



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

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Article Received on  
21 Oct. 2019,

Revised on 11 Nov. 2019,  
Accepted on 01 Dec. 2019,

DOI: 10.20959/wjpps201912-15234

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### SUMMARY

Atherosclerosis is an inflammatory disease which progresses over years. Mast cell plays an important role in its progression. Autopsy based studies are of great value for studies of atherosclerosis in aorta. Previously no other study in autopsy is done on mast cells profile and its correlation with atherosclerosis in large vessels like aorta. Mast cell count is important to analyse the atherosclerotic lesion progression.

**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. **Result:** Mean mast cell count in non-atherosclerotic part of aorta was (3.4  $\pm$  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 $\pm$ 2.4), (7.8 $\pm$ 3.4), (12.7 $\pm$ 5.1), (19.1 $\pm$ 2.6) and (28.2 $\pm$ 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 $\pm$ 2.4), (8.7 $\pm$ 3.4), (13.3 $\pm$ 5.3), (18.6 $\pm$ 1.03) and (19.1 $\pm$ 6.7) cells/10hpf respectively. The results are statistically significant (p value < 0.001). **Conclusion:** From the present study we conclude that as there is a progression in the Type/Grade of the atherosclerotic lesion, the mast cell count also increases.

**KEY WORDS:** Atherosclerosis, Aorta, Mast cell, Inflammatory, Autopsy.

### 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.<sup>[1]</sup> The size of the cholesterol-rich lipid core and the thickness of the fibrous cap over it are the main determinants of plaque stability.<sup>[2]</sup> The smooth muscles and the ECM

produced by the cap maintains its strength and thickness.<sup>[3]</sup> A plaque lesions which are vulnerable to rupture have a large size lipid core and a thin cap which leads to acute Athero - thrombotic events, such as MI and stroke.<sup>[3,4]</sup>

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.<sup>[7,8]</sup> 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.<sup>[9,10,11]</sup> Several studies have reported that mast cells play a very significant role in the development and in the progression of atherosclerosis. Several studies have been done on the role of mast cells in atherosclerotic lesions in vessels such as coronary arteries. However, very few studies have been done till date on the 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.

## **MATERIALS AND METHODS**

The present 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 at the same period.

## **HISTOLOGICAL PREPARATION**

Aortas were collected and fixed in the 10% formalin. The thoracic, abdominal and the non atherosclerotic part of the individual aortas were separately dissected and the atherosclerotic lesions were identified.

Bits were taken from the suspicious or definite atherosclerotic lesions. All of these bits were processed by routine tissue processing and stained with Hematoxylin and Eosin stain and 2% Toluidine blue stain.

## **PATHOLOGICAL ASSESSMENT AND MORPHOLOGICAL ANALYSIS**

The aortas were assessed by gross examination and were graded as no lesion, fatty streak and plaque. The microscopic examination was done according to the AHA grading system. The

mast cell count was done by using 2% Toluidine blue as special stain under 40X/10 high power field. The type of the atherosclerotic grading/ typing was done and correlated with the mast cell count. The atherosclerotic lesions were divided into Type I, Type II, Type III, Type IV, Type V, and Type VI.

### STATISTICAL ANALYSIS

Statistical analysis was done using Chi square Test and Annova Test in SPSS software.

### RESULTS

In the present study, the ages ranged from 15 to 80 years and mean age was  $43.77 \pm 16.39$  years. Number of males and female were 50(71%) & 20 (29%) respectively. The majority of cases 19 (27%) belong to the age distribution of 40-49 years .The most common cause of death was road traffic accidents 21(49%), followed by poisoning 17(24%), then hanging 9(13%), sudden death 6(9%), drowning 5(7%) and burns 4(6%). In this study it is observed that Type III, Type IV and Type V lesions are only reported in the age group of  $\geq 40$  years while Type I and Type II lesions are more common in the age of 10-39 years both in the thoracic and abdominal aorta (Table 1 & Table 2).

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

**Table 1: Comparison of microscopic findings of thoracic aorta with the age distribution.**

Age groups (in years)	Microscopic Examination of Thoracic aorta					Total	
	Type I	Type II	Type III	Type IV	Type V		
10-39	14 (50.0%)	14 (50.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	28 (40.0%)	
$\geq 40$	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%)	

