STUDY OF MAST CELL PROFILE IN ATHEROSCLEROTIC LESION OF AORTA: AN AUTOPSY STUDY

By

Dr. SULAGNA MANNA

DISSERTATION SUBMITTED TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, KOLAR, KARNATAKA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF MEDICINE IN PATHOLOGY

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ABBREVIATIONS

ABCA-A1	→	Adenosine Binding Cassette Transporter A1
AHA	→	American Heart Association
ATP	→	Adenosine Triphosphate
BFGF	→	Basic Fibroblast Growth Factor
BP	→	Blood Pressure
cm	→	centimetre
CETP	→	Cholesteryl Ester Transfer Protein
COX	→	Cyclooxygenase
CTK	→	Cytokines
CK	→	Chemokines
CRP	→	C-Reactive Protein
ECM	→	Extracellular Matrix
FcεR1 Fc	→	epsilon receptor 1- high affinity Ig E receptor
FGF	→	Fibroblast derived Growth Factor
GM-CSF	→	Granulocyte Macrophage Colony Stimulating Factor
GNRH	→	Gonadotropin Releasing Hormone
H & E	→	Hematoxylin and Eosin
HCL	→	Hydrochloric Acid
HDL	→	High Density Lipoprotein
HIV	→	Human Immunodeficiency Virus
ICAM	→	Intercellular Adhesion Molecule
IDDM	→	Insulin Dependent Diabetes Mellitus

Ig	→	Immunoglobulin
IHC	→	Immunohistochemistry
IHD	→	Ischaemic Heart Disease
IL	→	Interleukin
Inf	→	Inflammatory
LDL	→	Low Density Lipoprotein
LIF	→	Leukemia Inhibitory Factor
LOX	→	Lipooxygenase
LT	→	Leukotriene
LP	→	Lipoprotein
mg/dl	→	milligrams per decilitre
MI	→	Myocardial Infarction
MIF	→	Macrophage Inhibiting Factors
mmol/dl	→	miLlimoles per decilitres
MMPS	→	Matrix MetalloProteinase
MTHFR	→	Methylene Tetrahydrofolate Reductase
NF-κB	→	Nuclear Factor-kappaB
NCEP	→	National Centres for Environmental Prediction
NGF	→	Nerve Growth Factor
NO	→	Nitric Oxide
NPAL	→	Non-progressive Atherosclerotic Lesion
PAF	→	Platelet Activating Factor
PAL	→	Progressive Atherosclerotic Lesion

PAMPS	→	Pathogen Associated Molecular Patterns
PDGF	→	Platelet Derived Growth Factor
PG	→	Prostaglandin
PLP	→	Phospholipase
PLTP	→	Phospholipid Transfer Protein
RTA	→	Road Traffic Accident
SCF	→	Stem Cell Factor
SRS	→	Scavenging Receptors
TGF alpha	→	Transforming Growth Factor Alpha
TGF beta	→	Transforming Growth Factor Beta
Th	→	T-helper cells
TLRS	→	Toll-like receptors
TNF	→	Tutor Necrosis Factor
VEGF	→	Vascular Endothelial Growth Factor

ABSTRACT

Background

Atherosclerosis is an inflammatory disease which progresses over years by the cholesterol accumulation in the intimal layer of the arterial wall and thereby forming atherosclerotic plaques. Mast cell plays a very important role in its progression. They have a very significant role in various processes through the production of potent mediators such as histamine, heparin, proteases, LT, and a broad spectrum of multifunctional CTK .Autopsy based studies are of real value when undertaken selectively, as study of atherosclerosis and its progress is difficult in living population. Mast cell count is important to analyse the atherosclerotic lesion progression and its analysis is important to benefit patients.

Objective

To compare the results of mast cell count in atherosclerotic lesion with non-atherosclerotic part of the same aorta and to correlate the results of mast cell count in atherosclerotic lesion with the grading/type of atherosclerosis.

Materials and Methods

The study was carried from February 2016 to August 2017 at The Department of Pathology, in coordination with R. L. Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College Tamaka, Kolar. It includes 70 autopsy cases in the same period. Aortic specimens were taken from the cases who underwent autopsy during this period. Thoracic and abdominal parts were assessed according to the conventional AHA

criteria. These aortic samples were processed and stained by 2% toluidine blue which is special stain for mast cells and the mast cell count was done.

Results

The number of males was 50(71%) and females were 20(29%). Age ranged from10-80 years of age with a mean age of 43.77+-16.39 years. The most common cause of death was Road Traffic Accident 21(49%) followed by poisoning 17(24%) and hanging 9(13%). On gross examination comparison of thoracic and abdominal aorta it is observed that fatty streaks 31(54.3%) are more common in thoracic aorta while plaque lesion are more common in abdominal aorta 28(51.8%).On microscopic examination it was observed that Type I,III, and Type V lesions were more common in the abdominal aorta than the thoracic aorta. Mean mast cell count in non-atherosclerotic part was of aorta was (3.4±2.4) cells/10hpf. Mean mast cell count in atherosclerotic part of thoracic aorta in Type I, II, III, IV and Type V was (3.4±2.4)/10hpf, (7.8±3.4) /10hpf, (12.7±5.1)/ 10hpf, (19.1±2.6)/10hpf and (28.2±1.09)/ 10hpf respectively. Mean mast cell count in atherosclerotic part of abdominal aorta in Type I, II, III, IV and V was (3.4±2.4)) /10hpf, (8.7±3.4))/10hpf, (13.3±5.3))/10hpf, (18.6±1.03))/10hpf and (19.1±6.7))/10hpf respectively. It is observed that as the type of the atherosclerotic lesion increases there is progressive increase in the mast cell count. This is statistically significant finding with the p value< 0.001.

Conclusion

Atherosclerotic lesions are more common in male population and the type/ grade of the lesions increase as the age advances. Increase in mast cell count is seen with the progress in the type / grade of atherosclerotic lesions in aorta.

Keywords: Atherosclerosis, Aorta, Mast cell

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INTRODUCTION

Atherosclerosis is an inflammatory disease which progresses over years by the cholesterol accumulation in the intimal layer of the arterial wall and thereby forming atherosclerotic plaques.[1] The size of the cholesterol-rich lipid core and the thickness of the fibrous cap over it are the main determinants of plaque stability.[2] The smooth muscles and the ECM produced by the cap maintains its strength and thickness.[3] A plaque lesions which are vulnerable to rupture have a large size lipid core and a thin cap which leads to acute atherothrombotic events, such as MI and stroke.[3,4] The main cause of the destabilisation of the plaque is by the mechanism of inflammation which is induced by various modified LP and exuberated by various shear stress conditions.[2] Plaque shoulder region is infiltrated by macrophages, T-lymphocytes and mast cell and are highly prone for atherosclerotic events and are prone for plaque rupture and erosion.[5,6,7]

Mast cells play a role in various biological processes such as allergic diseases, acute and chronic inflammatory disorders, fibrotic conditions, wound healing, tissue remodelling, and host responses to parasites and neoplasms.[7,8] Mast cells have a very significant role in various processes through the production of potent mediators such as histamine, heparin, proteases, LT, and a broad spectrum of multifunctional CTK.[8] Mast cell have a role to play in the inflammatory fibroproliferative process during the atherosclerotic plaque development.[9] There are several special stains and sensitive IHC techniques developed to identify mast cell that have demonstrated both increased numbers and accumulations in atherosclerotic plaque and in the adventitia of human

aortas and coronary arteries.[9,10,11] Several studies have reported that mast cells play a very significant role in the development and in the progression of atherosclerosis.[12]

Autopsy studies are of real value when undertaken selectively, as study of atherosclerosis and its progress is difficult in living population.[13] The limited ability of imaging techniques to visualise the vessel wall, its lumen and define the various stages of atherosclerosis makes autopsy as an important tool for several research purposes and future prognosis.[14,15]

Several studies have been done on role of mast cells in atherosclerotic lesions in vessels such as coronary arteries. However, very few studies have been done till date on role of mast cells in atherosclerotic lesions in major vessel like aorta. Hence this autopsy based study was undertaken in Kolar to find out the grade or the type of the atherosclerotic lesion in the population and to determine the role of mast cells in the atherosclerotic lesion.

OBJECTIVES

- To compare the mast cell count in atherosclerotic lesion with non-atherosclerotic part of the same region of aorta.

- To evaluate the atherosclerotic lesions in thoracic and abdominal aorta and correlate the mast cell count in atherosclerotic lesion with the grading/type of atherosclerosis.

REVIEW OF LITERATURE

By definition, the meaning of atherosclerosis is "hardening" of the arteries.[16,17]

The term atherosclerosis was introduced by Marchand.[18] In 1858, Virchow analysed that the term "atheroma" is dermal cyst.[14] Atherosclerosis is a chronic degenerative vascular condition of arteries causing significant cardiovascular morbidity and mortality and responsible for more than 25% deaths in Indians.[19]

Mast cell is a highly potent immune cell which was first described by Paul Erlich in 1876, who called it 'Mastzelle'(the German word "Mastung denoting suckling" in the belief that they had taken up nutrients and stored them in their cytoplasmic storage granules).[20]

Mast cells are the type of inflammatory cells which participates in the immediate host defence.[21] These cells liberate cytoplasmic granules having preformed mediators and thus provides mast cells the ability to participate in the innate immunity.[8,21] Besides, these also play important role in various allergic reactions, host defence and tissue homeostasis.[21] Mast cells also have a role in several pathophysiological conditions such as asthma, autoimmune disease and atherosclerosis.[21]

NORMAL ANATOMY OF AORTA[22,23]

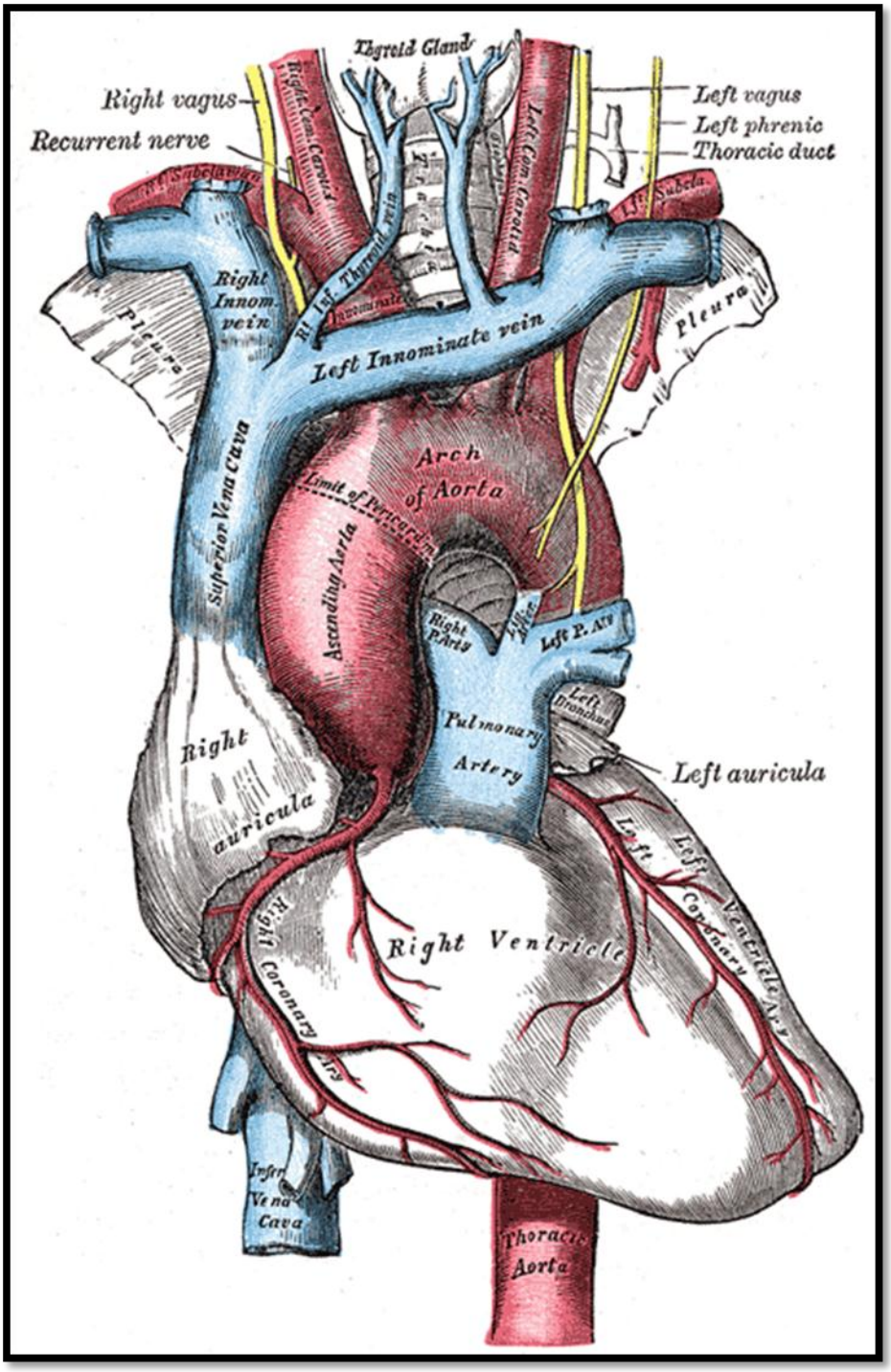

Figure 1: Normal anatomy of aorta[22]

Aorta is the main vascular trunk for multiple vessels and provides oxygenated blood to the tissues of the body. It originates from the left ventricle and ascends for a small distance and then forms an arch and to the left side, over the root of the left lung. It then descends within the thorax on the left side of the vertebral column, enters the abdominal cavity through the aortic hiatus in the diaphragm and then bifurcates opposite the lower border of the fourth lumbar vertebra, into the right and left common iliac arteries.

Thus, it is divided into various portions such as, the ascending aorta, the arch of the aorta, and descending aorta (the thoracic and abdominal aorta).

Ascending Aorta (Aorta Ascendens): It is approximately 5 cm in length and originates at the base of the left ventricle and passes obliquely upward, forward, and to the right, upto the upper border of the second right costal cartilage. At its origin, three small dilatations called the aortic sinuses are present. The ascending aorta lies within the pericardium, and is enclosed in a tube of the serous pericardium, along with the pulmonary artery.

Arch of aorta: It lies in the superior mediastinum and begins at the level of second sternocostal joint slightly to the right side. It then ascends diagonally and posterior to the left over the anterior surface of trachea and then posteriorly along the left side of trachea and finally descending to the left of fourth thoracic vertebrae terminating at the level of second left costal cartilage.[22,23]

Various branches arising from arch of aorta are:

- **Brachiocephalic trunk**- It is the largest branch of arch of aorta and arises from convexity of arch anterior to trachea and posterior to manubrium. It later gives terminal branches, right common carotid and right subclavian arteries.
- **Left common carotid artery**– It arises from arch of aorta to the left side and ascends upto the level of sternoclavicular joint from where it then enters the neck.
- **Left subclavian artery**- It arises from arch of aorta separately from the left common carotid artery and arises to the left of it.

Descending thoracic aorta: It begins from the level of lower end of fourth thoracic vertebrae and lies in posterior mediastinum. It continues upto the twelfth thoracic vertebrae where it enters the aortic hiatus. Various branches arising from descending thoracic aorta are:

- Pericardial branches
- Bronchial arteries- these are one on right side which usually arises from third posterior intercostals branch while on left these are two in number and arises from thoracic aorta.
- Oesophageal branches
- Mediastinal branches
- Phrenic branches
- Posterior intercostals branches
- Subcostal branches

Abdominal Aorta- The abdominal aorta begins at the aortic hiatus of the diaphragm, opposite the twelfth thoracic vertebra and ends at the level of fourth lumbar vertebra, by bifurcation into the right and left common iliac arteries. It diminishes in size, owing to the multiple large branches arising from it.[22,23]

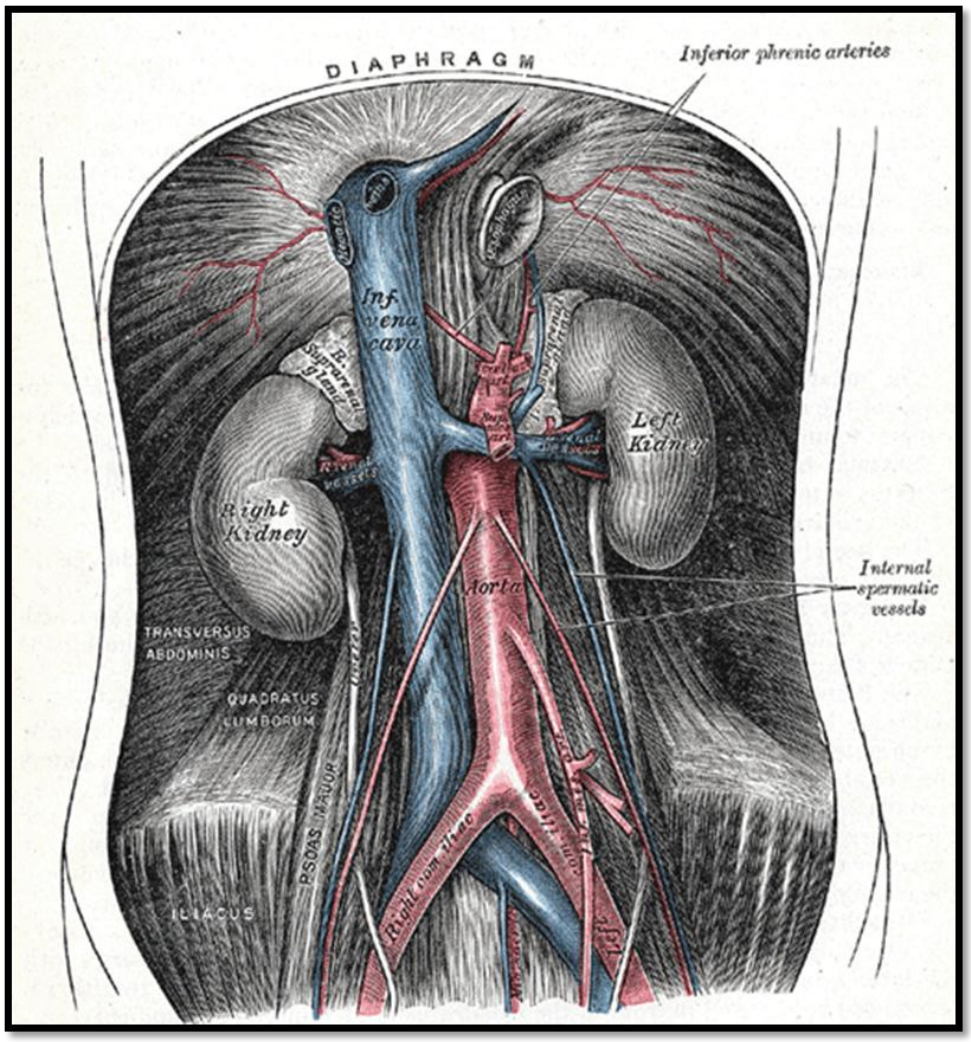

Figure 2: Anatomy of abdominal aorta[22]

NORMAL HISTOLOGY OF ARTERY

A vessel wall is primarily composed of two types of cell, the endothelial cells and the smooth muscle cells.[17] The endothelial cells originate from the yolk sac and these cells organise in a cluster to form vascular primordial.[17] The vascular primordial recruits the mesenchymal cells which subsequently convert into smooth muscle cell of the media and fibroblasts of the adventitia. In the arterial wall the two cell structure organise to form layers called 'tunica'. The intima layer is the innermost layer and is composed of collagen, proteoglycans and elastin and is thicker in aorta. Tunica media, the middle layer is mainly composed of smooth muscle cell. Tunica adventitia, the outermost arterial layer is composed of fibroblasts, vasa vasorum and nerves. Aorta and the other large vessels of the body are composed of elastic fibres which are along with the smooth muscle cells. Atherosclerosis mainly involves the tunica intimal layer of the of the vessel.[16,17,24]

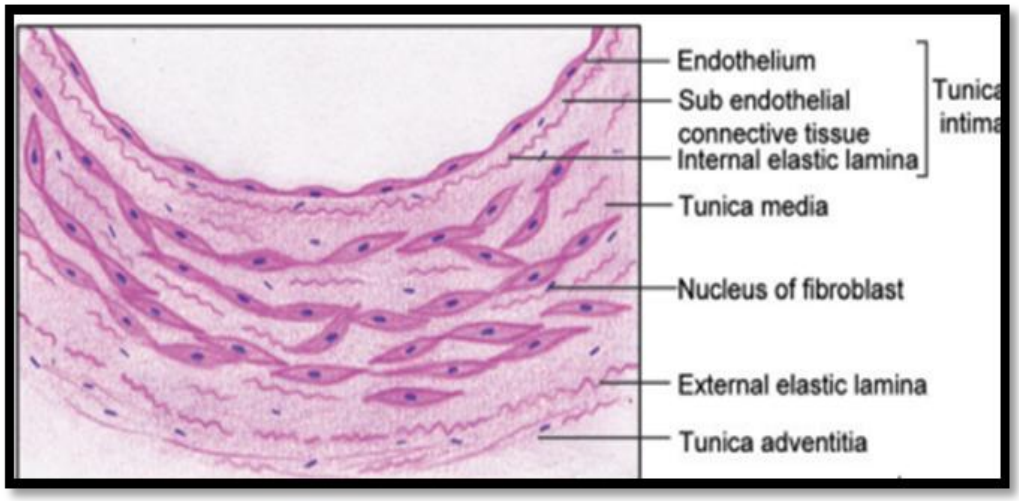

Figure 3: Normal histology of muscular ateries[24]

RISK FACTORS FOR ATHEROSCLEROSIS:

There were several studies done for determining the risk factors of atherosclerosis. Some studies revealed that the risk of the atherosclerosis in the rural areas has less prevalence than the urban areas.[25] Also several studies noted that South Asians have been at a twice the risk of atherosclerosis when compared to the Caucasian.[26] The various risk factors are classified into constitutional/non modifiable risk factors and acquired/modifiable risk factors.[27]

CONSTITUTIONAL/NON MODIFIABLE RISK FACTOR

- **Age**: Very important factor for atherosclerosis and risk of atherosclerosis rises with every decade.[28,29,30,31]
- **Sex**: Males are more prone for atherosclerosis. Postmenopausal women are also at a greater risk.[30,32]
- **Genetics**: Family history is among the most important risk factor for atherosclerosis. There are multifactorial causes for the familial predisposition to atherosclerosis.[16,17]

Modifiable Risk Factors

- **Cigarette Smoking**: Smoking of one packet of cigarette or more daily increases the death rate to double. It is analysed in several studies that the fatty streaks in the abdominal aorta is greater in the smokers than the non-smokers.**33,34**
- **Hyperlipidaemia**: Hypercholesterolemia is a very important risk factor for atherosclerosis. With the increased levels of the LDL and decreased levels of HDL the risk of atherosclerosis increases more.[33,34] HDL is known as good

cholesterol, it mobilises cholesterol of the tissues and helps in the transportation of the cholesterol to the liver for its excretion in the bile. Familial hypercholesterolemia is a condition having mutation in a gene which encodes for the receptor for LDL.

