develop signs and symptoms. Even patients who had stenosis above the critical level, they did not have remarkable signs or symptoms.

Carotid arteries were more involved than the lower limb arteries. Most of the patients had isolated carotid or lower limb involvement. A very few cases had multiple involvements.

Total number of involvement of PAD increased with severity of coronary artery disease, i.e. with number of coronary artery involvements, though isolated carotid and lower limb arteries stenosis were not related to severity of coronary artery disease. The study revealed that severity of PAD was also not related to severity of coronary artery disease.

Measurement of CIMT can be a good predictor of severity of coronary artery disease as a linear upwards trend of increasing the mean CIMT was seen with number of coronary vessels affected. IMT of internal carotid artery did not show such a relation.

Risk factors significantly associated with PAD were habit of taking regular red meat, hypertension, diabetes, high serum cholesterol level, high serum triglyceride level and obesity. Diabetes and high serum cholesterol level were significantly associated with severity of PAD. Positive family history of heart disease was associated with CAD involvement in younger age group below 40 years. Independent predictor factors for PAD were red meat diet, hypertension, diabetes mellitus, high BMI and high serum triglyceride level.

5.2 Limitation of the study

To see the burden of CAD the designation of single, double, triple vessel and involvement of left main coronary artery was used. This simple scoring system is limited in its ability to stratify patient with different level of disease risk. A more comprehensive scoring system can be done on the basis of Intravascular ultrasound (IVUS) findings (Gensini 1983). IVUS is a powerful tool for the evaluation of atherosclerosis and is more accurate and

reproducible than coronary angiography for the assessment of atherosclerotic burden, because unlike angiography it measures wall atheroma and not just luminal encroachment (Nicholls *et al.* 2006, Tobis *et al.* 2007). IVUS is still not available in Bangladesh.

Regarding risk factors smoking, diabetes mellitus, hypertension, dyslipidenia , obesity, physical activity and family history were only assessed. Some other risk factors like plasma homocystene levels, hypothyrodism, microllbuminuria were not included in this study due to limited laboratory facilities during the study.

History regarding diet was taken in a very simplified way. No categorization was done between processed and unprocessed red meat intake. No cumulative average of food intake was measured.

Search was confined only on carotid and lower limb arteries in this study. Upper limb arteries, renal arteries, mesenteric arteries were not included in this study. A further extensive study on all the peripheral arteries may be conducted to say exact nature of extra coronary artery involvement in CAD patients.

5.3 Recommendation

The recommendation from the findings of present study is that probability of PAD should not be ruled on the basis of history of intermittent claudication only. Although intermittent claudication is the classic symptom of PAD, the vast majority of those affected are asymptomatic. As PAD is part of the

atherosclerotic disease process and can coexist with CAD and cerebrovascular disease, a screening for PAD by duplex ultrasound should be done in all CAD and cercbrovascular patients. Screening of CAD patient for PAD with duplex ultrasound should be a routine procedure.

With the social change in recent years, Bangladesh is now experiencing a fast food culture, both western styles and traditional styles. Research is needed to elucidate the role of dietary issues in causation of atherosclerosis.

The association between atherosclerosis and role of genetic factor in Bangladeshi people has not been studied enough. Research on genetic risk factors predisposing to atherosclerosis is needed.

Identifying CAD at an early stage is important to prevent fatal events. Carotid IMT is a highly reproducible technique for quantifying atherosclerotic burden. Routine use of this technique can improve our ability to decide on preventive steps to reduce the development of sudden serious catastrophic cardiovascular events. A normal value of IMT for Bangladeshi people is still not known. A case control study in a large scale on this matter will be helpful to predict atherosclerosis for the people of this region.

This study was on prevalence of PAD on CAD patients. Further study to see the prevalence of CAD on PAD patient is recommended to find out the exact correlation between two diseases.

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APPENDICES

Appendix i: Letter to patients asking consent for participation in the study (In local language : Bangla).

জনাব,

আমি জেনে দুঃখিত যে, সম্প্রতি আপনার হৃৎপিন্ডের ধমনীতে এক বা একাধিক ব্লক ধরা পড়েছে। তবে আশার কথা এই যে, বর্তমান উন্নতি প্রযুক্তির কারণে আপনার বড় কোন ক্ষতি হবার আগেই এই অসুবিধা নির্ণয় করা সম্ভব হচ্ছে এবং ভবিষ্যতে অন্য কোন সমস্যা হবার আগেই তা প্রতিহত করার ব্যবস্থা নেয়া সম্ভব হবে।