- **Diabetes**: The patients with diabetes has a increased risk of hypercholesterolemia and has a marked increased risk of atherosclerosis.[33,35] Glucose accelerates process of atherosclerosis by causing activation of NFKB which induces expression of several adhesion molecules responsible for adhesion of monocytes to the endothelial cells.[36]
- **Hypertension**: it is considered to be associated with increased plaque burden in the aortic region.[31,33]

Many cardiovascular events occur without any particular risk factor. Some factors causing atherosclerosis are given below:

1. **Inflammation**: All the stages of atherosclerosis is associated with inflammation and mainly associated with plaque formation and rupture. CRP is regarded as the sensitive marker to correlate with the risk of IHD.[16,28]
2. **Metabolic Syndrome**: Metabolic syndrome associated with glucose intolerance, insulin resistance, hypertension and central obesity. All these factors are the known causes for atherosclerosis.

According to NCEP criteria[37], a diagnosis of 3 or more of the following biological and physiological abnormalities is required:

- Elevated triglycerides (≥150 mg/dl [≥1.7 mmol/L]).
- Low HDL cholesterol (men <40 mg/dl [<1.03 mmol/L]; women <50 mg/dl [<1.29 mmol/L]).
- Impaired fasting glucose (≥110 mg/dl).
- High BP (≥130/85 mm Hg), and
- Increased waist circumference (men >40 inches [>102 cm]; women >35 inches [>88 cm].

3. **Hyperhomocysteinemia**: It has a strong relation with the serum homocysteine levels. Homocysteine is formed during demethylation of methionine. Its degradation takes place via remethylation and/or transsulfuration. Altered homocysteine metabolism increases the risk for atherosclerosis, cerebrovascular disease, and peripheral vascular disease. Several factors responsible for hyperhomocysteinemia include genetic causes (MTHFR, heterozygous cystathionine synthase), vitamin like folic acid, B12, B6 deficiency, and altered renal function. Also, several studies have suggested direct correlation between homocysteine and risk factors such as smoking, diabetes, obesity, and hypertension.[37,38]
4. The levels of the homocysteine gets elevated either by the low folic acid and vitamin B12 intake.[39]

5. **Lipoprotein (LP)a-** It is associated with coronary and cerebrovascular disease. The levels are independent of the total cholesterol or the LDL levels. LP(a) is formed by joining a LP to a carbohydrate-rich, hydrophilic protein called apo(a). Lp(a) particles contain apo(a) and apo B in a 1:1 molar ratio. Apo(a) contains a single domain and a carboxyl-terminal domain with 85% amino acid identity with the plasminogen protease domain.[28]
6. **Obesity**: The prevalence of obesity has short and long-term consequences because it is associated with an abnormal lipid profile i.e. increased concentrations of total cholesterol, triglycerides and LDL, and decreased HDL.[16,40]
7. Lack of exercise[16]
8. **Stressful lifestyle**: Chronic life stress, social isolation and anxiety increase the risk of MI and stroke.[37]
9. **Infections**: Herpes virus, Cytomegalovirus and Chlamydia pneumonia have been reported to be detected in the atherosclerotic plaque.[16,41]

Table 1: Old, Old/New and New Risk Factors for Atherosclerosis[37]

Old	Old/New	New
Sex(men>women)	High-normal blood pressure	Apolipoprotein B; Apolipoprotein A-1
Age	Metabolic syndrome	Triglycerides
Family history of premature cardiovascular disease	Diabetes mellitus, impaired glucose tolerance	Small, dense LDL, Oxidized LDL; Antibodies against oxidized LDL
Total cholesterol; LDL Cholesterol, HDL cholesterol (negative risk factor)		Lipoprotein (a)
Hypertension		Homocysteine
Smoking		CRP
Overweight/obesity		

The rapid globalisation had lead to the dramatic shift in the diet and living behaviours. Now the adverse dietary changes, sedentary lifestyle, increase of tobacco chewing has comparatively increased the atherosclerosis in population in comparison to other countries.[42]

PATHOGENESIS OF ATHEROSCLEROSIS

Atherosclerotic lesion is one of the most common acquired abnormalities of the blood vessels. The endothelial cells, leukocytes, intimal smooth muscle and macrophages play a very important role in the pathogenesis of the atherosclerosis.[16,17,43]

Hypothesis for the atherosclerosis

1) **Insudation Hypothesis**: Accumulation of the fat in the intima is one of the most critical events in the atherosclerosis. This fat is derived from the plasma LP. The complete pathogenesis of the atherosclerosis is not explained by this hypothesis.[16,17]

2) **Encrustation Hypothesis**: There are several materials from the blood which are deposited in the inner and this is the reason of thickening of the intimal layer.[16,17]

3) **Response to injury hypothesis**: It states that the atherosclerosis is a chronic inflammatory process and is the healing response of the arterial wall to the endothelial injury.

Following pathologic events takes place for the atherosclerotic process:

- **Endothelial Injury**: Caused by the hypertension, hyperlipidaemia, cigarette smoking, homocysteine and infectious agent. As the endothelium gets injures it leads to vascular permeability, leukocyte adhesion and thrombosis.[16,17,18,43]
- **Accumulation of the LP in the vessel wall**: as the vascular permeability increases it leads to the entry of lipids and results in the production of oxygen free radicals, damages the tissue and results in the oxidation of LDL to oxidized LDL. These oxidized LDL is then taken up by the macrophages and the smooth muscles to form foam cells.[16,17]
- **Inflammation**: Several inflammatory pathways contributes to the progression ad complication of the atherosclerotic lesion. Macrophages act as the main players of the atherosclerotic lesion they get converted into

foam cells and also act as the store house for CTK and Ck. These attract various other inflammatory cells and macrophages and thereby producing MMPS. These MMPS produced dissolve the connective tissue matrix resulting in the rupture of the plaque.[16,17]

- **Smooth Muscle Proliferation**: The combination of intimal smooth muscle proliferation and ECM deposition convert a fatty streak into mature atheroma and progresses the growth of the atherosclerotic lesion. There are several growth factors that play a vital role like PDGF, FGF, and TGF alpha. These growth factors cause the proliferation of the smooth muscles and ECM synthesis.[16,17]

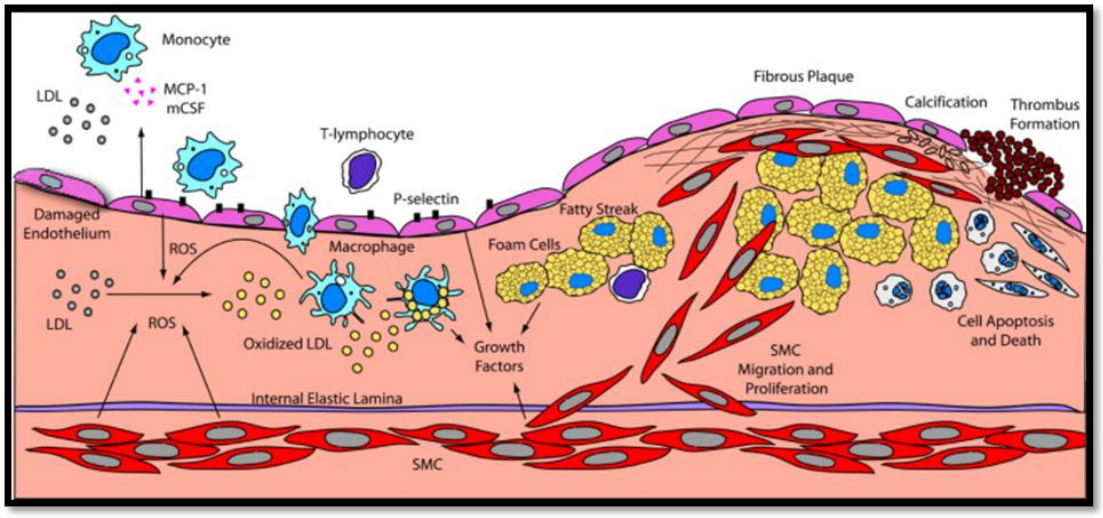

Figure 4: Development of atherosclerosis[44]

ATHEROSCLEROTIC LESION CLASSIFICATION ACCORDING TO AHA

According to the histological classification of the atherosclerotic lesions the class I and the class II lesions are regarded as the early lesion, class III is the intermediate lesion and class IV, V, and VI are the advanced lesion.[45]

MORPHOLOGY OF THE ATHEROSCLEROTIC LESIONS[45]

Fatty streaks

The earliest appearing lesions are the fatty streaks composed of mainly the lipid filled foamy macrophages which appear as flat yellow spots which generally coalesce to form into elongated streaks. Microscopically they show macrophage derived foam cells in the intimal layer.

Class III lesion (pre atheroma)

It is the intermediate lesion between the early and the advanced lesion. The thickness of the intimal layer increases slightly without obstruction of the blood flow. Pools of the extracellular lipid accumulate in the smooth muscle layer thereby separating the intimal layer. The extracellular lipids replace the proteoglycans and increase the ECM.

Class IV lesion (atheroma)

It comprises of lipid core with extracellular lipid which forms in the musculo elastic part of the intima. Inflammatory cells are found in numerous numbers in the periphery of the core. Capillaries forms the border of the lipid core.

Atherosclerotic Plaque

Grossly appears as mainly white or yellow coloured patches in the intimal layer.

The plaques are mainly formed by three different components:

- Cells mainly smooth muscles, macrophages and T cells
- Intracellular and extracellular lipid
- ECM which includes elastic fibres, collagen and proteoglycans.

Microscopically: Plaque is composed of mainly fibrous cap which is made up of smooth muscle cell and dense collagen. The shoulder area of the cap i.e. the side of the cap is mainly composed of macrophages, T cells and smooth muscle cell. The peripheral area of the cap mainly shows neovascularisation. Now some of the plaques are composed of exclusively smooth muscles and fibrous tissue and are known as fibrous plaques. Often these plaques undergo calcified and forms complicated plaques.

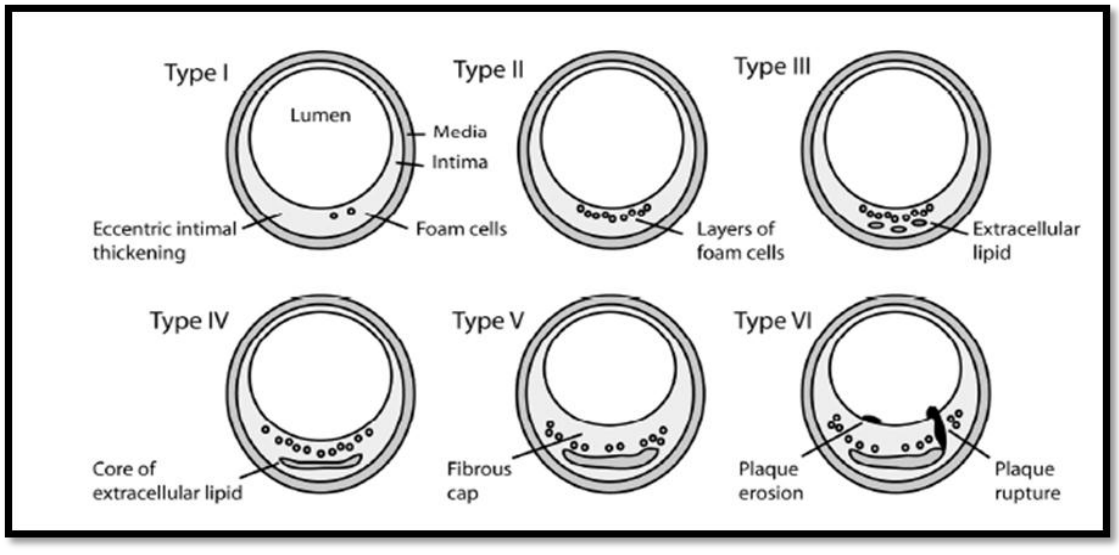

Figure 5: Progression of atherosclerotic lesion characterisation according to AHA[45]

OUTCOME OF THE ATHEROSCLEROTIC LESIONS[16,17]

- Atherosclerotic Stenosis: Mainly the smaller vessels get occluded. In these cases patients develop angina on exertion.
- Acute Plaque Change: Plaque rupture or erosion is followed by thrombosis which results in the acute tissue infarction.
- Plaques are composed of foam cells and extra cellular lipids and those in the fibrous caps are mainly thin.
- Hemorrhage into the plaque: Hemorrhage causes the expansion of the plaque and causes its rupture.
- Aneurysm: Due to the pressure of the underlying media there is loss of the elastic tissue thereby causing aneurysmal dilation and the rupture of the plaque.

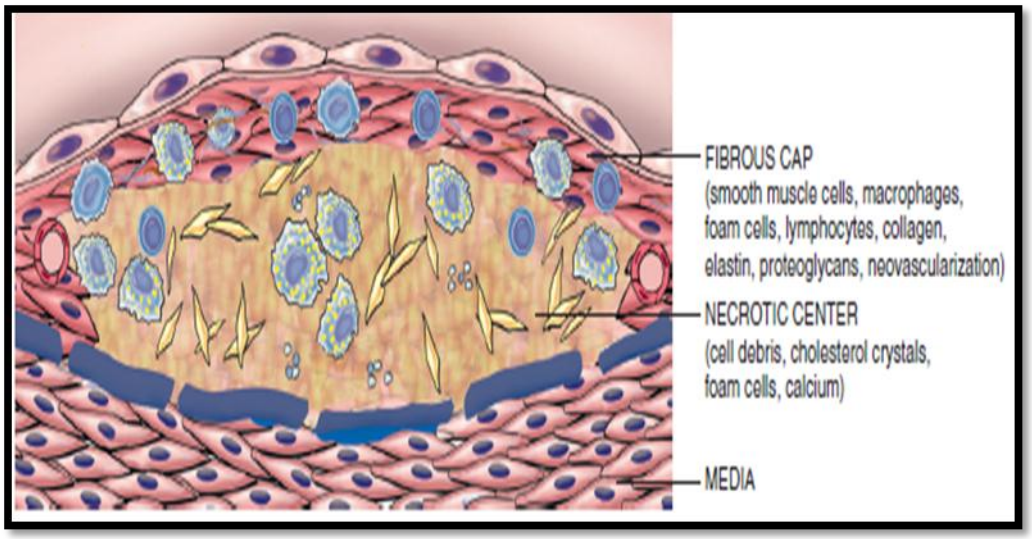

Figure 6: Components and appearance of fibrous cap atheroma[16]

Table 2: Current AHA classification[14,16]

AHA CRITERIA FOR GRADING OR TYPING OF ATHEROSCLEROTIC LESION (1994)[16]

Terms for Atherosclerotic Lesions In Histological Classification		Other Terms For The Same Lesions Often Based on Appearance To Unaided Eye	
Type I lesion	Initial Lesion		
Type II lesion		Fatty dot or streak	Early Lesion
IIa	Progression prone Type II Lesion		
IIb	Progression resistant Type II Lesion		
Type III Lesion	Intermediate Lesion (Pre-atheroma)		
Type IV Lesion	Atheroma	Atheromatous Plaque /Fibro-lipid Plaque/ Fibrous Plaque,/Plaque	
Va	Fibroatheroma (type V lesion)	Atheromatous Plaque /Fibro-lipid Plaque/ Fibrous Plaque,/Plaque	
Vb	Calcific Lesion (Type VII lesion)	Calcified Plaque	Advanced Lesion Raised Form
Vc	Fibrotic Lesion Type VIII	Fibrous Plaque	
Type VI Lesion	Lesion with surface defect and/hematoma/hemorrhage/ Thrombotic Deposit	Complicated lesion, Complicated Plaque	

Table 3: Modified AHA Classification[14]

	DESCRIPTION	THROMBOSIS
NON-ATHEROSCLEROTIC INTIMAL LESIONS		
Intimal thickening	The normal accumulation of smooth muscle cells (SMCs) in the intima in the absence of lipid or macrophage foam cells	Absent
Intimal xanthoma, or "fatty streak"	Luminal accumulation of foam cells without a necrotic core or fibrous cap. Based on animal and human data, such lesions usually regress.	Absent
PROGRESSIVE ATHEROSCLEROTIC LESIONS		
Pathological intimal thickening	SMCs in a proteoglycan-rich matrix with areas of extracellular lipid accumulation without necrosis	Absent
Erosion	Luminal thrombosis; plaque same as above	Thrombus mostly mural and infrequently occlusive
Fibrous cap atheroma	Well-formed necrotic core with an overlying fibrous cap	Absent
Erosion	Luminal thrombosis; plaque same as above; no communication of thrombus with necrotic core	Thrombus mostly mural and infrequently occlusive
Thin fibrous cap atheroma	A thin fibrous cap infiltrated by macrophages and lymphocytes with rare SMCs and an underlying necrotic core	Absent; may contain intra plaque hemorrhage/fibrin
Plaque rupture	Fibroatheroma with cap disruption; luminal thrombus communicates with the underlying necrotic core	Thrombus usually occlusive
Calcified nodule	Eruptive nodular calcification with underlying Fibrocalcific plaque	Thrombus usually non-occlusive
Fibrocalcific plaque	Fibrocalcific plaque	Absent

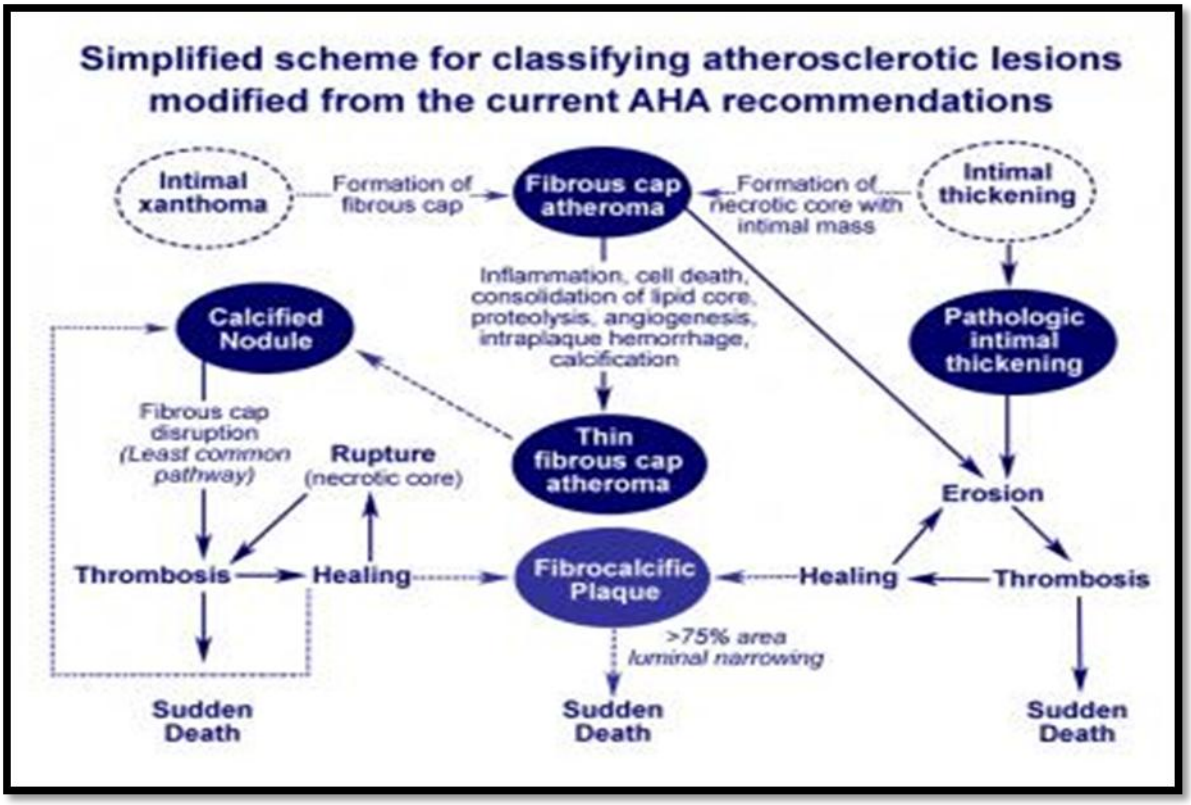

Figure 7: Progression of lesion according to modified AHA classification based on morphological description[14]

MAST CELL

Mast cells are inflammatory cells which participates in immediate host defence.[21]

These cells are round to elongated in shape and have characteristic cytoplasmic granules which are metachromatic stain positive. These cells have narrow and elongated folds on their surface. They have ovoid and non-segmented nucleus. The cytoplasm contains usual cellular granules like Golgi bodies, mitochondria and endoplasmic reticulum. However cytoplasmic granules are the dominant structures in the cytoplasm.

These cells on stimulation release these secretory granules in the surrounding. This process is known as degranulation.[46]

It is present at the interfaces of the body, such as skin, airways, gastrointestinal tract and vasculature.[21] Mast cells are able to function against foreign invaders very rapidly, i.e. Within minutes.[21] This property originates from liberation of cytoplasmic granules containing preformed mediators and gives mast cells their unique ability in innate immunity.[21] The mast cells have an important role in allergic reactions, host defence and tissue homeostasis. They play an important role in immunity by causing amplification of immunological reaction through activation of mast cells with other immunological molecules such as complement proteins and causing recruitment of various cells like neutrophils.[21] Mast cell have been found to be associated with several autoimmune disease such as multiple sclerosis, rheumatoid arthritis, IDDM, bullous pemphigoid and chronic idiopathic urticarial.[47]

PHYSIOLOGICAL ROLE OF MAST CELL

- **Angiogenesis** – they secrete various pro angiogenic factors such as VEGF, BFGF, TGF-beta, TNF-alpha and IL-8. It also releases protease and heparin. The histamine released by mast cells also induces angiogenesis.[48]
- **Homeostasis** – In immune system mast cells acts as first line defence against various antigens. It also plays important role against commensal bacteria's of gut. It helps in differentiation of follicular helper T cells via ATP signaling resulting in its role of IgA maturation causing homeostasis of gut bacteria.

- **Innate and adaptive immunity** – It recognizes various harmful antigens by binding to two pathogens directly in association with PAMPS on the surface of mast cell. The various mediators are released from mast cells increasing vascular permeability and smooth muscle constriction helping in expulsion of parasites from gastrointestinal tract by inducing vomiting or diarrhea.
- **Activation and mediator release** –Mast cells are present in different population of tissues and contains histamine rich granules.[10,20,49] Mast cells are associated with IgE associated disorders. The IgE sensitized mast cells are activated when it encounters with specific antigens which is recognised by antigen specific IgE molecule bound to FCϵRI receptors on mast cell.[49,50] Degranulation of mast cells occur after cross linking resulting in the release of inflammatory mediators stored in mast cell.
- Besides, it also has a role in various proinflammatory, immunoregulatory and biological processes such as mitogenesis, ECM degradation and tumour spread by recruiting certain growth factors and CTK.[51,52]

Mast cell is the derivatives from the specific bone marrow progenitor cells which migrate to tissues where their maturation depends on the micro environmental condition.[53]

Types of Mast Cell[11,54,55]

1) Type 1 contains the neutral proteases, tryptase and chymotryptic proteinase, and is known as TC mast cell.
2) The type 2 contains only tryptase and is termed the T mast cell.

NEWLY GENERATED MAST CELL MEDIATORS

Lipid Mediators

PG, thromboxane, and LT are the Eicosanoids which are derived from arachidonic acid. Various enzymes like COX and LOX metabolise Arachidonic acid into PG D2 and E2 and thromboxane A2, and into LT, such as LT B4 and C4.

PG D2 act as coronary vasoconstrictor, peripheral vasodilator, platelet aggregation inhibitor and chemo-attractant to neutrophils.[56] LT cause arterial, arteriolar constriction, and a prolonged 'wheal and flare' response in skin by causing increased vascular permeability.[57] A precursor for PAF is formed when PLP A2 generates arachidonic acid from phosphatidylcholine. PAF aggregates platelets, increases vascular permeability, and is a chemo-attractant for neutrophils.[58] All described lipid mediators are also broncho constrictors. In addition to PG D2 and LT C4, mast cell may also contain PG E2, LT B4, and PAF.[59]

CTK and CK

Mast cell releases a large variety of newly synthesized CTK and CK. CTK include pro inflammatory mediators

- TNF, IL-1α, IL-1β, IL-6, IL-18,GM-CSF, LIF, IFN-α and IFN-β;
- Th- 2-type CTK IL-3, IL-4, IL-5, IL-9, IL-13, IL-15, and IL-16; Th 1-type CTK IL-12 and IFN-γ
- Anti-inflammatory, immune modulatory or angiogenesis regulating IL-10, TGF-β, and VEGF.[60]
- In addition, releases MIF, SCF, GNRH-I, BFGF and NGF.[61]

- CK released from mast cell include CC ligands CCL-2, CCL-3, CCL-4, CCL-5, CCL-11, and CCL-20 as well as cxc-chemokine ligands CXCL-1, CXCL-2, CXCL-8, CXCL-9, CXCL-10, and CXCL-11,

These mediators have a role in recruiting other effector cells and in regulating immune response.[60] Overall, the mediators released from mast cell, notably various CTK, exhibit variation in their properties.[62]

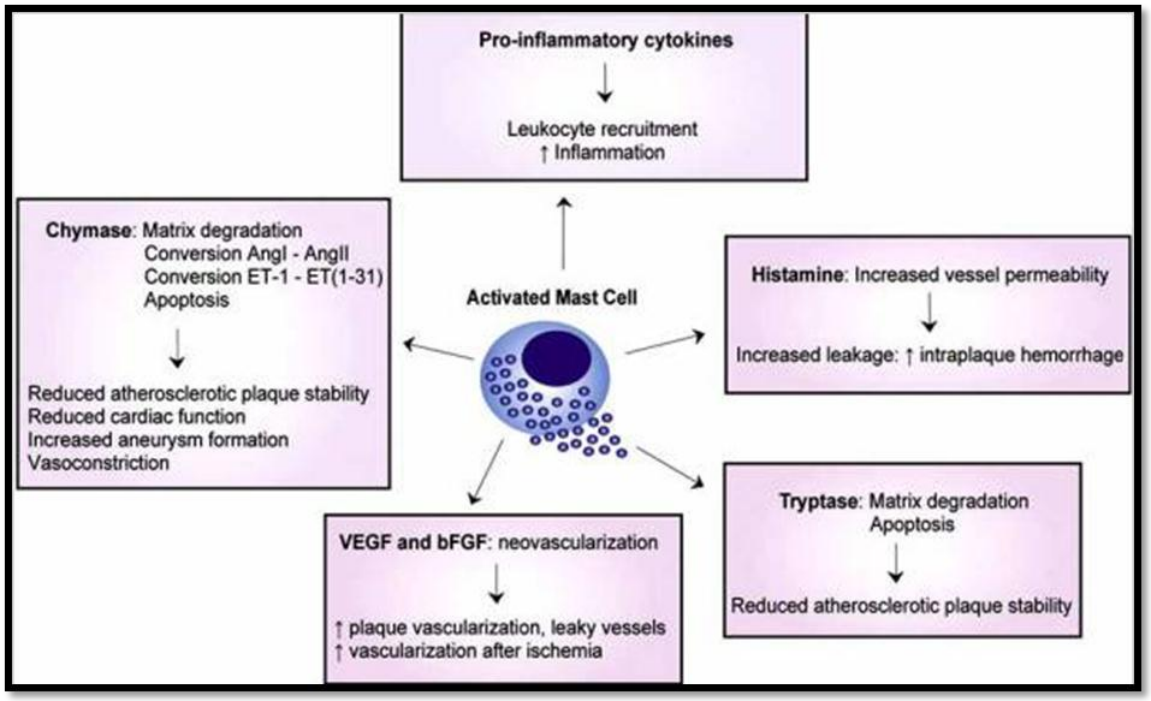

Figure 8: Important mast cell mediators in cardiovascular disease[63]

FUNCTION OF MAST CELL[8,64,65,66,67,68,69,70]

- Mast cells play important roles in various physiological processes in the body.
- Wound healing, tissue remodeling and homeostasis of the body.
- Innate immunity by phagocytosis and killing of bacteria. It produces antimicrobial peptides like cathelicidins, NO and superoxide radicals.
- It participates in wound healing.
- Mast cells have a role in many pathological conditions such as allergy and asthma, rheumatoid arthritis, atherosclerosis, and cancer, and they may even have a role in controlling diet-induced obesity.
- Type I hypersensitivity reactions induced by IgE mediated allergic inflammation. Anaphylaxis is a type of allergic reaction associated with the release of mediators from mast cell and basophils.
- Allergic disorders such as asthma, allergic rhinitis, and atopic dermatitis.
- Mast cells also participate in chronic inflammatory and autoimmune disorders that are induced by dysregulated activation of Mast cell .The activation of Mast cells is mediated through auto-antibodies in bullous pemphigoid and rheumatoid arthritis, and by deposited immune complexes in glomerulonephritis.
- Heart diseases such as heart failure and atherosclerosis. It also has role in stroke and aortic aneurysms.

- Role in cancer by promoting angiogenesis with subsequent tumour growth and/or formation of metastasis.

- Mast cells serves as a reservoir for HIV through latent infection with mast cell activation through TLRS 2, 4, and 9 triggering viral replication.

- It also contributes to bone remodelling and hair follicle cycling.

Mast cell effector mechanisms

Several mediators of the mast cells have the capacity to exert several effector mechanisms. Mast cells act as effector cells and to induce plaque progression. Once the mast cells are activated their mediators are released.[52] The primary plaque destabilising effects is mediated by the mast cell specific proteases tryptase and chymase.[52,71]

Table 4: Potential mast cell activator[55]

Activator	Described mediators in releasate
IgE	Degranulation via crosslinking of FcεRs, resulting in release of proteases, growth factors, cytokines etc.
oxLDL-IgG immune complexes	Cytokine release, specifically of TNFα, IL-8 and CCL2 via Fcγ receptors.
OxLDL	Induces albumin leakage *in vivo*, possibly via histamine release, and induces the release of TNFα, IL-6 and CCL2 via TLR4 *in vitro*.
LPS	Cytokines such as IL-6, but also some release of proteases via TLR4.
LPA	Release of tryptase and CCL2 via LPA_1.
C5a	Release of tryptase and CCL2 via C5aR.
Substance P	Chymase and tryptase via NK_1R.
Neuropeptide Y	Tryptase and IL-6.

Chymase plays important role in atherosclerotic plaque progression and destabilisation. Chymase also contributes to atherosclerosis by modifying HDL, thereby affecting its cholesterol efflux ability.[72,73]

MAST CELL IN ATHEROSCLEROSIS

The number of the mast cell is increased in the atherosclerotic arteries compared to that of the normal arteries.[9] In the initial events of atherosclerosis, mast cell increases the accumulation of LDL in the intima of the vessel.[74] Histamine released from locally activated mast cell increases endothelial permeability and thus the trans endothelial transport of LDL into tissues. Heparin proteoglycans from activated mast cell granules binds to LDL and facilitates granule-bound chymase, together with carboxypeptidase A, to proteolyze apoB-100 on LDL. LDL is fused by this mechanism and the formation of larger lipid droplets on mast cell granules.[75] These particles containing fused LDL and granule remnants are then taken up by macrophages and smooth muscle cells resulting in the formation of foam cells.[75,76,77] Mast cells inhibits macrophage-induced oxidation of LDL which may lead to atheroprotection.[78]

Chymase degrades apoA-I in pre-β-HDL as well as apoA-II, apoA-IV and apoE, and thus reduces the removal of cholesterol by HDL from macrophage foam cells.[79,80] The mechanism includes the inhibition of the ABCA1 efflux pathway.[81] Tryptase is also capable of degrading apoLP in HDL thus blocking its function as a cholesterol acceptor.[82] In addition, chymase is also capable of inhibiting the activities of PLTP and CETP.[83,84,85,86,87]

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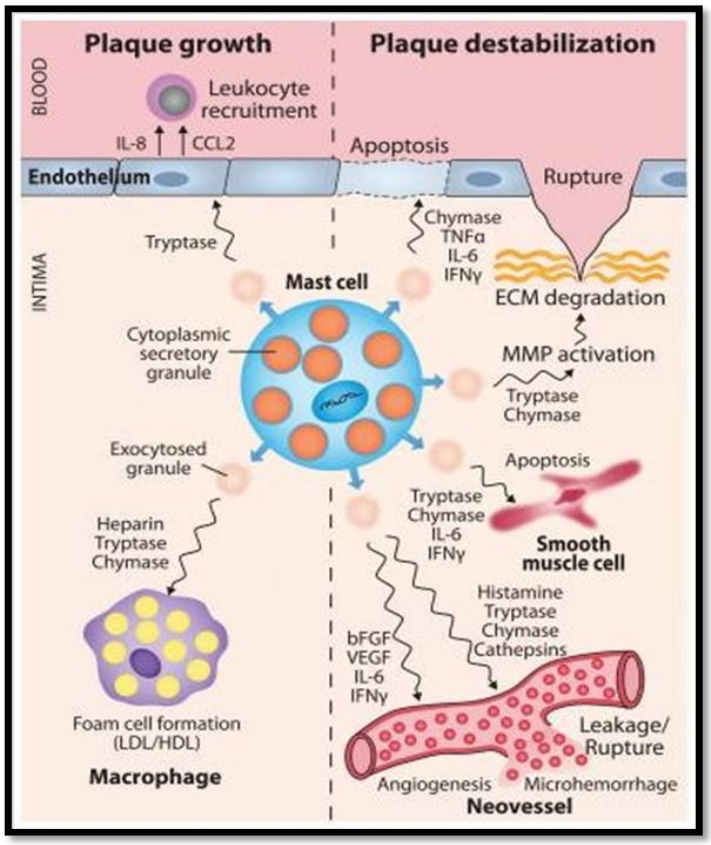

Figure 9: Role of activated mast cell in growth and destabilisation of an atherosclerotic plaque[55]

Integrins are used by mast cells for interaction with ICAM-1 from vascular endothelium. Mast cells enter the sub-endothelial space via the cell surface chemokine receptors and target tissue Ck[monocyte chemotactic protein-1, eotaxin, and RANTES (regulated upon activation, normal T-cell expressed and secreted).[89,90] Mast cells releases Various mediators such as histamine, heparin, proteoglycans, serotonin, CTK [interferon-g (IFN-g), IL-6, and TNF-a, chemotactic factors, and angiogenic factors such

as FGF, VEGF, and Angiopoietin-1.[82] Mast cells secretes proteases, such as chymase and tryptase. These are secreted as active compounds by the mast cell granules, convert pro matrix Metalloproteinases to MMPS (especially MMP-1, -2, and -9) that degrade matrix molecules.[91] Cathepsin also causes degradation of Collagen, elastin, and fibronectin/vitronectin, which are essential for adhesion of smooth muscle cells and endothelial cells .The degraded matrix molecules also play a role in leukocyte recruitment and release angiogenic factors.[51] Chymase activate pro-IL-1b and lead to production of TGF-b1which induces Smooth muscle cell migration and proliferation.

LDL is altered by proteases so there is increased macrophage LDL uptake and conversion to foam cells; at the same time.[9,51,72]

Degradation of the HDL3 occurs which, results in destruction of its ability to efflux cholesterol from foam cells. Lp-PLA2, LP-associated phospholipase A2; oxidized LDL; TCR, T-cell receptor; VCAM, vascular cell adhesion molecule.[82]

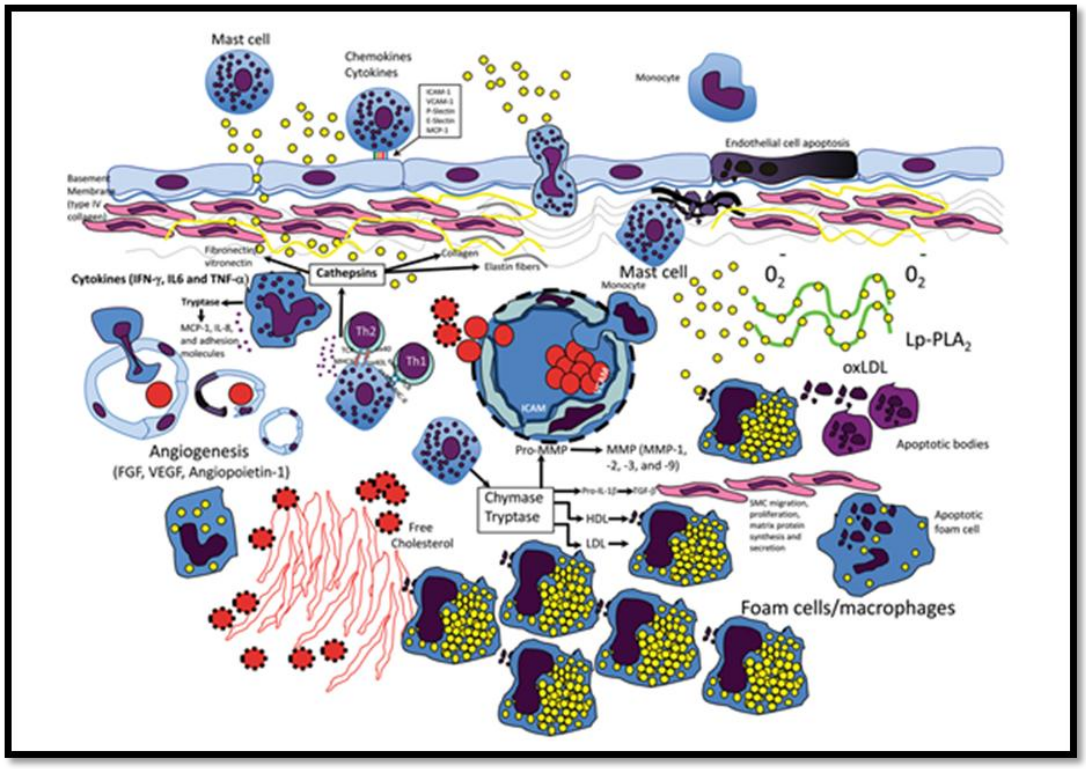

Figure 10: Mast cell function in atherosclerosis[89]

SPECIAL STAINS TO IDENTIFY MAST CELL[47,63,92,93]

- Toluidine Blue, cresyl Violet, Azure A, Methylene Blue and Modified thionine acridine orange- these are the basic aniline dyes for staining the metachromatic granules of the Mast cell.
- Demonstration of the enzymes by histochemical stains.
- Monoclonal antibodies like anti tryptase are stained by Immunoperoxidase.

METHODOLOGY

Study duration

The study was conducted from February 2016 to August 2017 for a period of 18 months.

Study design

Cross-sectional study

Inclusion criteria

Patients of any age on whom medico-legal autopsies have been done to ascertain the cause of death.

Exclusion criteria

- Autopsies on exhumed bodies.
- Autopsies on poorly preserved bodies that are more than 3 days old where autolytic changes would have taken place.
- All cases below 10 years of age.

Sample size

Sample size was estimated using mean and standard deviation of Mast cells count in atherosclerotic plaque from the pilot study on 10 autopsies.

Mean Mast cell count was:

51.90±6.35 cell/ mm^{sq}.

SD= 6.35, d=2% error, z alpha =2.51 at 99%CI.

N= $(2.58)^2 x (6.35)^2/2^2$

N= 70 autopsy cases

Statistical analysis

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square was used as test of significance. Continuous data was represented as mean and standard deviation. Independent t test was used as test of significance to identify the mean difference between two groups. P value <0.05 was considered as statistically significant.

Study procedure and Method of collection of Data

Aortas were collected from autopsies conducted at R. L. Jalappa and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar in coordination with Department of Forensic Medicine.

Prior to the study ethical clearance was obtained from the ethical clearance board of the institution.

The study included total 70 cases from which the aortas were collected and fixed in formalin in Department of Pathology.

The segments were identified and gross examination was done. From the 70 cases each aorta were divided into thoracic, abdominal and non atherosclerotic portion of the Aorta. Thoracic segment of the aorta included the segment distal to the arch of aorta. Abdominal segment of the aorta included the segment just proximal to the bifurcation of the aorta to the iliac arteries. Non atherosclerotic bit was taken as a random bit from the aorta where macroscopically no lesion was identified. Bits from the suspicious or definite lesions were taken for tissue processing and these processed tissue were embedded in paraffin blocks.

These paraffin blocks were used for H & E Staining and Toluidine blue staining. The H & E sections of the thoracic, abdominal and non-atherosclerotic part of the aorta were first examined at low magnification (x40 and x100 magnification) using (Olympus CX 21i) microscope and graded/typed according to the AHA classification of atherosclerosis.

The toluidine blue staining was done to identify the mast cells. Mast cells were counted in 10 high power fields (40X) in the adventitial layer of aorta. The average number of Mast cells per 10 high field were determined. Toluidine blue should stain Mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Toluidine blue staining was done on skin biopsies which acted as control for staining. Review of slides was done by two pathologists, to eliminate the observer bias.

HEMATOXYLIN AND EOSIN STAINING PROCEDURE

Procedure

- Deparaffinise the section in 3 changes of xylene (5 minute each).
- Two changes of isopropyl alcohol (5 minute each)
- Wash in tap water (1 minute)
- Keep in Harris Haematoxylin (5-7 minutes).
- Rinse in running tap water (3 minutes)
- Differentiate in 1% acid alcohol (10 seconds)
- Wash well in tap water / lithium carbonate 5 minutes (until bluing)
- Dip in eosin (20 seconds)
- Rinse in tap water (1 minute)
- Dehydrate in two changes of isopropyl alcohol (10 seconds each)
- Clear the section with dip in xylene (10 seconds)
- Mount in Distyrene, Plasticizer and Xylene (DPX).

TOLUIDINE BLUE STAIN KIT FOR MAST CELLS

Reagents supplied in the kit

- Potassium Permanganate Solution 0.5% 100ml.
- Sodium Metabisulfite 2% w/v Solution 100ml.
- 0.02% Toluidine Blue Solution 100ml.

Staining Procedure

- Deparaffinise and hydrate to distilled water.
- Potassium permanganate solution for 2 minutes.
- Rinse in two changes of distilled water.
- Sodium metabisulfite solution for 1 minute.
- Wash in running tap water for 3 minutes.
- Rinse in two changes of distilled water.
- Toluidine Blue solution for 15 minutes.
- Rinse in three changes of distilled water.
- Dehydrate in increasing concentration of alcohols and clear in xylene
- Mount in mounting media
- Observe under microscope

Staining Results:

Mast cell granules ---------- purple (metachromatic)

Acid mucopolysaccharides ---------- red to pink

Nuclei ---------- blue

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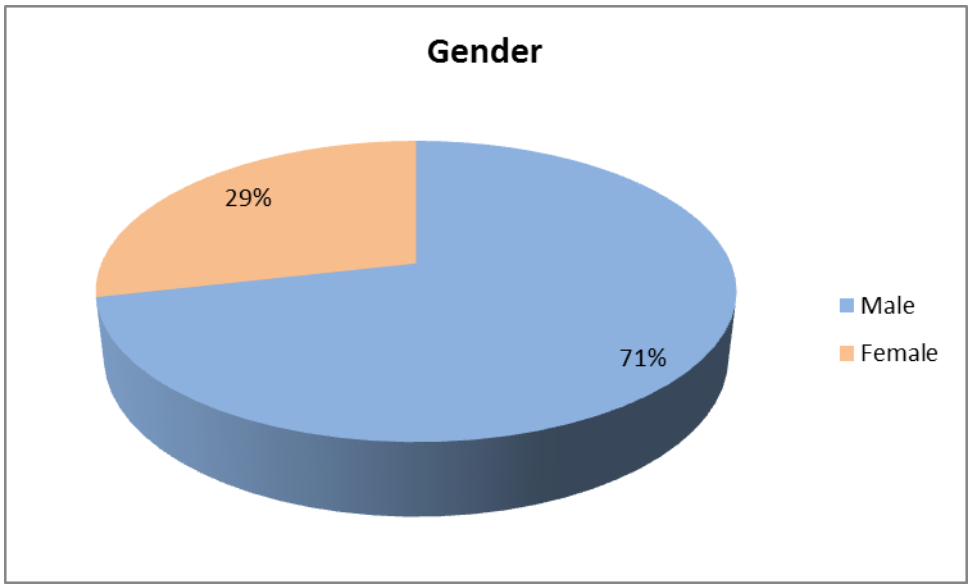
Va	Fibroatheroma (type V lesion)	Atheromatous Plaque/Fibro-lipid Plaque/Fibrous Plaque/Plaque	
Vb	Calcific Lesion (Type VII lesion)	Calcified Plaque	Advanced Lesion Raised Form
Vc	Fibrotic Lesion Type VIII	Fibrous Plaque	
Type VI Lesion	Lesion with surface defect and/hematoma/hemorrhage/ Thrombotic Deposit	Complicated lesion, Complicated Plaque	

RESULTS

The duration of the present study is from February 2016 to August 2017, for a period of 18 months. All cases which satisfied the inclusion criteria are included in the present study. A total of 70 cases are studied.