আপনি হয়তো জানেন যে, অতিরিক্ত ধূমপান, রক্তচাপ, রক্তে চর্বির অত্যাধিক মাত্রা ইত্যাদি কারণে হৃৎপিন্ডের ধমনীতে এক ধরনের প্রলেপ পড়ায় ধমনী সরু হয়ে যায় এবং ক্রমান্বয়ে ব্লক হয়ে পড়ে। ধারণা করা যেতে পারে যেহেতু হৃৎপিন্ডের ধমনীতে এই ঘটনা ঘটেছে, শরীরের অন্যান্য ধমনীতেও একই ঘটনা ঘটতে পারে এবং আপনার শরীরের ও অন্যান্য ধমনীতে এই ঘটনা ঘটেছে কিনা তা কে বলতে পারে।

আমি সম্প্রতি একটা গবেষণায় লিপ্ত হয়েছি যে, যাদের হৃৎপিন্ডে ব্লক ধরা পড়েছে তাদের শরীরের অন্যান্য ধমনীতে কোন ব্লক আছে কিনা এবং থাকলে কতখানি ক্ষতিকর তা নির্ধারণ করা।

আমি আপনাকে এই গবেষণায় অংশগ্রহণ করার জন্য বিনীত অনুরোধ জানাচ্ছি। আপনি সম্মত থাকলে আমি ডপলার আল্ট্রাসনোগ্রাফির মাধ্যমে আমি আপনার শরীরের বিশেষ কিছু মূল্যবান ধমনীর রক্ত চলাচল সংক্রান্ত পরীক্ষা করব। এই একটা ব্যথাহীন পরীক্ষা, যা ১৫ থেকে ৩০ মিনিট সময় নেবে। কোন ইনজেকশন দেবার প্রয়োজন নেই। কোন রেডিয়েশন ব্যবহার করা হবে না। এর কোন পার্শ্বপ্রতিক্রিয়া নেই। এই পরীক্ষার কোন খরচ আপনাকে বহন করতে হবে না। আমি আপনার স্বাস্থ্য সম্পর্কে হয়তো কিছু প্রশ্ন করব যা সম্পূর্ণ গোপনীয় দলিল হিসেবে বিবেচিত হবে।

এই পরীক্ষায় অংশগ্রহণের জন্য আপনি একটি সুবিধা পাচ্ছেন যে, আপনি নিজে আপনার শরীরের অন্যান্য ধমনীর অবস্থা সম্পর্কে আগাম তথ্য পাচ্ছেন এবং দুর্ভাগ্যজনকভাবে যদি কোন খারাপ অবস্থার পূর্বাভাস পাওয়া যায় তাহলে আগে থেকেই ব্যবস্থা নেবার সুযোগ পাচ্ছেন।

আপনি সম্মতি থাকলে আমি আপনাকে পরীক্ষা-নিরীক্ষার সম্ভাব্য সময় জানিয়ে দেব।

জয়দীপ ভাদুড়ী
পি.এইচ.ডি ফেলো
ই,বা,সা,
রাজশাহী বিশ্ববিদ্যালয়,
রাজশাহী।

Appendix ii

INFORMED CONSENT FORM

(In local language : Bangla)

গবেষণা কর্মে অংশগ্রহণের সম্মতি পত্র

আমি নিম্নস্বাক্ষরকারী সজ্ঞানে সম্মতি প্রদান করছি যে, উপরোক্ত গবেষণামূলক কর্মকাণ্ডে অংশগ্রহণে আমি রাজি আছি। আমাকে গবেষণার বিষয়বস্তু, পদ্ধতি, তার সুবিধা-অসুবিধা, পরীক্ষা-নিরীক্ষার ধরন সবকিছু ব্যাখ্যা করা হয়েছে। আমাকে আরও জানানো হয়েছে গবেষণা চলাকালে যে কোন মুহূর্তে আমার সম্মতি এবং অংশগ্রহণ প্রত্যাহার করার সুযোগ আমার রয়েছে। আমাকে এই মর্মে আশ্বস্ত করা হয়েছে যে, এই গবেষণায় অংশগ্রহণে আমার কোন শারীরিক ক্ষতি হবার সম্ভাবনা নেই।

উল্লেখিত বিষয়ে বিস্তারিত জেনে আমি সজ্ঞানে এবং স্বেচ্ছায় এই গবেষণায় অংশগ্রহণের সম্মতি প্রদান করলাম।

নাম:

ওয়ার্ড: বেড:

রাজশাহী চিকিৎসা মহাবিদ্যালয় হাসপাতাল
হৃদরোগ বিভাগ

Appendix iii : Letter to the patients asking consent for participation in the study (English version).

Sir

We are sorry to know that some blockages have been detected in the artery of your heart. But the good news is, because of advanced technology it has been detect very early before it could have caused you any harm. Now it is also possible to take further necessary action before anything serious problem may occur in the future.

You may know that, too much smoking, high blood pressure, diabetes, sedentary life style and excess level of cholesterol in blood cause a deposit of a layer in the artery of the heart which causes the artery to become narrower and eventually blockage takes place.