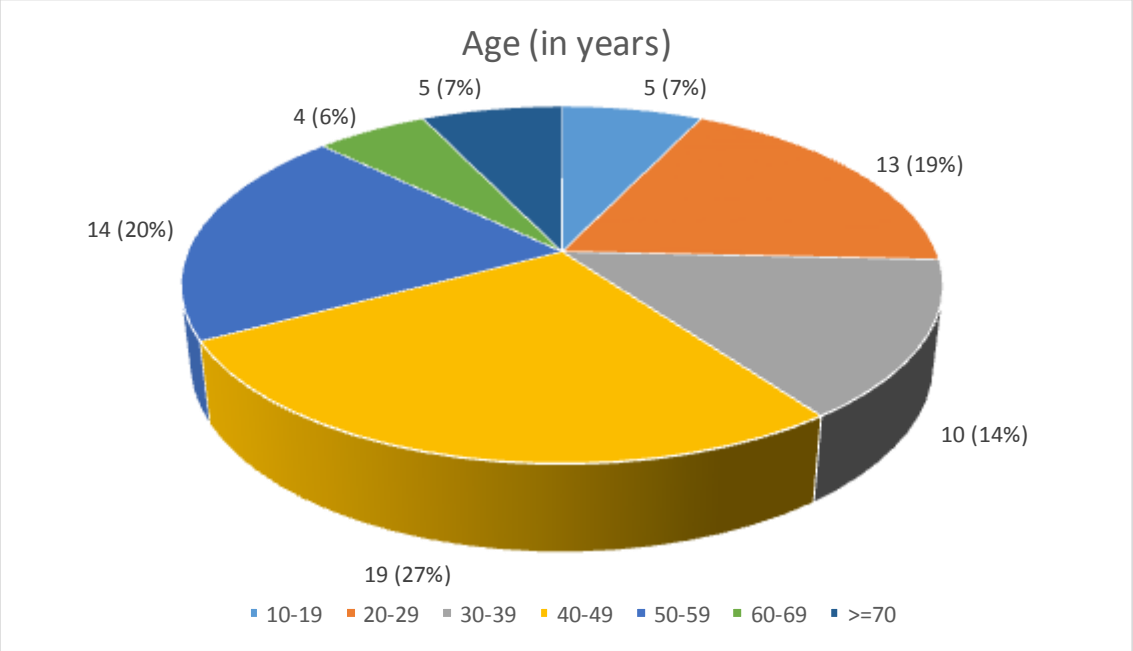
GENDER DISTRIBUTION OF THE CASES



Graph 1: Pie chart showing gender distribution of the cases

In the present study, number of males are 50(71%) and number of females are 20(29%). Male: Female ratio = 5:2.

AGE DISTRIBUTION OF THE CASES



Graph 2: Pie chart showing age distribution of the cases

In the present study, the age group ranged from 10-80 years.

Majority of the cases belong to the age group of 40-49 years which consisted of 19 (27%) cases. Followed by 50-59 years which consisted of 14 (20%) cases, 13(19%) of the cases belonged to the age group of 20-29 years,10(14%) cases belonged to the age group of 30-39 years,5(7%) cases belonged to the age group of 10-19 years, 5(7%) of the cases belonged to the age group of ≥70 years and 4(6%) cases belonged to the age group of 60-69 years.

The maximum number of cases that is 19(27%) belonged to the age group of 40-49 years of age.

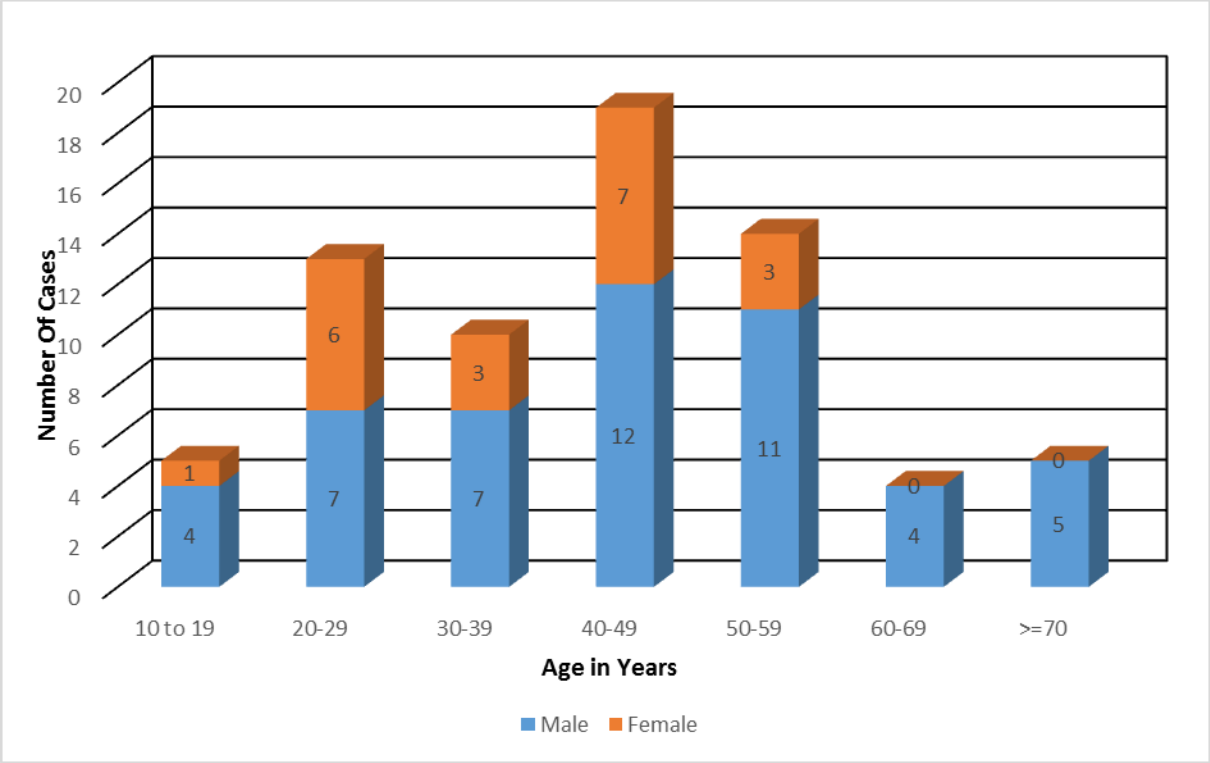
DEMOGRAPHIC ANALYSIS OF AGE WITH GENDER DISTRIBUTION

Table 5: Demographic analysis of age with gender distribution

Age groups (in years)	Sex		Total N (%)
	Male N (%)	Female N (%)	
10 –19	4 (80.0%)	1 (20.0%)	**5 (7.1%)**
20 – 29	7 (53.8%)	6 (46.2%)	**13 (18.6%)**
30– 39	7 (70.0%)	3 (30.0%)	**10 (14.3%)**
40 – 49	12 (63.2%)	7 (36.8%)	**19 (27.1%)**
50 – 59	11 (78.6%)	3 (21.4%)	**14 (20.0%)**
60– 69	4 (100.0%)	0 (0.0%)	**4 (5.7%)**
≥70	5 (100.0%)	0 (0.0%)	**5 (7.1%)**
Total	**50 (71.4%)**	**20 (28.6%)**	**70 (100.0%)**

In the present study, 4(80%) cases are males and 1(20%) is female population in the age group of 10-19 years. In the age group of 20-29 years, 7 (53.8%) cases are male and 6(46.2%) of the cases are female. In the age group of 30-39 years, 7(70%) cases are male and 3 (30%) cases are female. In the age group of 40-49 years, 12(63.2%) cases are male and 7(36.8%) cases are female. In the age group of 50-59 years, 11(78.6%) cases are male and 3(21.4 %) cases are female. In the age group of 60-69 years, 4 (100%) cases are male. In the age group of ≥ 70 years 5(100%) cases are male. Hence, it is seen that in the age group of ≥ 60 the cases in the study are males.

CORRELATION OF AGE WITH THE GENDER



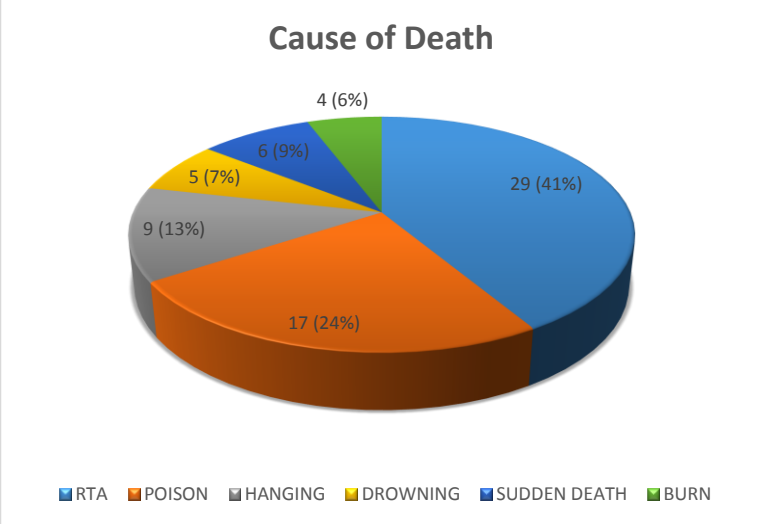
Graph 3: Bar diagram showing correlation of age with the gender

In the bar diagram, it is depicted that the maximum number of cases 19(27%) are in the age group between 40-49 years. Male cases are 12(63.2%) and females cases are 7(36.8%) and both of them are maximum in 40-49 years. In the age group of >60 years (100%) cases are males.

CLINICAL / FORENSIC CAUSE OF DEATH

Table 6: Clinical / forensic cause of death

CAUSE OF DEATH	NUMBER OF CASES
Road Traffic Accident	21(49%)
Poisoning	17(24%)
Hanging	9(13%)
Sudden Death	6(9%)
Drowning	5(7%)
Burns	4(6%)
Total	70(100%)



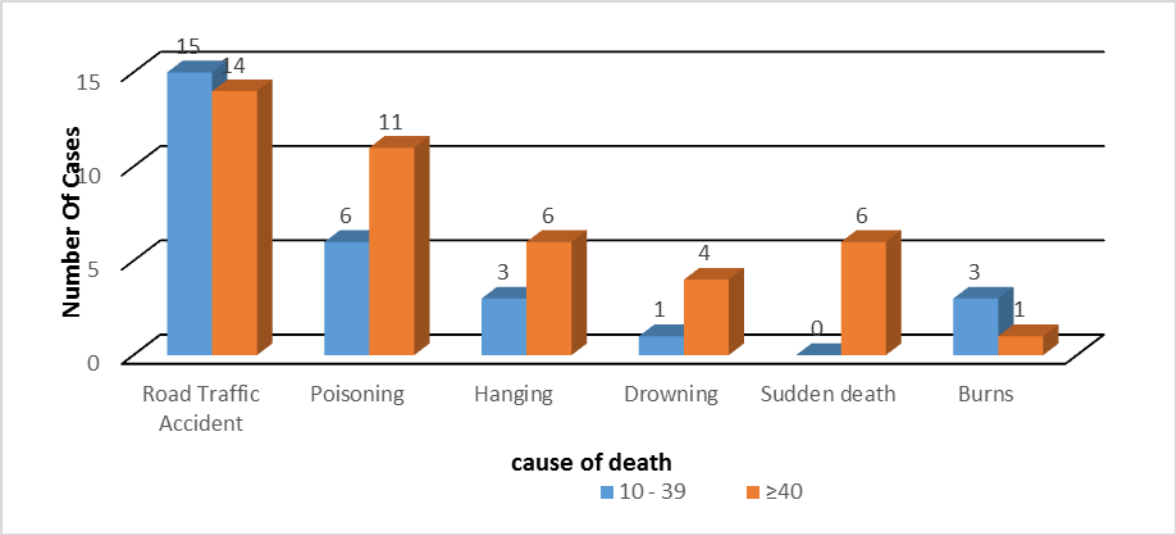
Graph 4: Pie chart showing cause of death of cases

In the current study, the most common cause of death is Road Traffic Accident as it includes 29 (41%) cases, followed by poisoning 17(24%), then hanging 9(13%),6(9%) are sudden death,5(7%) cases are of drowning and 4(6%) cases are of burns.

CAUSE OF DEATH WITH THE AGE DISTRIBUTION

Table 7: Comparison of cause of death with the age distribution

Cause of death	Age groups (in years)		Total N (%)
	10 – 39 N (%)	≥40 years N (%)	
Road Traffic Accident	15 (51.7%)	14 (48.3%)	**29 (41.4%)**
Poisoning	6 (35.3%)	11 (64.7%)	**17 (24.3%)**
Hanging	3 (33.3%)	6 (66.7%)	**9 (12.9%)**
Drowning	1 (20.0%)	4 (80.0%)	**5 (7.1%)**
Sudden death	0 (0.0%)	6 (100.0%)	**6 (8.6%)**
Burns	3 (75.0%)	1 (25.0%)	**4 (5.7%)**
Total	**28 (40.0%)**	**42 (60.0%)**	**70 100.0%**



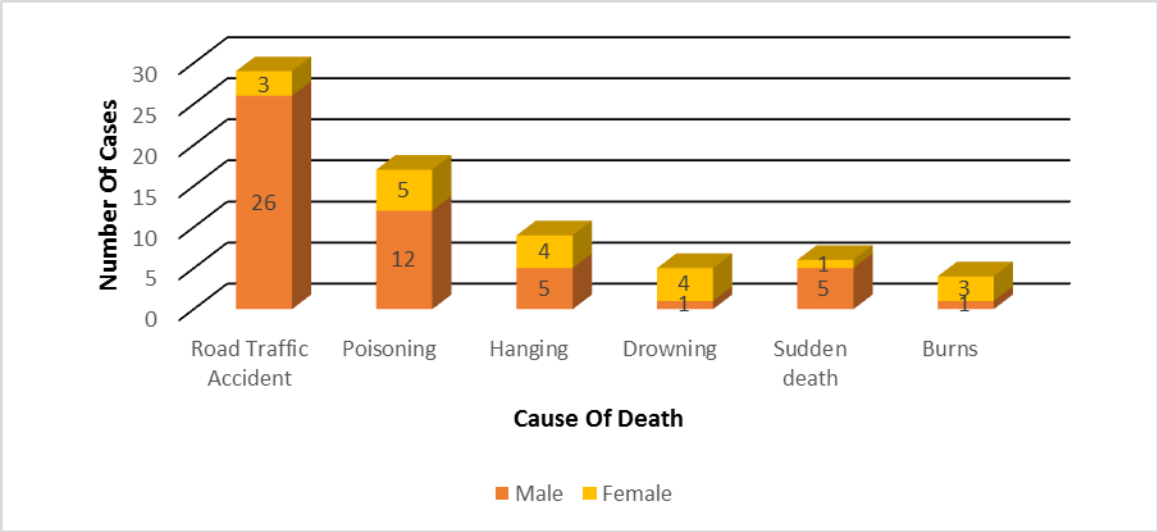
Graph 5: Bar diagram showing cause of death with age distribution

In this study, the most common cause of death is road traffic accidents which consist of total 29(41.4%) cases of which 15(51.7%) cases are in the age group of 10-39 years while 14(48.3%) cases are ≥40 years of the age. Following, it 17(24.3%) cases are of poisoning of which 6(35.3%) cases are in the age group of 10-39 years and 11(64.7%) cases are in the age group of ≥40 years. 9(12.9%) cases are of hanging of which 3(33.3%) cases are in the age group of 10-39 years and 6(66.7%) cases are in age group of ≥40 years.5(7.1%) cases are of drowning of which 4(80%) cases are in age group of ≥40 years. 6(8.6%) cases are of sudden death and all of these cases 6(100%) are in the age group of ≥ 40 years of age. 4(5.7%) cases are of burns of which 3(75.0%) cases are in age group of 10-39 years and 1(25.0%) of the cases are ≥40 years.

CLINICAL/FORENSIC CAUSE OF DEATH WITH GENDER DISTRIBUTION

Table 8: Comparison of clinical/forensic cause of death with gender distribution

Cause of death	Sex		Total N (%)
	Male N (%)	Female N (%)	
Road Traffic Accident	26 (89.7%)	3 (10.3%)	**29 (41.4%)**
Poisoning	12 (70.6%)	5 (29.4%)	**17 (24.3%)**
Hanging	5 (55.6%)	4 (44.4%)	**9 (12.9%)**
Drowning	1 (20.0%)	4 (80.0%)	**5 (7.1%)**
Sudden death	5 (83.3%)	1 (16.7%)	**6 (8.6%)**
Burns	1 (25.0%)	3 (75.0%)	**4 (5.7%)**
Total	**50 (71.4%)**	**20 (28.6%)**	**70 (100.0%)**



Graph 6: Bar diagram showing clinical/forensic cause of death with gender distribution

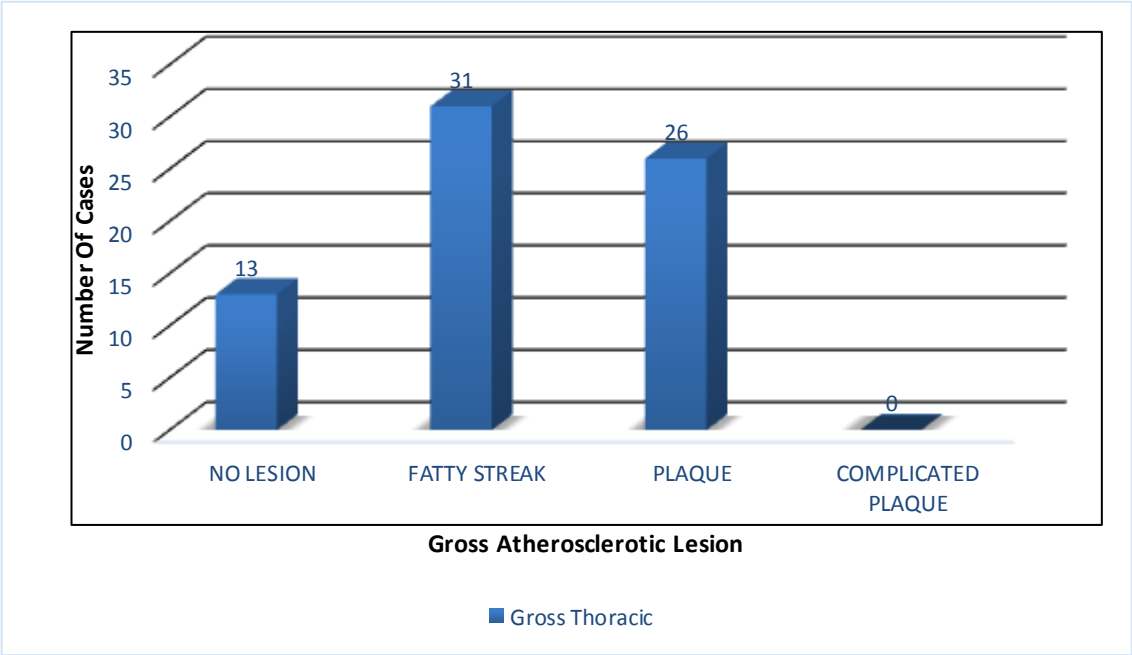
In this study, the maximum deaths are due to road traffic accidents which are 29(41.4%). Among these cases 26(89.7%) cases are male and 3(10.3%) cases are females. Following it 17(24.3%) cases died of poisoning and of which 12(70.6%) cases are males and 5(294%) cases are females.9(12.9%) cases died of hanging of which 5(55.6%) cases are males and 4(44.4%) cases are females.5(7.1%) cases died of drowning of which 1(20.0%) of the cases is male and 4(80%) cases are females.6(8.6%) cases died of sudden death of which 5(83.3%) cases are males and only 1(16.7%) case is female.4(5.7%) cases died of burns in it 1(25.0%) case is male and 3(75.0%) cases are females.

It is observed that majority of the cases those who died of road traffic accident are males 26(89.70%) cases. In cases related to the sudden death 5(83.3%) cases are males. and 1(16.7%) case is female. The cause of death due to drowning and burns are common in female population.

GROSS EXAMINATION OF THORACIC AORTA

Table 9: Gross examination findings of thoracic aorta

GROSS EXAMINATION	NUMBER OF CASES
NO LESION	13 (18.57%)
FATTY STREAK	31 (44.28%)
PLAQUE	26 (37.14%)
COMPLICATED PLAQUE	00
TOTAL	70(100%)



Graph 7: Bar diagram depicting gross examination findings of thoracic aorta

In the gross examination of the thoracic aorta 13(18.57%) cases showed no lesion, the lesions with fatty streaks are 31(44.28%) cases and 26(37.14%) cases showed plaques. No cases of the thoracic aorta showed complicated plaques. Fatty streaks are the most common lesion seen consisting of 31(44.28%) of the cases followed by the plaque lesion 26(37.14%) cases.

GROSS EXAMINATION OF THORACIC AORTA WITH AGE

Table 10: Comparison of gross examination findings of thoracic aorta with age

Age Category (in years)	Gross Examination of Thoracic Aorta			P value
	No lesion	Fatty Streak	Plaque	
10 – 39	10 (35.7%)	15 (53.6%)	3 (10.7%)	
≥40	3 (7.1%)	13 (31.0%)	26 (61.9%)	<0.001
Total	**13 (18.6%)**	**28 (40.0%)**	**29 (41.4%)**	

*Chi-square test

In this study, in the age group of 10-39 years it is observed that 10(35.7%) cases had no lesion, 15(53.6%) cases had fatty streak and 3(10.7%) cases had plaque lesion. It is observed that in age group of ≥40 years 3(7.1%) cases had no lesion, 13(31.0%) cases had fatty streak and 26(61.9%) cases had plaque lesion.