It is estimated that since the incidence is taking place in the artery of the heart, the process might also take place in the other parts of your body and who knows if such things have happened in the other arteries of your body as well.

I have been under a research program where I would like to study if the people who have blockage in their arteries of heart may have blockage in any other arteries of their body as well and if they have, what is the extent of the disease and how harmful it is.

I cordially request you to take part in my research program. If you allow, I shall examine the blood flow in some of the important arteries of your body through Doppler ultrasonography. This is a painless procedure which will take 30-45 minutes. Some water based gel like substance will be applied over the skin of

your neck and entire thighs and legs which might be little inconvenient to you. The gel is non allergic, non sticky and easily washable. There is no need for any injection or radiation for the procedure and it does not cause any side effect. Yes, I would like to get your blood sample to test your cholesterol level, diabetic status and some other relevant tests. About 5ml blood will be taken from your vein for the purpose. You don't have to bear any cost for this. I might have some enquiries regarding your health which will strictly be kept confidential.

You will get a benefit from this study and that is you'll get some advanced information about the current state of the arteries of your body and if, unfortunately, any problem can be detected, preventive steps can be taken before it gets worst.

You will have full right to refuse to participate or withdraw your consent and discontinue participation in the study at any time during the procedure.
If you allow, I shall let you know the probable time schedule for the test.

Sincerely yours

Joydeep Bhaduri
PhD Fellow,
Institute of Biological Sciences,
University of Rajshahi, Rajshahi,
Bangladesh.

Appendix iv

INFORMED CONSENT FORM

Consent for participation in the research work : Duplex Ultrasonographic Studies on Peripheral Arteries in Patient Having Coronary Artery Disease.

I have been explained clearly about the reason for doing this study, reason for selecting me as a subject in the study. I also have been explained about the risks, benefits and confidentiality of the study. I am willing to attend the investigation procedure for the purpose of the study. Freedom is given to me for the participation in the study or to discontinue participation at any time without prejudice.

All the above explained in detail to me clearly in my own language. I am giving consent voluntarily for inclusion of me in the study as a subject.

Name and signature of the Participant

Ward No- Bed no-

Cardiology Department,

Rajshahi Medical College Hospital,

Rajshahi, Bangladesh.

Appendix v : Questionnaire and Data Collection Form :

Duplex Ultrasonographic Studies on Peripheral Arteries in Patient Having Coronary Artery Disease .

Si No : **Date :**

A. Particulars of Patient :

Name : Hospital/Clinic Reg. No:

Age:

Sex : 1.Male 2.Female ☐

Address .

B. Personal History :

Marital status 1.Married 2.Unmarried ☐

Occupation 1.Service holder 2.Retired from service 3.Unemployed

4. Housewife 5. Laborer 6.Farmer 7. Businessman 8. Professional 9. Others ☐

Smoking / Tobacco - 1.No 2.Yes ☐

Pack year ☐

Quite for yrs. ☐

Alcohol Consumption 1. No 2.Yes, Occational 3.Yes, Regular ☐

Dietary Habit 1.Vegitarian. 2. Non-vegetarian but occasional red Meat eater.

3. Regular Red Meat eater ☐

Economical Status :

1.Below poverty line (<3000tk/month) 2.Poor (3000-10000tk/month) ☐

3.Middle Class (10000-20000tk/month) 4. Riich (>20000tk/month)

Literacy Level : ☐

1.Illiterate 2. Up to primary level of education.

3. Primary to High School. 4. Above high school level.

Physical Activity : 1.Sedentary 2. Moderate 3.Hard / Manual labor ☐

Regular exercise 1.Walking 2. Swimming 3.Jim 4.Others 5.No ☐

C. Family History of heart disease : 1.Yes 2.No

D. Past History:

History of Hypertension	1.No	2.Not Known	3.Yes ,
History of Diabetes	1. No	2. Not Known	3.Yes
History of Dyslipidaemia	1. No	2.Not Known	3.Yes

E. Relevant Complaints or Symptoms:

Central Chest Pain :	1.Yes :	2.No :
Sweating :	1.Yes :	2.No :
Breathlessness	1.Yes :	2.No :
Vertigo	1.Yes :	2.No :
Leg pain	1.Yes :	2.No :
Intermittent Claudication	1.Yes :	2.No :
Leg Ulcer	1.Yes :	2.No :

F. Clinical Examination Findings :

Weight in kg: .....

Height in cm:.....

Waist :.....

Hip :.....

Waist/Hip ratio :.....

Pulse Rate :..... per minute

Blood Pressure: Systolic.....mm of Hg

Diastolic.....mm of Hg

Carotid Bruit		1. Present	2.Absent
Peripheral Pulse :	Femoral	1. Present	2.Absent
	Popliteal	1. Present	2.Absent
	Dorsalis Pedis	1. Present	2.Absent

F. Investigations

Fasting Blood Sugar .....mmol,

ECG abnormalities : 1. Non significant 2. Acute change 3.Old MI

ETT findings : 1.Significant 2.Non significant 3.Not done

Lipid Profile

Total Cholesterol	..... mg/dl	1.Normal	2.High
TG Cholesterol	..... mg/dl	1.Normal	2. High
LDL Cholesterol	..... mg/dl	1.Normal	2.High
HDL Cholesterol	..... mg/dl	1.Normal	2.Low

Echocardiography :

LVID : ..... mm

LVIDS : ..... mm.

EF ..... %

G . Coronary Angiogram Findings :

Name of arteries involvement		**Site of involvement**		**Degree of involvement**
LMCA : Y/N -1/2		*1.Proximal. 2 Middle. 3.Distal*		%
LAD : Y/N -1/2		*1.Proximal. 2 Distal. 3. Distal*		%
LCX : Y/N -1/2		*1.Proximal. 2 Distal. 3. Distal*		%
RCA : Y/N -1/2		*1.Proximal. 2 Distal. 3. Distal*		%

H. Duplex Ultrasound findings of peripheral arteries :

Right Common Carotid Artery:

Intima Media Thickness

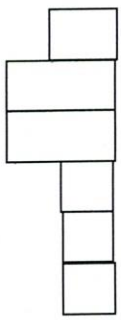
PSV

EDV

Presence of plaque

Degree of stenosis

Wave pattern 1.Normal 2.Turbulence



Left Common Carotid Artery:

Intima Media Thickness

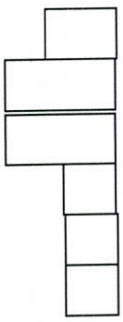
PSV

EDV

Presence of plaque

Degree of stenosis

Wave pattern 1. Normal 2. Turbulence



Right Internal Carotid Artery:

Intima Media Thickness

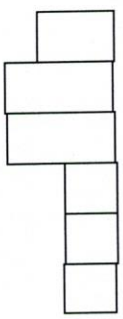
PSV

EDV

Presence of plaque

Degree of stenosis

Wave pattern 1.Normal 2.Turbulence



Left Internal Carotid Artery:

Intima Media Thickness

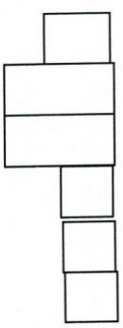
PSV

EDV

Presence of plaque

Degree of stenosis

Wave pattern 1. Normal 2.Turbulence



Right Common Femoral Artery

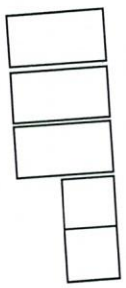
PSV

RI

PI

Wave pattern 1.Normal 2. Turbulence

Degree of stenosis



Left Common Femoral Artery

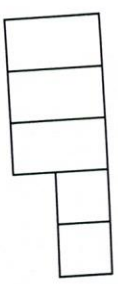
PSV

RI

PI

Wave pattern 1.Normal 2. Turbulence

Degree of stenosis



Right Popliteal Artery

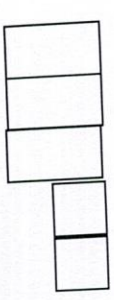
PSV

RI

PI

Wave pattern 1.Normal 2. Turbulence

Degree of stenosis



Left Popliteal Artery

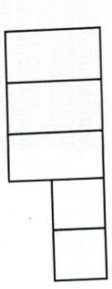
PSV

RI

PI

Wave pattern 1. Normal 2.Turbulence

Degree of stenosis



Right Anterior Tibial Artery

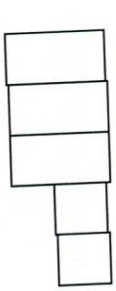
PSV

RI

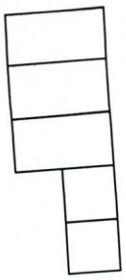
PI

Wave pattern 1.Normal 2. Turbulence

Degree of stenosis



Left Anterior Tibial Artery



PSV

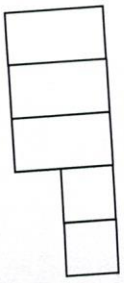
RI

PI

Wave pattern 1. Normal 2.Turbulence

Degree of stenosis

Right Posterior Tibial Artery



PSV

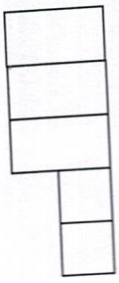
RI

PI

Wave pattern 1.Normal 2. Turbulence

Degree of stenosis

Left Posterior Tibial Artery



PSV

RI

PI

Wave pattern 1. Normal 2.Turbulence

Degree of stenosis

Signature of the Investigator