Hence, it is observed in our study that fatty streaks are more common in 10-39 years while plaque lesion is more common in ≥40 years.

Here we conclude that with the age ≥40 years there is increase in the number of plaque lesions.

P value is <0.001 which is statistically significant.

GROSS EXAMINATION OF THORACIC AORTA WITH SEX DISTRIBUTION

Table 11: Comparison of gross examination of thoracic aorta with sex distribution

Gender	Gross Examination of thoracic Aorta			P value
	No lesion	Fatty Lesion	Plaque	
Male	08 (16.0%)	20 (40.0%)	22 (44.0%)	
Female	05 (25.0%)	08 (40.0%)	07 (35.0%)	0.651
Total	**13 (18.6%)**	**28 (40.0%)**	**29 (41.4%)**	

*Fischer's Exact test

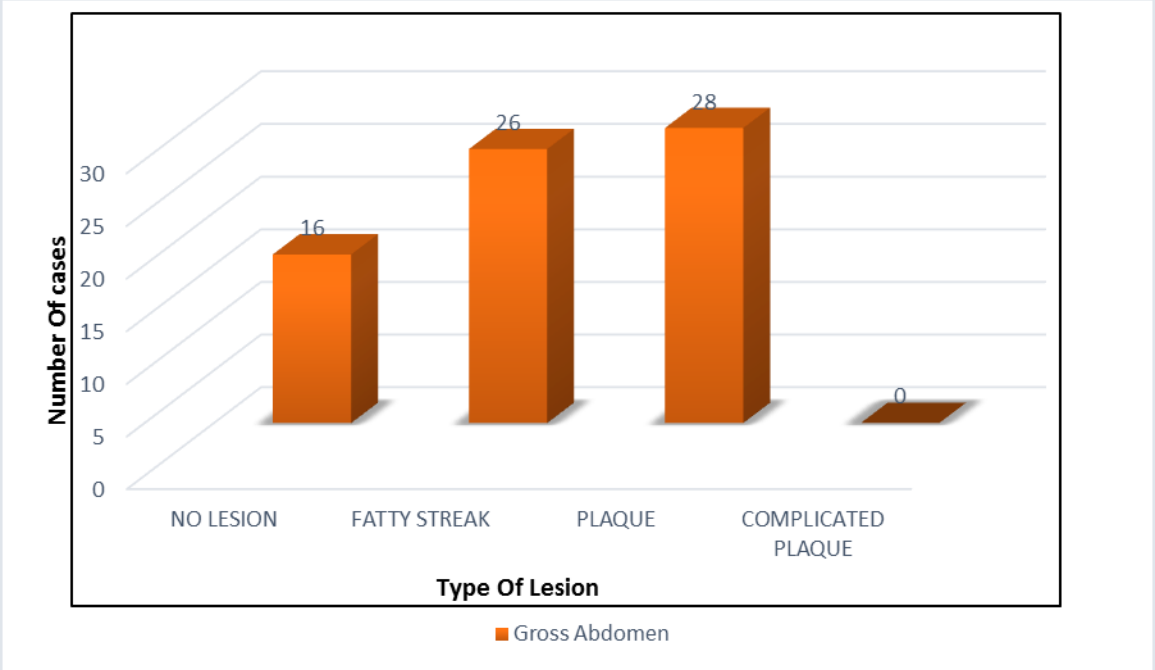
In the comparison of the gross examination of the thoracic aorta with the sex distribution it is observed that in male population, 8(16%) cases had no lesion, 20(40.0%) cases had fatty streak and 22(44%) cases had plaque lesion. While in female population, 5(25%) cases had no lesion, 8(40%) cases had fatty streak and 7(35%) cases had plaque lesion.

Hence, it is observed in our study that in male population most common lesion was plaque while in female population fatty streaks were found to be more common. However, there was no significant correlation between the gender type and the gross examination of the thoracic aorta (p value <0.651).

GROSS EXAMINATION OF ABDOMINAL AORTA

Table 12: Gross examination findings of abdominal aorta

GROSS EXAMINATION OF ABDOMINAL AORTA	NUMBER OF CASES
NO LESION	16(22.8%)
FATTY STREAK	26(37.1%)
PLAQUE	28(40%)
COMPLICATED PLAQUE	00
TOTAL	70(100%)



Graph 8: Bar diagram showing gross examination findings of abdominal aorta

In the gross examination of the abdominal aorta 16(22.8%) cases showed no lesion, the lesions with fatty streaks are 26(37.1%) cases and 28(40%) cases showed plaques. No cases of the thoracic aorta showed complicated plaques.

Plaque are the most common lesion seen consisting of 28(40%) of the cases followed by the fatty streaks 26(37.14%) cases.

GROSS EXAMINATION OF ABDOMINAL AORTA WITH AGE

Table 13: Comparison of gross examination findings of abdominal aorta with age

Age Category (in years)	Gross Examination of Abdominal Aorta			P value
	No lesion	Fatty Lesion	Plaque	
10 – 39	10 (35.7%)	15 (53.6%)	3 (10.7%)	
>40	6 (14.3%)	11 (26.2%)	25 (59.5%)	<0.001
Total	**16 (22.9%)**	**26 (37.1%)**	**28 (40.0%)**	

*Chi-square test

In this study, in the age group of 10-39 years it is observed that 10(35.7%) cases had no lesion, 15(53.6%) cases had fatty streak and 3(10.7%) cases had plaque lesion. It is observed that in age group of ≥40 years 6(14.3%) cases had no lesion, 11(26.2%) cases had fatty streak and 25(59.5%) cases had plaque lesion.

Hence, it is observed in our study that fatty streaks are more common in 10-39 years while plaque lesion is more common in ≥40 years.

Here we conclude that with the age ≥40 years there is increase in the number of plaque lesions.

P value is <0.001 which is statistically significant.

GROSS EXAMINATION OF ABDOMINAL AORTA WITH SEX DISTRIBUTION

Table 14: Comparison of gross examination findings of abdominal aorta with sex distribution

Gender	Gross Examination of Abdominal Aorta			P value
	No lesion	Fatty Lesion	Plaque	
Male	09 (18.0%)	20 (40.0%)	21 (42.0%)	
Female	07 (35.0%)	06 (30.0%)	07 (35.0%)	0.345
Total	**16 (22.9%)**	**26 (37.1%)**	**28 (40.0%)**	

*Fischer's Exact test

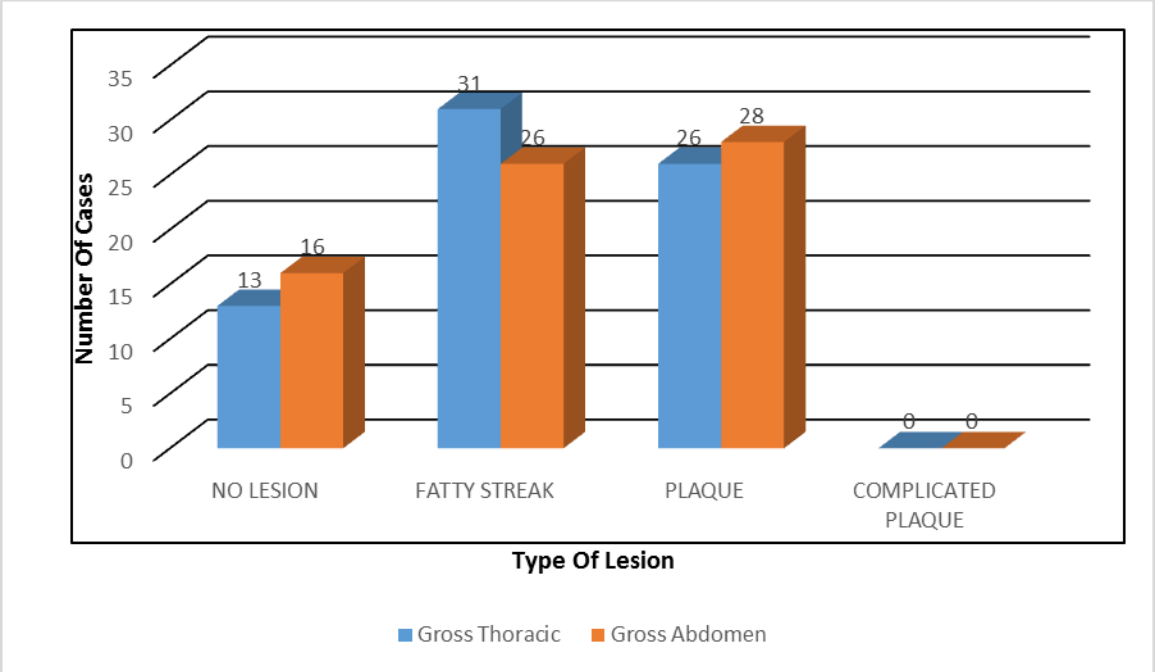
In the comparison of the gross examination of the abdominal aorta with the sex distribution it is observed that in male population,9(18%) cases had no lesion, 20(40.0%) cases had fatty streak and 21(42%) cases had plaque lesion. While in female population, 7(35%) cases had no lesion, 6(30%) cases had fatty streak and 7(35%) cases had plaque lesion. Hence, it is observed in our study that in both male and female population most common lesion was plaque.

However, there was no significant correlation between the gender type and the gross examination of the abdominal aorta (P value <0.345).

COMPARISON OF THE GROSS EXAMINATION OF THORACIC AND ABDOMINAL AORTA

Table 15: Comparison of the gross examination findings of thoracic and abdominal aorta

	No Lesions	Fatty Streak	Plaque	Complicated Plaque	Total
Thoracic	13(44.82%)	31(54.3%)	26(48.14%)	-	70(100%)
Abdomen	16(55.17%)	26(45.6)	28(51.8%)	-	70(100%)
Total	29	57	54	-	140



Graph 9: Bar diagram showing comparison of the gross examination of thoracic and abdominal aorta

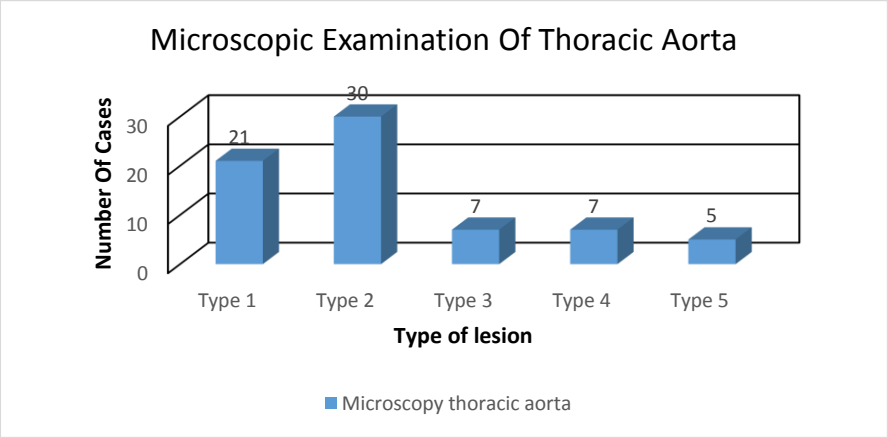
In the study, on comparison of the gross examination between thoracic and abdominal aorta it is observed that fatty streaks are more common in thoracic aorta while plaque lesions are slightly more common in abdominal aorta compared to the thoracic aorta. Fatty streaks was found in 31(44.28%) cases in thoracic aorta and 26(37.1%) cases in abdominal aorta. While 26(37.14%) cases of plaque lesions were seen in thoracic aorta and 28(40%) lesions seen in abdominal aorta.

There was no statistical significant difference noted between the gross examination of thoracic and abdominal aorta.

MICROSCOPIC EXAMINATION OF THORACIC AORTA ACORDING TO AHA CRITERIA

Table 16: Comparison of the gross examination findings of thoracic and abdominal aorta

TYPE OF LESION	NUMBER
TYPE I	21(30%)
TYPE II	30(42.86%)
TYPE III	7(10%)
TYPE IV	7(10%)
TYPE V	5(7.14%)
TYPE VI	00



Graph 10: Bar diagram showing microscopic examination of thoracic aorta according to AHA criteria

On microscopic examination of thoracic aorta done according to the AHA criteria, 21(30%) cases are included in Type I, 30(42.86%) cases are included type II lesions, 7(10%) cases are included in type III lesion, 7(10%) cases are included in type IV lesion and 5(7.14%) of cases are included in type V lesion.

Hence, in our study it is observed that type II lesions are most common on microscopic examination of the thoracic aorta.

MICROSCOPIC COMPARISON OF THORACIC AORTA WITH THE GENDER DISTRIBUTION

Table 17: Comparison of the microscopic findings of thoracic aorta with the gender distribution

Gender	Microscopic examination of thoracic aorta					Total	P value
	Type I	Type II	Type III	Type IV	Type V		
Male	11 (22.0%)	25 (50.0%)	7 (14.0%)	4 (8.0%)	3 (6.0%)	**50 (71.4%)**	
Female	10 (50.0%)	5 (25.0%)	0 (0.0%)	3 (15.0%)	2 (10.0%)	**20 (28.6%)**	**P<0.050**
Total	**21 (30.0%)**	**30 (42.9%)**	**7 (10.0%)**	**7 (10.0%)**	**5 (7.1%)**	**70 (100.0%)**	

*Fischer's Exact test

In the comparison of the microscopic examination of the thoracic aorta with the gender distribution it is observed that in male population,11(22%) cases had Type I lesion, 25(50%) cases had Type II lesion , 7(14%) cases had Type III lesion, 4(8%) had Type IV lesion and 3(6%) cases had Type V lesion. While in female population, 10(50%) cases had Type I lesion, 5(25%) cases had Type II lesion, no cases were reported in Type III lesion, 3(15%) cases had Type IV lesion and 2(10%) cases had Type V lesion.

Hence, it is observed in our study that all the types of the atherosclerotic lesions of thoracic aorta are more common in male population.

There is a significant correlation between the gender type and the gross examination of the thoracic aorta (p value<0.05).

MICROSCOPIC COMPARISON OF THORACIC AORTA WITH THE AGE DISTRIBUTION

Table 18: Comparison of microscopic findings of thoracic aorta with the age distribution

Age groups (in years)	Microscopic Examination of Thoracic aorta					Total	
	Type 1	Type 2	Type 3	Type 4	Type 5		
10-39	14 (50.0%)	14 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**28 (40.0%)**	
≥40	07 (16.7%)	16 (38.1%)	07 (16.7%)	07 (16.7%)	05 (11.9%)	**42 (60.0%)**	**P<0.001**
Total	**21 (30.0%)**	**30 (42.9%)**	**07 (10.0%)**	**07 (10.0%)**	**05 (7.1%)**	**70 (100.0%)**	

* Fischer's Exact test

In this study, on Microscopic examination of thoracic aorta in the age group of 10-39 years it is observed that 14(50%) cases had type I and 14(50%) cases are Type II lesion, while Type III, Type IV and Type V are not reported. It is observed that in age group of ≥40 years 7(16.7%) cases had type I lesion, 16(38.1%) cases had type II lesion, 7(16.7%) cases had type III lesion, 7(16.7%) cases had type IV lesion while 5(11.9%) cases had Type V lesion.

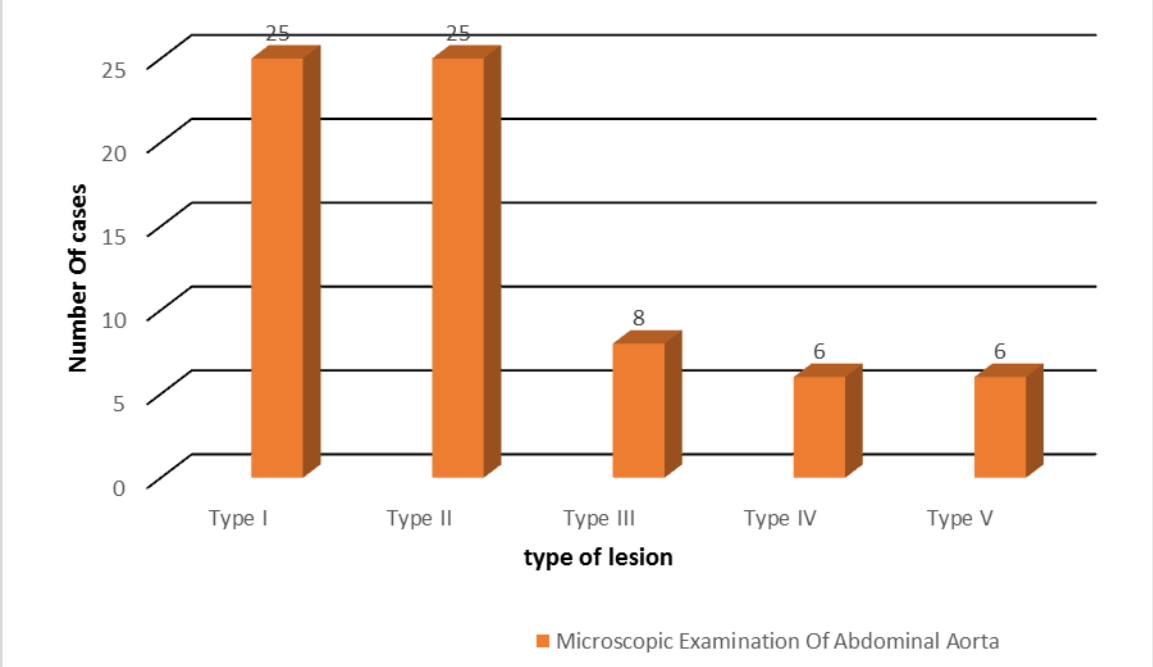
Hence, it is observed in our study that Type III, Type IV and Type V lesions are only reported in age group of ≥40 years while Type I and Type II lesion are more common in age group of 10-39 years.

Here we conclude that with the age ≥40 years there is progression in the type of lesion. P value is <0.001 which is statistically significant.

MICROSCOPIC EXAMINATION OF ABDOMINAL AORTA ACCORDING TO AHA CRITERIA

Table 19: Microscopic examination findings of abdominal aorta according to AHA criteria

TYPE OF LESION	NUMBER
TYPE I	25(35.71%)
TYPE II	25(35.71%)
TYPE III	8(11.42%)
TYPE IV	6(8.5%)
TYPE V	6(8.5%)
TYPE VI	00



Graph 11: Bar diagram showing microscopic examination findings of abdominal aorta according to AHA criteria

On microscopic examination of abdominal aorta done according to the AHA criteria, 25(35.71%) cases are included in Type I, 25(35.71%) cases are included type II lesions, 8(11.42%)cases are included in type III lesion, 6(8.5%)cases are included in type IV lesion and 6(8.5%)of cases are included in type V lesion.

Hence, in our study it is observed that type II and Type I lesions are most common on microscopic examination of the abdominal aorta.

MICROSCOPIC COMPARISON OF ABDOMINAL AORTA WITH THE GENDER DISTRIBUTION

Table 20: Comparison of microscopic findings of abdominal aorta with the gender distribution

Gender	Microscopic Examination of Abdominal Aorta					P value
	Type I	Type II	Type III	Type IV	Type V	
Male	14 (28.0%)	22 (44.0%)	7 (14.0%)	3 (6.0%)	4 (8.0%)	
Female	11 (55.0%)	3 (15.0%)	1 (5.0%)	3 (15.0%)	2 (10.0%)	0.050
Total	**25 (35.7%)**	**25 (35.7%)**	**8 (11.4%)**	**6 (8.6%)**	**6 (8.6%)**	

* Fischer's Exact test

In the comparison of the microscopic examination of the abdominal aorta with the gender distribution it is observed that in male population,14(28%) cases had Type I lesion, 22(44%) cases had Type II lesion, 7(14%) cases had Type III lesion, 3(6%) had Type IV lesion and 4(8%) cases had Type V lesion. While in female population,11(55%) cases had Type I lesion, 3(15%) cases had Type II lesion, 1(5%) case in Type III lesion, 3(15%) cases had Type IV lesion and 2(10%) cases had Type V lesion.

Hence, it is observed in our study that all the types of the atherosclerotic lesions of abdominal aorta are more common in male population.

There is a significant correlation between the gender type and the gross examination of the thoracic aorta (P value <0.05).

MICROSCOPIC COMPARISON OF ABDOMINAL AORTA WITH THE AGE DISTRIBUTION

Table 21: Comparison of microscopic findings of abdominal aorta with the age distribution

Age groups (in years)	Microscopic Examination of Abdominal Aorta					Total	
	Type I	Type II	Type III	Type IV	Type V		
10 – 39	20 (71.4%)	08 (28.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	**28 (40.0%)**	
≥40	05 (11.9%)	17 (40.5%)	08 (19.0%)	06 (14.3%)	06 (14.3%)	**42 (60.0%)**	**P<0.001**
Total	**25 (35.7%)**	**25 (35.7%)**	**08 (11.4%)**	**06 (8.6%)**	**06 (8.6%)**	**70 (100.0%)**	

* Fischer's Exact test

In this study, on Microscopic examination of abdominal aorta in the age group of 10-39 years it is observed that 20(71.4%) cases had type I and 8(28.6%) cases are Type II lesion, while Type III, Type IV and Type V are not reported. It is observed that in age group of ≥40 years 5(11.9%) cases had type I lesion, 17(40.5%) cases had type II lesion, 8(19.0%) cases had type III lesion, 6(14.3%) cases had type IV lesion while 6(14.3%) cases had Type V lesion.

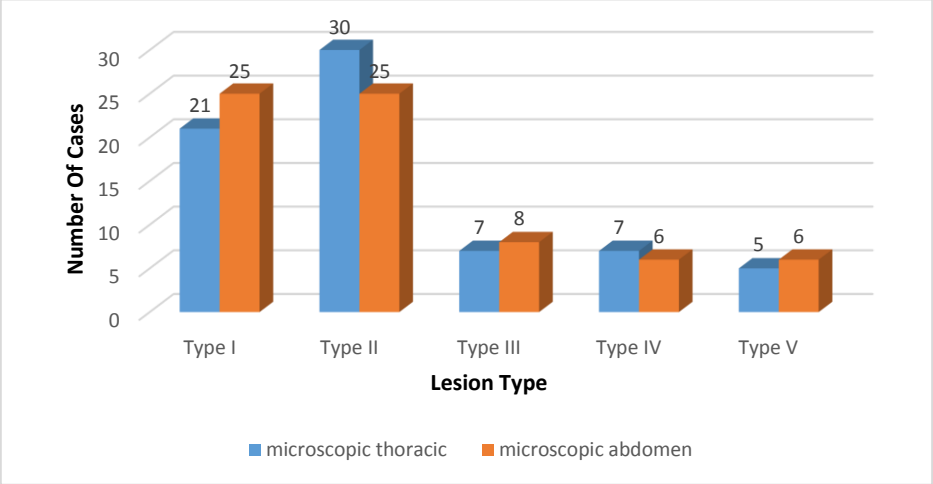
Hence, it is observed in our study that Type III, Type IV and Type V lesions are only reported in age group of ≥40 years while Type I and Type II lesion are more common in age group of 10-39 years.

Here we conclude that with the age ≥40 years there is progression in the type of lesion. P value is <0.001 which is statistically significant.

COMPARISON IN MICROSCOPY BETWEEN THORACIC AND ABDOMINAL AORTA

Table 22: Comparison of microscopic findings between thoracic and abdominal aorta

Type of Lesion	Thoracic Aorta	Abdominal Aorta
Type I	21(30%)	25(35.71%)
Type II	30(42.86%)	25(35.71%)
Type III	7(10%)	8(11.42%)
Type IV	7(10%)	6(8.5%)
Type V	5(7.14%)	6(8.5%)
Type VI	00	00



Graph 12: Bar chart showing comparison in microscopy between thoracic and abdominal aorta

In this study, it is seen that the Type I lesion were more common in abdominal aorta, Type II and type IV lesion more common in thoracic aorta ,Type III and Type V lesions more common in abdominal aorta.

COMPARISON OF GROSS AND MICROSCOPIC FINDINGS OF THORACIC AORTA

Table 23: Comparison of gross and microscopic findings of thoracic aorta

Microscopic Examination Of Thoracic aorta		Gross examination of Thoracic Aorta			Total	
		No lesion	Fatty Lesion	Plaque		
Type 1		12	7	2	**21**	
		(57.1%)	(33.3%)	(9.5%)	**(30.0%)**	
Type 2		1	21	8	**30**	
		(3.3%)	(70.0%)	(26.7%)	**(42.9%)**	
Type 3		0	0	7	**7**	
		(0.0%)	(0.0%)	(100.0%)	**(10.0%)**	**P<0.001**
Type 4		0	0	7	**7**	
		(0.0%)	(0.0%)	(100.0%)	**(10.0%)**	
Type 5		0	0	5	**5**	
		(0.0%)	(0.0%)	(100.0%)	**(7.1%)**	
Total		**13**	**28**	**29**	**70**	
		(18.6%)	**(40.0%)**	**(41.4%)**	**(100.0%)**	

* Fischer's Exact test

On comparison of the gross examination of the thoracic aorta with the microscopic examination, it was noticed that 12(57.1%) cases with no lesion on gross examination were reported to be Type I lesion on microscopy. Similarly 21(70%) cases with fatty streaks on gross examination were reported to be Type II lesion on microscopy. While all cases of Type III, Type IV and Type V lesions had plaque lesions.

There was a significant correlation noted between the microscopy and the gross findings of thoracic aorta ($p<0.001$)

In type IV lesions, the tissue layer between the lipid core and the endothelial surface is mainly the intima always occurs before the development of the lesion, when the fibrous tissue mainly the collagen increases in the “nearly normal” cover of a lipid core the lesion is then labelled type V. In conventional 5-micron thick histological sections, or with the unaided eye, the upper intimal layer of a type IV lesion is indistinguishable from the fibrotic cover (fibrous cap) of a type V lesion, which is why both type IV and type V lesions are indiscriminately labelled fibrous plaque.

COMPARISON OF GROSS AND MICROSCOPIC FINDINGS OF ABDOMINAL AORTA

Table 24: Comparison of gross and microscopic findings of abdominal aorta

Microscopy Examination Of Abdominal Aorta		Gross Examination Abdomen Aorta			Total	
		No lesion	Fatty Lesion	Plaque		
Type I		15	10	0	**25**	
		(60.0%)	(40.0%)	(0.0%)	**(35.7%)**	
Type II		1	16	8	**25**	
		(4.0%)	(64.0%)	(32.0%)	**(35.7%)**	
Type III		0	0	8	**8**	
		(0.0%)	(0.0%)	(100.0%)	**(11.4%)**	**P<0.001**
Type IV		0	0	6	**6**	
		(0.0%)	(0.0%)	(100.0%)	**(8.6%)**	
Type V		0	0	6	**6**	
		(0.0%)	(0.0%)	(100.0%)	**(8.6%)**	
Total		**16**	**26**	**28**	**70**	
		(22.9%)	**(37.1%)**	**(40.0%)**	**(100.0%)**	

* Fischer's Exact test

On comparison of the gross examination of the abdominal aorta with the microscopic examination, it was noticed that 15(60.0%) cases with no lesion on gross examination were reported to be Type I lesion on microscopy. Similarly 16(64%) cases with fatty streaks on gross examination were reported to be Type II lesion on microscopy. While all cases of Type III, Type IV and Type V lesions had plaque lesions.

There was a significant correlation noted between the microscopy and the gross findings of abdominal aorta ($p<0.001$)

COMPARISON OF THE MAST CELL COUNT IN NON ATHEROSCLEROTIC AND ATHEROSCLEROTIC PART OF THORACIC AND ABDOMINAL AORTA

Table 25: Comparison of the mast cell count in non-atherosclerotic and atherosclerotic part of thoracic and abdominal aorta

	Non atherosclerotic part (Control)	Atherosclerotic part of aorta(case)	
		Thoracic	Abdominal
Mean Mast cell count per 10hpf	3.4±2.4	9.5±7.6	9.1±6.5

In the above table, it is seen that the mean mast cell is (3.4±2.4)/10hpf in the non-atherosclerotic segment of the aorta and the mean mast cell is (9.5±7.6)/10hpf in thoracic aorta and mean mast cell is(9.1±6.5)/10hpf in abdominal aortas.

It is noted that when mast cell count is compared between the non-atherosclerotic part and atherosclerotic part of the thoracic and abdominal aorta there is increase in the count of the mast cells.

COMPARISON OF THE TYPE/GRADE OF ATHEROSCLEROTIC LESION ACCORDING TO AHA CRITERIA OF THE THORACIC AORTA WITH THE MAST CELL COUNT

Table 26: Comparison of the type of atherosclerotic lesion according to AHA criteria of the thoracic aorta with the mast cell count

	Mean (IQR) of Mast cell Count/10 hpf					
M/E	**Type I**	**Type II**	**Type III**	**Type IV**	**Type V**	**P value**
Thoracic	3.4±2.4	7.8±3.4	12.7±5.1	19.1±2.6	28.2±1.09	<0.001

*Anova test

On comparison of the mast cell count with the Type of atherosclerotic lesion it is observed that in type I lesion of the thoracic aorta, the mean of mast cell count is (3.4±2.4)/10 hpf, while in type II lesion mean mast cell is (7.8±3.4)/10hpf, mean mast cell in type III lesion is (12.7±5.1)/10hpf, mean mast cell in type IV lesion is (19.1±2.6) / 10hpf and mean mast cell in type V is 28.2+-1.09/10hpf.

Hence, in our study it is observed that there is an increase in the mast cell count with increase in type of lesion which is a statistically significant finding (p value<0.001).

Hence, we conclude that with progression of atherosclerotic lesion there is progressive increase in the mast cell count.

COMPARISON OF THE TYPE/GRADE OF ATHEROSCLEROTIC LESION ACCORDING TO AHA CRITERIA OF THE ABDOMINAL AORTA WITH THE MAST CELL COUNT

Table 27: Comparison of the type/grade of atherosclerotic lesion according to AHA criteria of the abdominal aorta with the mast cell count

M/E	Mean (IQR) of Mast cell Count/10hpf					P value
	Type I	Type II	Type III	Type IV	Type V	
Abdomen	3.5±2.4	8.7±3.4	13.3±5.3	18.6±1.03	19.1±6.7	<0.001

On comparison of the mast cell count with the type of atherosclerotic lesion it is observed that in type I lesion of the abdominal aorta, the mean of mast cell count is (3.5±2.4) /10hpf, while in type II lesion mean mast cell is (8.7±3.4)/10hpf, mean mast cell in type III lesion is (13.3±5.3)/10hpf, mean mast cell in type IV lesion is (18.6±1.03)/10hpf and mean mast cell in type V is (19.1±6.7)/10hpf.

Hence, in our study it is observed that there is an increase in the mast cell count with increase in type of lesion in abdominal aorta .This finding is statistically significant with p value <0.001. Hence, we conclude that with progression of atherosclerotic lesion there is progressive increase in the mast cell count.

PHOTOGRAPHS

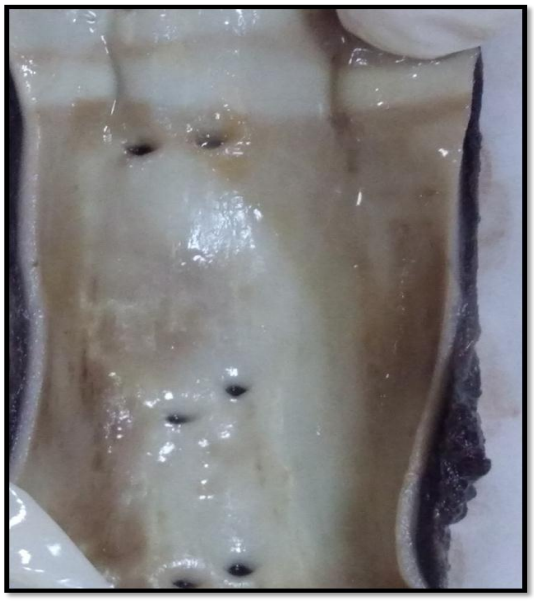


Figure 11: Gross photograph of an non-atherosclerotic aorta

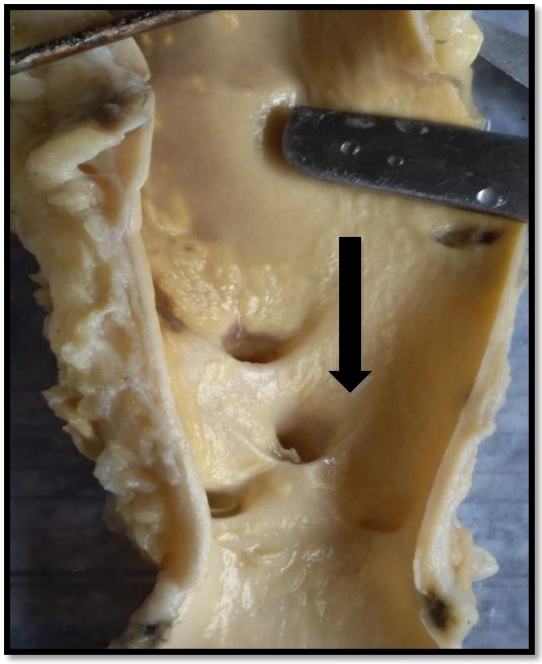


Figure 12: Macroscopy/gross photograph of a fatty streak in aorta.

Arrow pointing towards the fatty streak

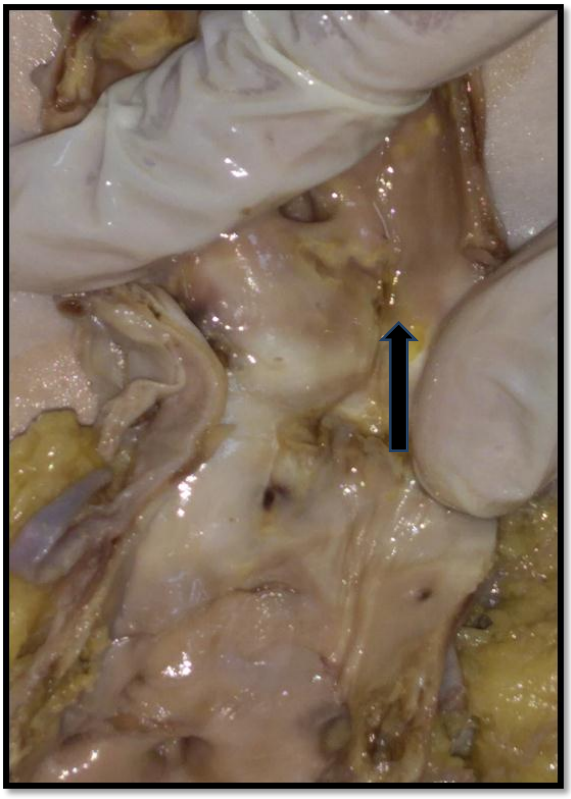


Figure 13: Gross photograph showing plaque lesion in aorta.

Arrow pointing towards the plaque lesion

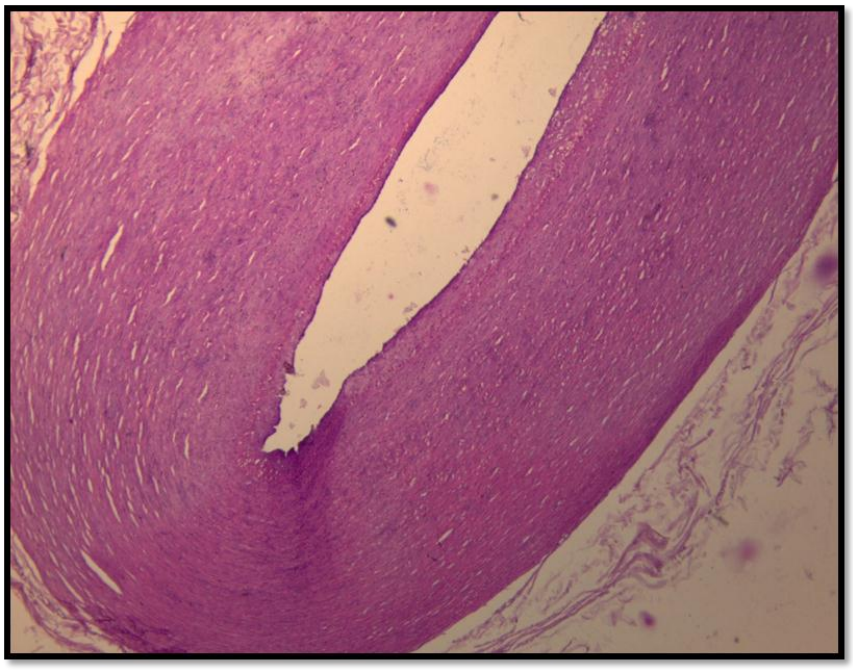


Figure 14: Microphotograph showing non-atherosclerotic aorta (H and EX4)

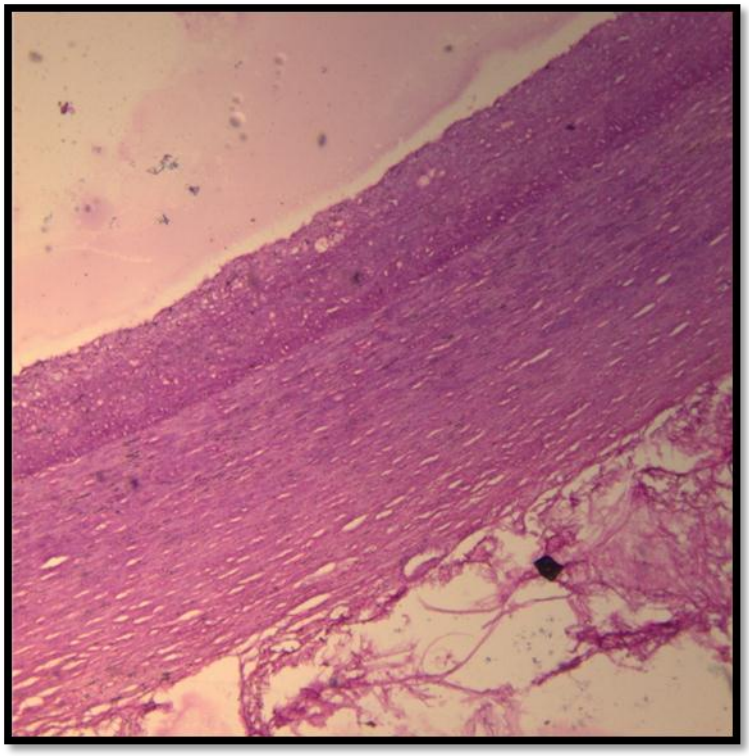


Figure 15: Microphotograph showing type II atherosclerotic aorta (H and EX10)

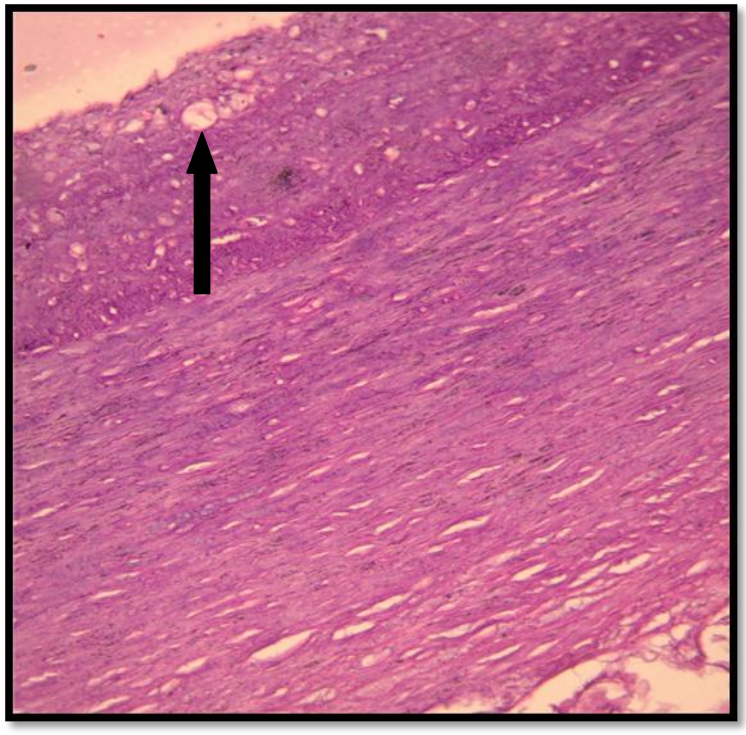


Figure 16: Microphotograph showing type II atherosclerotic aorta (H and EX40)

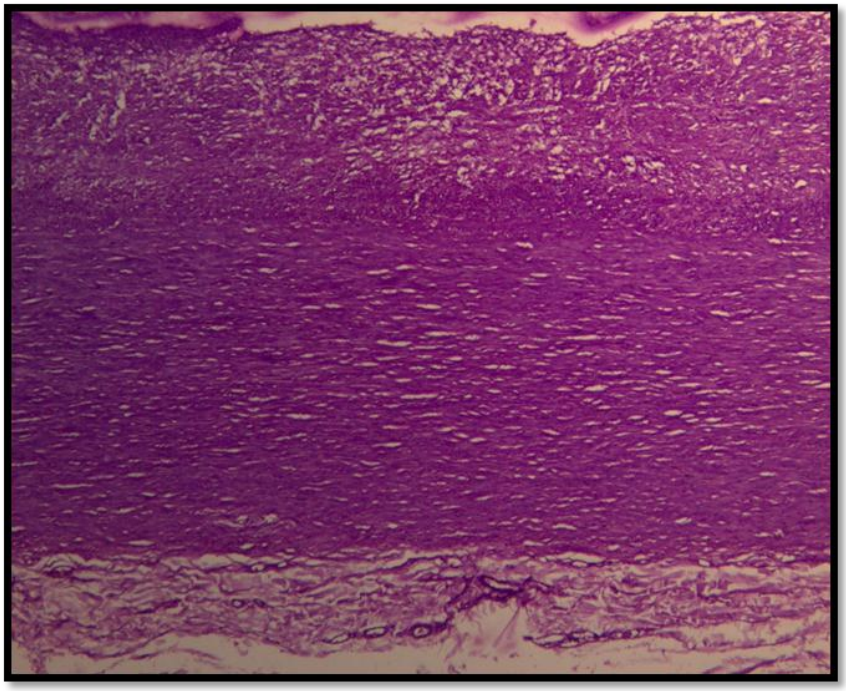


Figure 17: Microphotograph showing Type III atherosclerotic aorta (H and EX10)

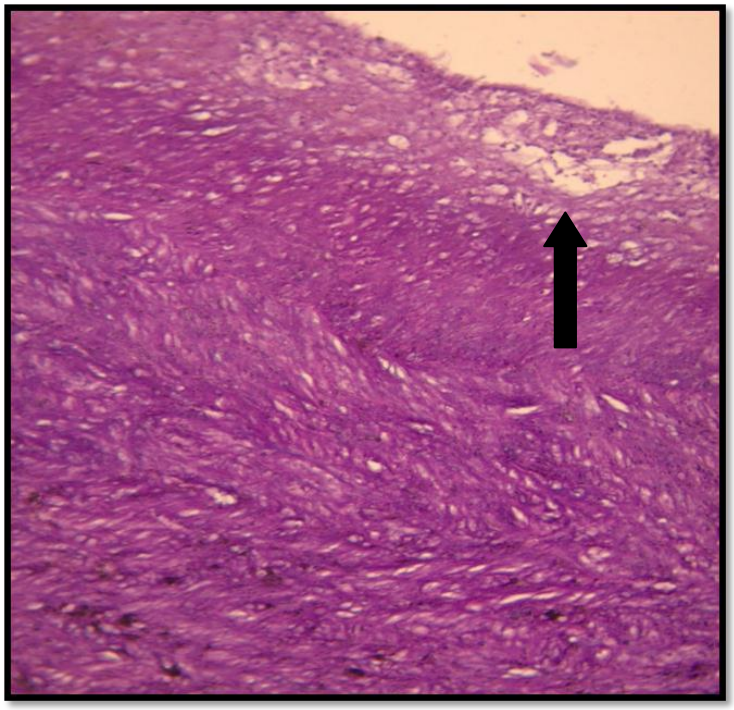


Figure 18: Microphotograph showing Type III (preatheromatous lesion) atherosclerotic aorta (Hand EX40). Arrow pointing area with accumulation of foam cells and macrophages

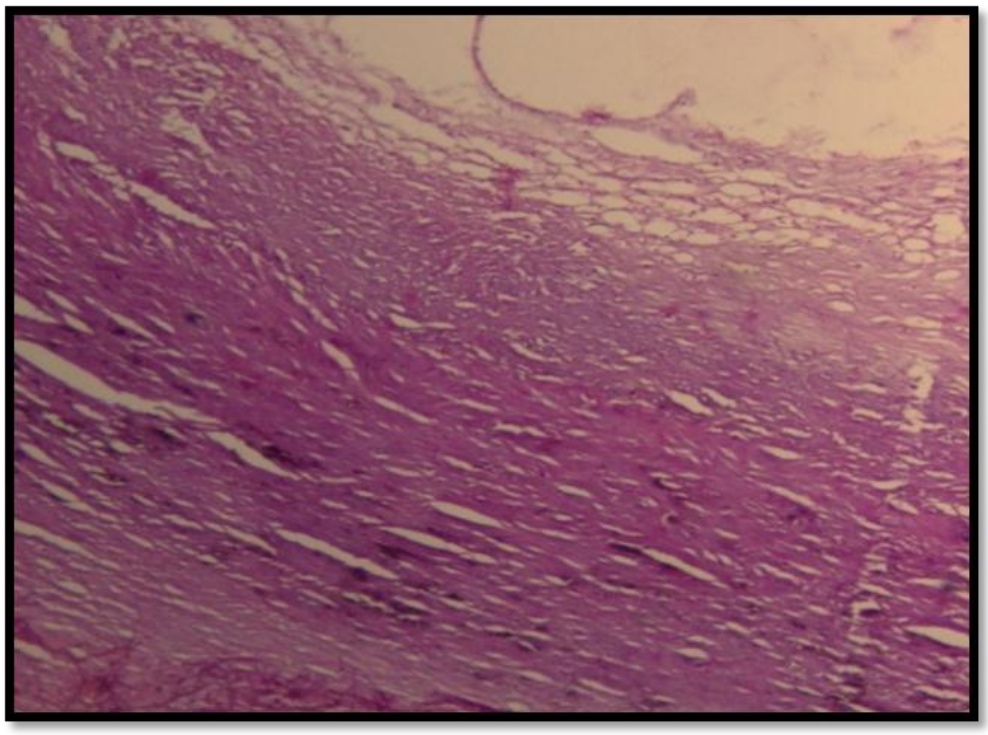


Figure 19: Microphotograph showing type IV atherosclerotic aorta H and EX10)

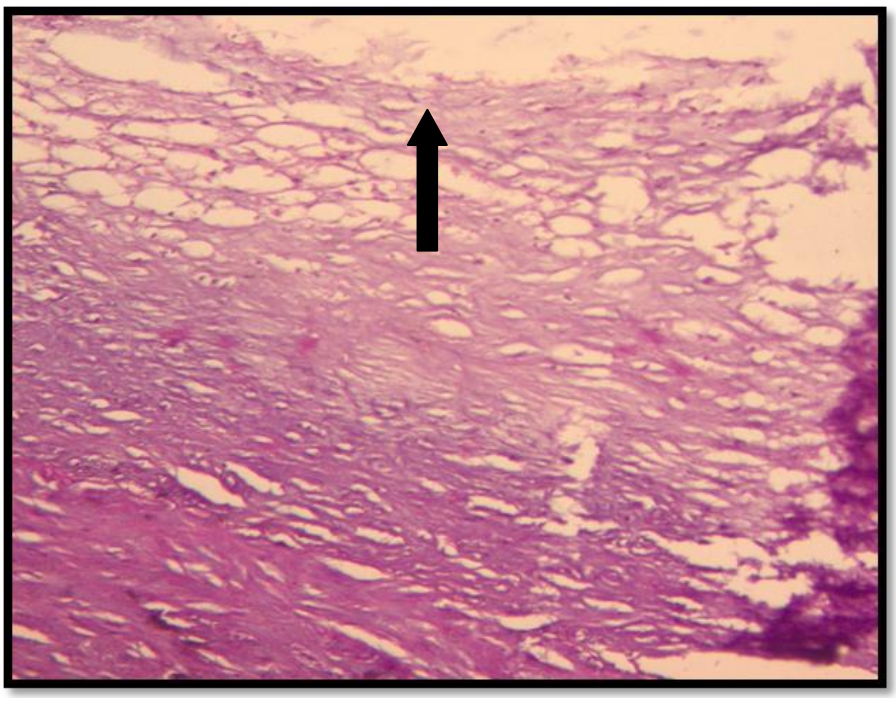


Figure 20: Microphotograph showing Type IV atherosclerotic aorta (H and EX40).

Arrow showing abundant foamy macrophages with inflammatory cells

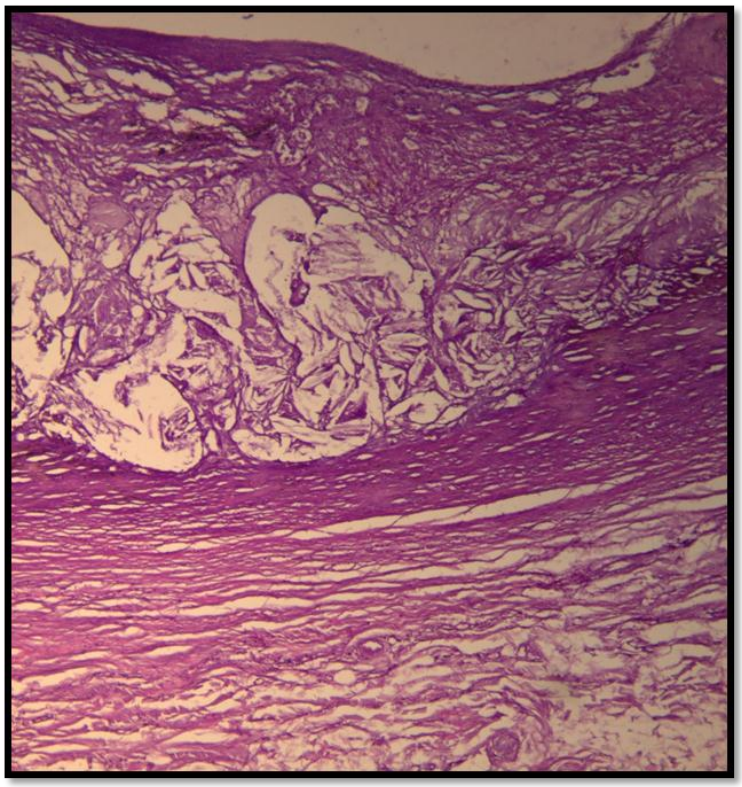


Figure 21: Microphotograph showing Type V atherosclerotic aorta (H and EX10)

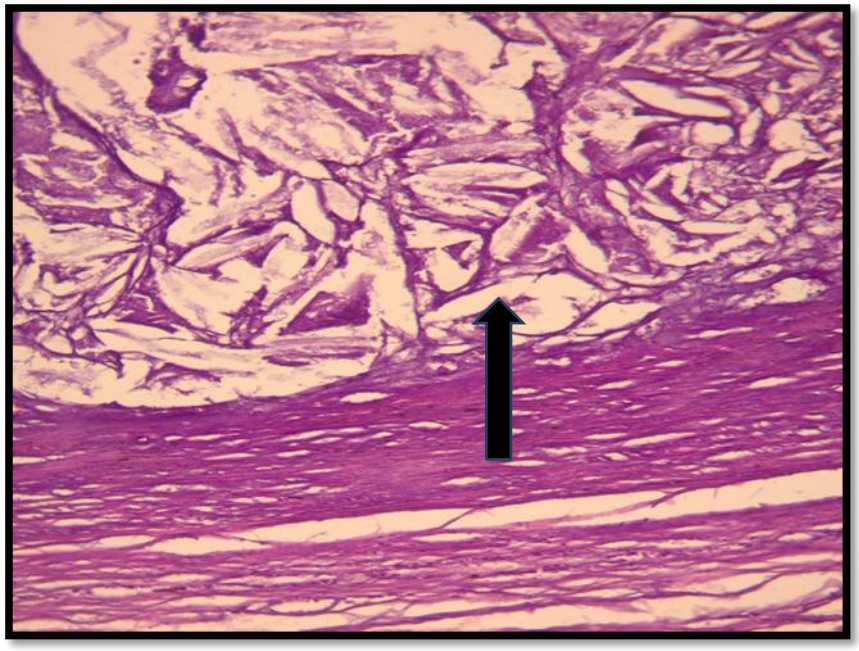


Figure 22: Microphotograph showing Type V atherosclerotic aorta (H and EX40).

Showing abundant cholesterol clefts

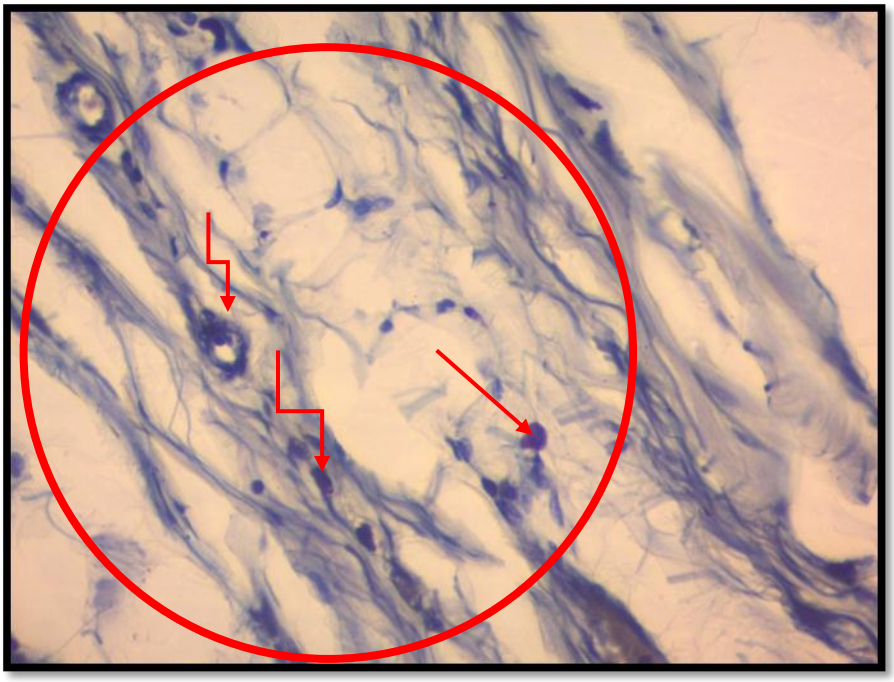


Figure 23: Microphotograph showing mast cell in atherosclerotic aorta in Type III lesion (Toludine Blue Stain X4)

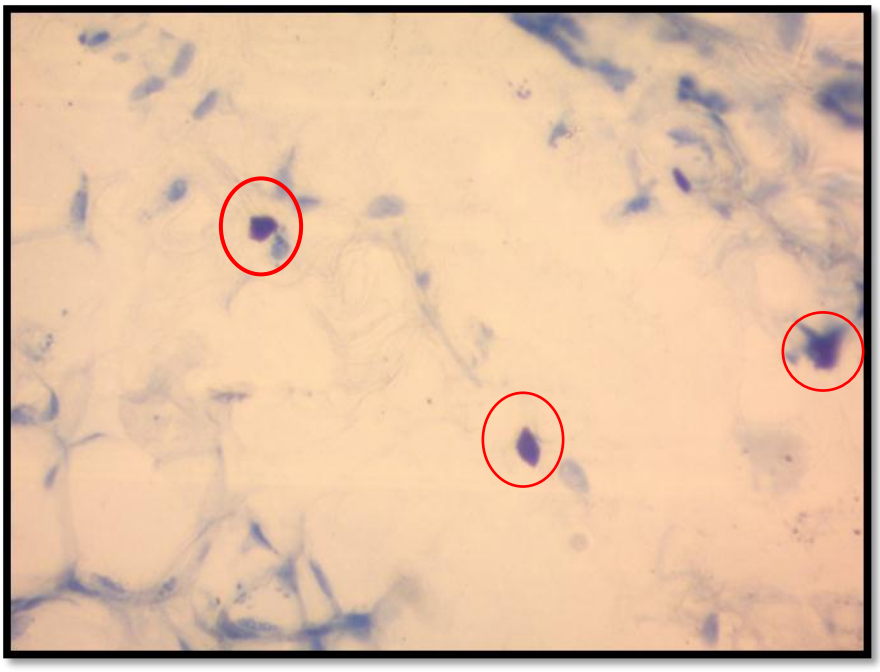


Figure 24: Microphotograph showing mast cell in atherosclerotic aorta in Type IV lesion (Toludine Blue Stain X40)

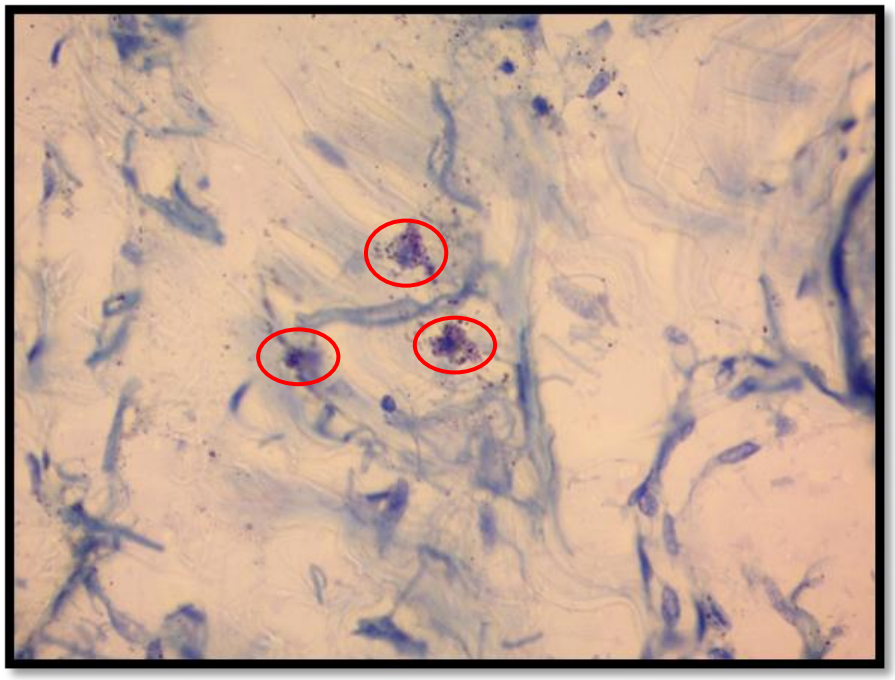


Figure 25: Microphotograph showing mast cell in atherosclerotic aorta in Type V lesion (Toludine Blue Stain X40)

DISCUSSION

Atherosclerosis is one of the most important cause of death and disability in India and many other countries. By 2020, cardiovascular disease would become the leading cause of death and disability over infectious diseases globally. Cardiovascular disease comprises of atherosclerotic vascular diseases like coronary heart disease (CHD), cerebrovascular disease (CBVD), and peripheral arterial diseases.[94,95]

In India, more than 10 million deaths were reported annually related to CVD. Around 20.3% and 16.9% of all these deaths were reported in men and women respectively.[96] It is reported that India has suffered from the highest loss in productive years due to the cardiovascular problems worldwide.[94] There are regional variations reported in the prevalence of CVD. Several studies have reported the prevalence of CAD in south Indian population to be around 11%. There is a 10 fold increase in the prevalence of CAD in urban India during the last 40 years. Prevalence of CHD in urban areas of Northern states like J&K, Delhi, U.P. and Rajasthan have a prevalence rate of 6-10%. The rates in the rural areas are 6-7% in Jammu and Kashmir, 3-5% in Himachal Pradesh and Punjab among the Northern states while in Rajasthan, it is 3-5%.[97]

Atherosclerosis is a chronic inflammatory process and various inflammatory cell types are involved. These inflammatory cells enter the plaque from the circulation and thereby creating an inflammatory environment surrounding the vessel wall.[19] The trend of increased number of the mast cell is observed in many chronic inflammatory processes.[89,90]

AGE BASED DISTRIBUTION OF ATHEROSCLEROSIS

Atherosclerosis is a chronic inflammatory process which shows progression of the lesions the age advances. The process of atherosclerosis starts when a person is young and worsens with age.

In the present study, the age of the cases range from 15-80years. The majority of cases 19(27%) belong to the age distribution of 40-49 years with the mean age of 43.77±16.39 years.

Another study done by Ferraz ML et al (2012) observed that the age of the patients range from 18 to 85 years.[98]

In another study by Ahmed S et al (n=50) 2013 the age of the cases range from 19-80 years. The majority of the cases were from 3rd to 4th decades of life forming 58% of total number of cases studied.[102]

Hence, the age range of the present study was in concordance with the studies by Ferraz et al and Ahmed S et al. However, the majority of the cases in the present study were found in fifth decade which is discordant to the study by Ahmed S et al in which majority of the cases were in fourth decade.

Table 28: Comparison of the age of patients with other studies

STUDY	PLACE	AGE (years)	Mean Age
Present Study(n=70)	Karnataka	15-80	43.77±16.39
Ferraz ML et al (n=141)[98]	Brazil	18-85	-
Thej et al (n=113)[29]	Karnataka	8-85	37.11±15.69
Luciana SR et al (n=44)[99]	Brazil	36-69	52±9.8
Ahmed S et al (n=50)[102]	Karnataka	19-80	-
Lokesh H et al (n=91)[100]	Karnataka	7-72	39.23±14.28
Porwal V et al (n=103)[103]	Ajmer	15-80	-
Garg et al (n=115)[104]	Haryana	29-80	52±14

In a study done by Garg et al in 2011,115 autopsy cases were studied, and was observed 52(45.2%) atherosclerotic lesions were found in the cases >40 years of age.[104]

Similarly, a study done by Thej et al in 2012 aortas were taken from 113 autopsy cases it is seen that the degree of atherosclerosis increases with the increase of age. 90% cases of atherosclerosis were seen in older (35-85 years) individuals.[29]

In the present study, it is noted that 83.3% of cases with atherosclerotic lesion belong to the age group of >40 years which is more than the percentage of atherosclerotic lesion in the similar age group in a study done by Garg et al 52(45.2%)[104] However, it is less than the study done by Thej et al (90%).[29]

Table 29: Comparison Of the percentage of atherosclerotic lesion with other studies

Study	Number of cases	% of atherosclerotic lesion
Present study	70	83.3%
Garg et al[104]	115	45.2%
Porwal et al[103]	103	41.7%
Thej et al[29]	113	90%

Another study done by Porwal et al in 2016, 103 cases were taken and it is seen that 41.7% showed atherosclerotic lesions in age group of>40 years. Out of these atherosclerotic lesions Type II atherosclerotic lesion were 1(25%), Type III were 7(16.6%), Type IV were 10(41%), Type V 9(64.2%) n the age group of >40 years.[103]

In the present study, it is observed that of all the Type II lesions, 16(53.3%) lesion were noted in the age group of >40 years. However, 100% of type III lesions, type IV lesions and type V lesions were noted in age group of > 40 years.

Hence, the present study is in concordance with the study done by Porwal et al which shows there is progressive increase in the type of atherosclerotic lesion, in age group > 40 years.[103]

Similar observation was also noted in a study done by Ferraz ML et al (2012) which mentioned that the degree of atherosclerosis is higher in the elderly age group.[98]

Similarly, the study done by Luciana et al also shows a positive and significant correlation with the degree of the atherosclerosis and age of patient (p=0.020).[99] This was in concordance with the present study which also showed positive correlation with degree of atherosclerosis and age of patient (p <0.050).

Atherosclerotic plaques shows various evidence of cellular senescence that is characterized by reduced cell proliferation, irreversible growth arrest and apoptosis, elevated DNA damage, epigenetic modifications, and telomere shortening and dysfunction. Not only is cellular senescence associated with atherosclerosis, but also it is seen cellular senescence promotes atherosclerosis.[101] Hence, from the comparison with several other studies it is seen that as the age progress the course of the atherosclerotic process also progress.

SEX BASED DISTRIBUTION OF ATHEROSCLEROSIS

Men and women have found to have variation in prevalence of atherosclerosis. Men have greater prevalence of atherosclerosis than females probably due to the protective effect of oestrogen in females.[105]

Table 30: Comparison of the sex ratio of the cases with other studies

STUDY	No. of cases	SEX ratio
Present Study	70	5:2
Thej et al[29]	113	2:1
Luciana SR et al[99]	44	6:5
AHMED S et al[102]	50	7:3
Lokesh H et al[100]	91	2.8:1

In the present study, the male: female ratio was 5:2.The incidence of atherosclerotic lesion in males in thoracic aorta was 39(78%). and in abdominal aorta was 36(72%). While in females it was 10(50%) in thoracic aorta and 9(45%) in abdominal aorta.

In a study done by Thej et al 2012 (n=113), the incidence of atherosclerotic lesion in males was 80% and females was 75%.[29]

In another study done by Ahmad S et al 2013 (n=50), the incidence of atherosclerotic lesion in males was 31 (77.5%) and females was 9(27.2%).[102]

In a study done by Luciana et al 1999(n=44) the incidence of atherosclerotic lesion in males was 24 (66.7%) and females was 20(33.3%).[99]

Hence, the present study is in concordance with the studies done by Ahmad S et al, Thej et al and Luciana et al which also mentions that atherosclerotic lesions are more common in the male.

However, in the present study population there was no significant correlation noted of atherosclerosis with the gender type (p value <0.651).

This indicates that the complexity of the atherosclerotic lesions depends on several other factors. These factors include regional location, family history, smoking and genetic makeup.[30]

COMPARISON OF THE CAUSE OF DEATH WITH OTHER STUDIES

In the present study, the most common cause of death is Road Traffic Accident (RTA) 21(49%) followed by Poisoning 17 (24%), hanging 9(13%), Drowning 5(7%), sudden death 6(9%) and Burns 4 (6%). Maximum victims of the RTA are males in the present study.

Table 31: Comparison of cause of death with other studies

STUDY	CAUSE OF DEATH						
	RTA	Poisoning	Hanging	Drowning	Sudden death	Burns	Others
Present study	21(49%)	17(24%)	9(13%),	5(7%)	6(9%)	4(6%)	-
Thej et al[29]	52(46%)		-	-	-	6 (5.3%)	55(48.7%)
Lokesh et al[100]	42 (46.1%)	21 (23.1%)	5(5.5%)	-	-	5(5.5%)	

In a study done by Thej et al (2012(most common cause of death was road traffic accidents (RTA) 52 (46%), which is in concordance with the present study.[29] The other causes of death mentioned by Thej et al (2012) are suicidal poisoning (37.1%), burns (5.3%), cut throat injuries, alcohol intoxication, ruptured uterus and hypertensive encephalopathy. It observed that maximum victims of RTA cases were males (40%) and maximum victims of burns were females (6.67%).[29]

Study done by Lokesh et al mentioned that most common cause of death was road traffic accidents 42(46.1%), followed by poisoning 21(23.1%), hanging and burns 5(5.5%) each. This study is in concordance with the present study.[100]

In the present study random cases were taken which included the cases that had come for medico-legal investigation so the cause of death with the atherosclerosis in the patients has no significant correlation.

MICROSCOPIC COMPARISON OF THE ATHEROSCLEROTIC LESIONS BETWEEN THORACIC AND ABDOMINAL AORTA BY AHA GRADING SYSTEM

It is well known fact that atherosclerosis is a focal disease that is manifested throughout the arterial vasculature. There is difference in the flow parameters within various vascular beds which results in the localization of atherosclerosis, which tends to occur at sites of low shear stress, turbulence, and oscillating flow.[106]

In the present study, type 1 lesion was more common in abdominal aorta 25(35.7%) compared to thoracic aorta 21(30%),Type II lesion is more common in thoracic aorta 30(42.8%) compared to abdominal aorta 25(35.7%), Type III is more common in abdominal aorta 8(11.4%) compared to thoracic aorta 7(10%), Type IV is more common in thoracic aorta 7(10%) compared to abdominal aorta 6(8.5%) while type V lesion is more common in abdominal aorta 6(8.5%) compared to 5(7.1%) in thoracic aorta. In the present study there was no significant difference noted between the atherosclerotic lesion types in thoracic aorta with the types of atherosclerotic lesion in abdominal aorta.

According to the modified AHA criteria Type I and Type II lesions are sometimes combined under Non-Progressive Atherosclerotic Lesions and Type III belongs to the intermediate group and, Type IV, Type V, Type VI lesions are included under Progressive Atherosclerotic Lesion According to Modified AHA criteria.

Table 32: Comparison of microscopic typing / grading with other studies for thoracic aorta

	NPAL	PAL
Present study	51(86%)	19(14.071%)
Thej et al[29]	22(19.4%)	91(80.5%)
Lokesh et al[100]	80.2%	18(19.8%)

In the present study, 7 (10%) cases were Type III lesion, 7(10%) cases were type IV lesion and 5(7.14%) cases were type V lesion and there were no Type VI lesion in thoracic aorta according to AHA classification. These lesions are Progressive atherosclerotic lesion according to the Modified AHA classification and comprised of 19(14.071%) cases. This study is in concordance with the study done by Lokesh et al where progressive atherosclerotic lesion (PAL) includes 18(19.8%) cases in thoracic aorta.[100]

In another study done by Thej et al in 2012 in 113 cases, 91(80.5%) cases were included Progressive Atherosclerotic Lesion in thoracic aorta.[29] It is in discordance with the present study where 19(14.071%) cases were included in Progressive atherosclerotic lesion in thoracic aorta.

Table 33: Comparing of microscopic typing / grading with other studies for abdominal aorta

	NAPL	PAL
Present study	71.43%	20(28.57%)
Thej et al[29]	18(15.9%)	95(84%)
Lokesh et al[100]	70.3%	27(29.7%)

In the abdominal aorta, 8(11.42%) cases were Type III lesion, 6(8.5%) cases were Type IV lesions, 6(8.5%) were type V lesions and no cases were seen with type VI lesions. Similarly, these lesions which includes 20(28.57%) cases comes under the category of PAL according to Modified AHA criteria. This study is in concordance with the study done by Lokesh et al where 27(29.7%) were under progressive atherosclerotic lesion (PAL).[100]

In another study done by Thej et al in 2012 in 113 cases, 95(84%) cases were included progressive atherosclerotic lesion in thoracic aorta.[29] It is in discordance with the present study where 20(28.57%) cases were included in progressive atherosclerotic lesion in thoracic aorta.

In another study done by Benvenuti LA, et al, Plaque ulceration was more frequently seen in the (80%) cases of abdominal aorta than in the thoracic aorta (17%).

In this study, it was seen that the abdominal atherosclerotic segments have a more complicated fat deposition which has a more obliterative finding than the segments of the thoracic aorta.[107]

This finding is similar to our study where Type III 8(11.42%) and Type V 6(8.50%) lesions are more common in abdominal aorta which itself signifies that the higher grade lesions are more common in the abdominal aorta.

Atherosclerosis is a disease that attacks various sites and regions of the arterial tree. Sites with low or oscillatory endothelial shear stress are mainly located near branch points and along inner curvatures, are most susceptible. The abdominal aorta, coronary arteries, ilio- femoral arteries, and carotid bifurcations are typically the most affected. Before the atherosclerosis is developed, these sites are characterized by the presence of adaptive intimal thickening .Adaptive intimal thickenings develop spontaneously after birth and may grow to be as thick as the underlying media. These thickened areas may provide a soil for initial lesion development and the rate of progression remains higher here than at other arterial sites.[106,108]

COMPARISON OF MAST CELL BETWEEN CONTROL (NON ATHEROSCLEROTIC LESION) AND CASES (ATHEROSCLEROTIC LESION) OF THORACIC AND ABDOMINAL AORTA WITH OTHER STUDIES

Mast cells have a very potential role in various inflammatory processes by the release of potent mediators such as histamine, heparin, protease leukotrienes and cytokines. It also contributes to the inflammatory fibro proliferative process of the development of various stages of atherosclerotic plaques.[12]

In the present study, the mean mast cell count in non-atherosclerotic part of aorta which is used as control is (3.4±2.4)/10hpf and the mast cell count in the atherosclerotic aorta (7.8±3.4)/10hpf in Type II lesion, (12.7±5.18)/10hpf in Type III lesion, (19.14±2.6)/10hpf in Type IV lesion, (28.2±1.09)/10hpf in Type V lesion

In a study done by Maria J et al 1997, carotid arteries were taken from 92 autopsy specimens, revealed presence of 2-3 mast cells in the intimal layer of the non-atherosclerotic portion of carotid vessel. In contrast the atherosclerotic lesions showed increased distribution of mast cells in all the layers of the vessel.[12]

Hence, the present study findings is similar to the findings in the study done by Maria J et al.[12]

COMPARISON OF THE MICROSCOPIC TYPING ACCORDING TO THE AHA CRITERIA AND THE MAST CELL COUNT AND ITS COMPARISON WITH OTHER STUDIES

Mast cells play a very important role in the process of the development of various atherosclerotic lesions. In various studies increased number of Mast cells is seen in the atherosclerotic plaque regions and in the adventitia of the human aorta.[12]

In this study, the staining of the mast cell is done by toluidine blue staining and they were found to be present in the adventitial layer of the aorta.

Here it is observed that the mean mast cells calculated is (7.8±3.4)/10hpf in Type II lesion, (12.7±5.18)/10hpf in Type III lesion, (19.14±2.6)/10hpf in Type IV lesion, (28.2±1.09)/10hpf in Type V lesion I the thoracic aorta. It is observed that as the type/ grade of the lesion increases the mean mast cell count also increases and this value is clinically significant $p<0.001$.

Mast cells calculated are (8.7±3.4)/10hpf in Type II lesion, (13.37±5.3)/10hpf in Type III lesion, (18.66±1.03)/10hpf in Type IV lesion, (19.167±6.7)/10hpf in Type V lesion in the abdominal aorta. It is observed that as the type/ grade of the lesion increases the median Mast cell count also increases and this value is clinically significant $p<0.001$.

On comparison of the mast cell count between the thoracic and abdominal aorta lesions it is observed that with the progress of the atherosclerotic lesion the mast cell count increases significantly.

In a study done by Pouchev et al 1965 the correlation of the mast cell with the progression of the atherosclerotic lesions was studied.[109]

The AHA grading system not applied but individual lesions were studied in the aortic specimens where Mast cells were counted in the adventitial layer of aorta per 40 high power field. It was seen that the unaffected vessel with the mean mast cell was 38/40hpf, lipid streaks showed 39/40hpf, atheroma 62/40hpf, atheromatous ulceration 84.5/40hpf. It was observed that as the atherosclerotic lesion grade increases the value of the mean mast cell increases.[109]

This study is in concordance with the present study where mean mast cell count is increased with the increases of the type / grade of lesion in both thoracic and abdominal aorta.

In a study done by Petri laine et al 2000, coronary arteries were taken from the 52 autopsy cases. Mast cells were stained by IHC marker tryptase. It was seen that (12±2%), (54±4), (79±12) and (104±5) in types 0-I, II, III, IV lesions respectively. It is stated that as the lesion complication increases the mast cell numbers also increase.[110]

There are several methods for studying the grade of the atherosclerotic lesions. In the study by Mara et al, the intimal thickness and endothelial thickness was measured and atherosclerotic lesions were graded comparing them with the gross findings. This method also helped them to quantify the degree of atherosclerosis

In this study by Ferraz et al in 2012, in 141 autopsy specimens of aorta IHC was used and the mast cell density in the aortic specimens were calculated.[98] This showed significantly positive correlation between the number of the Mast cell and the degree of the atherosclerosis.(r=0.214, p=0.01).

This finding of this study is in concordance with the present study where mean mast cell count in the non-atherosclerotic lesion is (3.4+-2.4)/10hpf while its count is increased with lesion progression.

In a study done by Kaartinen et al, 1994 studied the coronary arterial segments with the atherosclerotic lesion and mast cell percentage was studied. IHC was used and percentage of mast cells along with T cells and macrophages studied. It was observed that in normal intima mean mast cell percentage was 0.1 (0-0.8), while in Fatty streak it was 0.9 (0-4.8), Atheroma Cap 0.5 (0-1.7), Core 0.5 (0-3.0) Shoulder 1.1 (0-4.0). Hence, it was concluded that with the progress of the lesion the mast cell count is increased.[10]

CONCLUSION

The study was done to find out the role of mast cells in atherosclerotic lesions and to evaluate the gross and microscopic findings of the atherosclerotic lesions in the thoracic and abdominal aorta and correlating with mast cell count

In the present study, it is noted atherosclerotic lesions were more common in age group of>40 years and there is progressive increase in the type of atherosclerotic lesion in age group > 40 years. It is observed that while fatty streaks are more common in age group of 10-39 years, but in age group more than 40 years there is an increase in number of plaque lesions. This indicates the need for anti atherogenic preventive measures in younger age group so as to prevent future risk of morbid conditions like cardiovascular disease and strokes.

However, there was no significant difference noted between the atherosclerotic lesion types in thoracic aorta with the types of atherosclerotic lesion in abdominal aorta. This indicates that the presence of risk factors uniformly affect the regions of the aorta.

Mast cell count was studied in the non-atherosclerotic and atherosclerotic part of the thoracic and abdominal aorta by the toluidine blue staining method. The count was compared with the type of atherosclerotic and was found with the increase in the type of lesion the mast cell count increases.

SUMMARY

- **"STUDY OF MAST CELL PROFILE IN ATHEROSCLEROTIC LESION OF AORTA: AN AUTOPSY STUDY"** is a prospective based cross sectional study conducted in Department of Pathology, Sri Devraj Urs Medical College, Kolar from February 2016 to August 2017.

- A total of 70 cases were studied of which 50(71%) were males and 20(29%) were females with M: F ratio of 5:2.

- Majority of the patients belonged to 40-49 years and constitutes (27.1%).

- The most common cause of death in this study was RTA (41%), followed by poisoning (24%) and hanging (13%).

- The maximum victims of RTA were males (89.7%). The death by burns was more common in females (75%).

- Fatty streaks are more common in 10-39 years while plaque lesion is more common in ≥40 years in both thoracic and abdominal aorta.

- On gross examination comparison of thoracic and abdominal aorta it is observed that fatty streaks 31(54.3%) are more common in thoracic aorta while plaque lesion are more common in abdominal aorta 28(51.8%).

- On microscopic examination it was observed that Type I, III, and Type V lesions were more common in the abdominal aorta than the thoracic aorta

- Mean mast cell count in non-atherosclerotic part was of aorta was (3.4±2.4) cells/10hpf.

- Mean mast cell count in atherosclerotic part of thoracic aorta in Type I, II, III, IV and Type V was (3.4±2.4)cells/10hpf, (7.8±3.4)cells/10hpf,(12.7±5.1) cells/10hpf, (19.1±2.6) cells/10hpf and (28.2±1.09)cells/10hpf respectively

- Mean mast cell count in atherosclerotic part of abdominal aorta in Type I, II, III, IV and V was (3.4±2.4) cells/10hpf, (8.7±3.4) cells/10hpf, (13.3±5.3) cells/10hpf, (18.6±1.03) cells/10hpf and (19.1±6.7) cells/10hpf respectively.

- It is observed that as the Type of the atherosclerotic lesion increases there is progressive increase in the mast cell count. This is statistically significant finding with the p value< 0.001.

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ANNEXURES I: STUDY PROFORMA

TITLE: STUDY OF MAST CELL PROFILE IN ATHEROSCLEROTIC LESION OF AORTA: AN AUTOPSY STUDY

Name:

Age:

Sex:

Medical history/ cause of death:

Organs sent for history:

Gross examination: Aorta

Dimension:

Any vascular abnormalities:

	Atherosclerotic lesion site	**Non-atherosclerotic part site**
Thoracic aorta		
Abdominal aorta		

Microscopy:

Histopathological examination:

Grading of atherosclerotic lesion:

Mast cell count: toluidine staining (40x)

MAST CELL COUNT		
ATHEROSCLEROTIC PART OF THORACIC AORTA	**ATHEROSCLEROTIC PART OF ABDOMINAL AORTA**	**NON ATHEROSCLEROTIC PART**

ANY OTHER OBSERVATION:

ANNEXURES II: CONSENT FORM

STUDY OF MAST CELL PROFILE IN ATHEROSCLEROTIC LESION OF AORTA- AN AUTOPSY STUDY

I the next of the kin of the deceased _____ have given my consent to take the sample of aorta for research purpose during autopsy by the Department of Pathology, Sri Devaraj Urs Medical College, Tamaka, Kolar.

I have been explained that it is only for research and betterment of mankind.

Name of the deceased:

Autopsy no:

Name of the relative:

Relationship of the relative:

Signature:

Phone no:

Address:

ANNEXURE III: PATIENT INFORMATION SHEET

STUDY TITLE: STUDY OF MAST CELL PROFILE IN ATHEROSCLEROTIC LESION OF AORTA: AN AUTOPSY STUDY

Place of study: Sri Devaraj Urs Medical College attached to R. L. Jalappa Hospital and Research, Tamaka, Kolar.

The main aim is to study the mast cell count in atherosclerotic lesion and comparing it with non-atherosclerotic part of aorta and also to correlate the result of mast cell count in the atherosclerotic lesion with the grading/type of atherosclerosis.

Patient's relative/kin are requested to participate in a study conducted by the department of pathology as a part of dissertation. This study will be done on dead patients. Aortas will be collected from autopsies conducted at R. L. Jalappa and Research attached to Sri Devaraj Urs Medical College Tamaka, Kolar in coordination with Department of Forensic Medicine.

This study is approved by the institution ethical committee. The information collected will be used only for dissertation and publication. There is no compulsion to agree to participate in the study.

All information collected from the patient's relative/kin will be kept confidential and will not be disclosed to any outsider. Patient's identity will not be revealed. Relative/kin will not receive any monetary benefits of participating in the study.

This informed consent document is intended to give patient's relative/kin general background of study. Please read the following carefully and discuss with other family members. They can ask their queries related to study at any time during the study. If they are willing to participate in the study they will be asked to sign an informed consent form by which they are acknowledging that they wish to participate in the study and that entire procedure is explained to them by study doctor.

For further information they are free to contact the investigator:

PRINCIPAL INVESTIGATOR: **Dr SULAGNA MANNA**
Contact number: 8860142422
e-mail id: sulagna.manna@gmail.com

Introduction

Objectives

Review of Literature

Normal Anatomy

Normal Histology

Methodology

Results

Photographs

Discussion

Conclusion

Summary

Bibliography

Annexures

ANNEXURE V: KEY TO THE MASTER CHART

A NO.	→	Autopsy number
COD	→	Cause of death
G TH	→	Gross thoracic aorta
G AB	→	Gross abdomen aorta
H/P GD TH	→	Histopathological grading of thoracic aorta
H/P GD AB	→	Histopathological grading of abdominal aorta
MC TH	→	Mean mast cell count thoracic aorta
MC AB	→	Mean mast cell count abdominal aorta

ANNEXURE IV: MASTER CHART

Sl. No.	A NO	Age	Sex	COD	G TH	G AB	H/P GD TH	H/P GD AB	MC TH	MC AB
1	A/10/16	3	1	1	2	2	2	2	10	10
2	A/11/16	2	2	6	1	1	1	1	5	5
3	A/12/16	2	1	1	0	0	1	1	3	2
4	A/13/16	4	1	1	2	2	1	2	11	12
5	A/14/16	3	1	1	1	1	1	1	6	4
6	A/15/16	2	1	1	0	0	2	1	2	3
7	A/39/16	4	1	3	2	2	3	3	17	17
8	A/40/16	3	2	6	0	0	1	1	2	2
9	A/41/16	2	1	1	1	1	2	2	6	6
10	A/42/16	2	1	1	1	1	2	1	7	5
11	A/43/16	3	1	3	2	2	2	2	8	9
12	A/44/16	4	2	5	1	2	2	3	6	18
13	A/45/16	1	1	1	1	1	2	1	6	7
14	A/46/16	2	1	2	1	1	2	2	6	6
15	A/47/16	3	1	1	2	2	2	2	7	7
16	A/48/16	4	1	3	2	2	3	3	14	14
17	A/49/16	2	2	3	0	0	1	1	2	2
18	A/50/16	2	2	6	1	1	1	1	5	5
19	A/51/16	5	1	1	2	2	2	2	11	11
20	A/52/16	7	1	6	1	1	2	2	12	12
21	A/53/17	3	1	1	1	1	1	1	8	9
22	A/54/17	2	2	1	0	0	1	1	2	2
23	A/55/17	2	1	2	0	0	1	1	2	3
24	A/56/17	3	2	1	1	1	2	2	6	6
25	A/57/17	5	1	5	2	2	3	3	17	17
26	A/58/17	2	2	2	1	1	2	2	12	12
27	A/59/17	1	1	2	0	0	1	1	2	2
28	A/60/17	3	1	1	1	1	2	1	6	7
29	A/1/17	2	2	4	1	1	2	1	4	5

ANNEXURE IV: MASTER CHART

Sl. No.	A NO	Age	Sex	COD	G TH	G AB	H/P GD TH	H/P GD AB	MC TH	MC AB
30	A/2/17	4	2	1	2	2	2	2	12	12
31	A/39/16	4	1	2	2	2	2	2	12	13
32	A/4/17	2	1	1	1	1	2	1	8	9
33	A/5/17	1	1	1	0	0	1	1	3	3
34	A//6/17	1	2	2	0	0	1	1	3	2
35	A/7/17	1	1	2	0	0	1	1	2	2
36	A/8/17	5	1	3	2	2	3	3	3	2
37	A/9/17	5	1	1	2	2	2	2	18	18
38	A/10/17	3	2	3	1	1	1	1	5	6
39	A/11/17	3	1	1	1	1	2	2	5	8
40	A/12/17	6	1	1	2	2	4	4	17	18
41	A/13/17	4	1	2	1	1	2	2	6	7
42	A/14/17	5	1	4	2	2	3	3	17	17
43	A/15/17	4	1	3	1	1	2	2	6	7
44	A/16/17	4	2	2	0	0	1	1	3	4
45	A/17/17	4	1	2	0	0	1	2	2	1
46	A/18/17	5	1	5	1	1	2	2	7	6
47	A/19/17	5	2	4	2	2	4	4	17	18
48	A/20/17	6	1	1	1	1	2	2	5	7
49	A/21/17	7	1	1	2	2	5	5	27	12
50	A/22/17	5	1	2	2	2	4	4	22	20
51	A/23/17	4	2	3	2	2	5	5	28	27
52	A/24/17	4	1	1	2	2	3	3	11	12
53	A/25/17	4	1	2	1	1	2	2	12	11
54	A/26/17	5	1	1	1	1	2	2	12	11
55	A/27/17	4	2	2	2	0	1	1	3	0
56	A/28/17	4	1	2	0	0	1	1	1	1
57	A/29/17	5	1	5	1	1	2	2	6	7
58	A/30/17	5	2	4	2	2	4	4	17	18

ANNEXURE IV: MASTER CHART

Sl. No.	A NO	Age	Sex	COD	G TH	G AB	H/P GD TH	H/P GD AB	MC TH	MC AB
59	A/31/17	6	1	1	1	1	2	2	5	6
60	A/32/17	7	1	1	1	2	5	5	28	15
61	A/33/17	5	1	2	2	2	4	4	22	20
62	A/34/17	4	2	3	2	2	5	5	30	28
63	A/35/17	4	1	1	2	2	3	3	10	10
64	A/36/17	7	1	5	2	2	4	5	22	18
65	A/37/17	4	2	2	1	0	1	1	3	0
66	A/38/17	4	1	2	1	0	1	1	1	1
67	A/39/17	5	1	5	1	1	2	2	6	7
68	A/40/17	5	2	4	2	2	4	4	17	18
69	A/41/17	6	1	1	2	1	2	2	5	6
70	A/42/17	7	1	1	2	2	5	5	28	15

AGE	SEX	COD
10-19-1	MALE-1	RTA-1
20-29-2	FEMALE-2	POISON-2
30-39-3		HANGING-3
40-49-4		DROWNING-4
50-59-5		SUDDEN DEATH-5
60-69-6		BURN-6
>=-7		

G TH
NO LESION-0
FATTY STREAK-1
PLAQUE-2
COMPLICATED PLAQUE-3

G AB
NO LESION-0
FATTY STREAK-1
PLAQUE-2
COMPLICATED PLAQUE-3

H/P of Th	H/P Ab
Type I-1	Type I-1
Type II- 2	Type II- 2
Type III-3	Type III-3
TypeIV-4	TypeIV-4
Type V-5	Type V-5
Type VI-6	Type VI-6