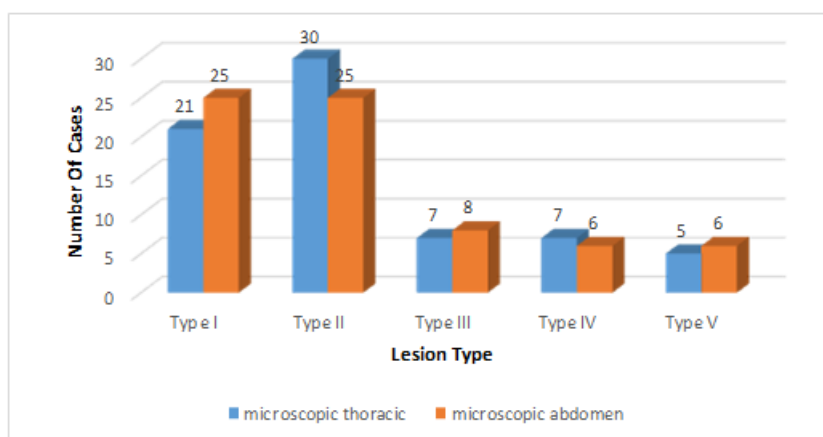
\* Fischer's Exact test

**Table 2: 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 the present study on comparison of microscopic examination (Gross Non-atherosclerotic, **Pic. 1 and Pic.2**) between thoracic and abdominal aorta (Gross plaque - **Pic. 3**) it is observed that Type II and type IV (**Pic. 4**) lesions are more common in the thoracic aorta where as Type I, Type III and Type V (**Pic.5**) lesions are more common in the abdominal aorta.



**Chart 1: Bar chart showing comparison in microscopy between thoracic and abdominal aorta.**

On comparison of the mean mast cell count between the non atherosclerotic part and the atherosclerotic part of the thoracic and abdominal aorta it is observed that the mean mast cell is  $(3.4 \pm 2.4)/10$  hpf in the non-atherosclerotic segment of the aorta and the mean mast cell is  $(9.5 \pm 7.6)/10$  hpf in the thoracic aorta and mean mast cell is  $(9.1 \pm 6.5)/10$  hpf in the abdominal aortas.

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 mast cell count is  $(3.4 \pm 2.4)/10$  hpf, while in type II lesion mean mast cell count is  $(7.8 \pm 3.4)/10$ hpf, mean mast cell in type III lesion is  $(12.7 \pm 5.1)/10$ hpf, mean mast cell in type IV lesion is  $(19.1 \pm 2.6) / 10$ hpf and mean mast cell in type V is  $(28.2 \pm 1.09)/10$ hpf (**Pic.6**) respectively.

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 mast cell count is  $(3.5 \pm 2.4) /10$ hpf, while in type II lesion mean mast cell is  $(8.7 \pm 3.4)/10$ hpf, mean mast cell in type III lesion is  $(13.3 \pm 5.3)/10$ hpf, mean mast cell in type IV lesion is  $(18.6 \pm 1.03)/10$ hpf and mean mast cell in type V is  $(19.1 \pm 6.7)/10$ hpf (Table-4).

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

**Table 4: Mean mast cell count with the Type of atherosclerotic lesion.**

	Mean (IQR) of Mast cell Count/10 hpf					
M/E	Type I	Type II	Type III	Type IV	Type V	P value
Thoracic	$3.4 \pm 2.4$	$7.8 \pm 3.4$	$12.7 \pm 5.1$	$19.1 \pm 2.6$	$28.2 \pm 1.09$	$<0.001$
Abdomen	$3.5 \pm 2.4$	$8.7 \pm 3.4$	$13.3 \pm 5.3$	$18.6 \pm 1.03$	$19.1 \pm 6.7$	

\*Anova test

## 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.<sup>[12,13]</sup>

In several other studies like Lokesh et al<sup>[14]</sup>, Thej et al<sup>[15]</sup>, it is observed as the age increases the atherosclerotic lesions are more common and this finding is similar to the finding of the present study.

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 5: Comparison of microscopic typing / grading with other studies for thoracic aorta.**

	NPAL	PAL
Present study	51(86%)	19(14.071%)
Thej et al <sup>[15]</sup>	22(19.4%)	91(80.5%)
Lokesh et al <sup>[14]</sup>	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 the thoracic aorta.<sup>[14]</sup>

In another study done by Thej et al.<sup>[15]</sup> in 2012 in 113 cases, 91(80.5%) cases were included Progressive Atherosclerotic Lesion in the thoracic aorta. It is in discordance with the present study where 19(14.071%) cases were included in Progressive atherosclerotic lesion in the thoracic aorta.

**Table 6: Comparison of microscopic typing / grading with other studies for abdominal aorta.**

	NAPL	PAL
Present study	71.43%	20(28.57%)
Thej et al <sup>[15]</sup>	18(15.9%)	95(84%)
Lokesh et al <sup>[14]</sup>	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 include 20(28.57%) cases come 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).<sup>[14]</sup>

In another study done by Thej et al.<sup>[15]</sup> in 2012 amongst 113 cases, 95(84%) cases were included progressive atherosclerotic lesion in thoracic aorta. It is in discordance with the

present study where 20(28.57%) cases were included in progressive atherosclerotic lesion in the thoracic aorta.

In another study done by Benvenuti et al.<sup>[16]</sup> Plaque ulceration was more frequently seen in (80%) cases of the 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.<sup>[16]</sup>

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

#### **Comparison of the microscopic typing according to the AHA Criteria and the mast cell count and its comparison with other studies**

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.<sup>[17]</sup>, the correlation of the mast cell with the progression of the atherosclerotic lesions was studied.

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.<sup>[17]</sup>

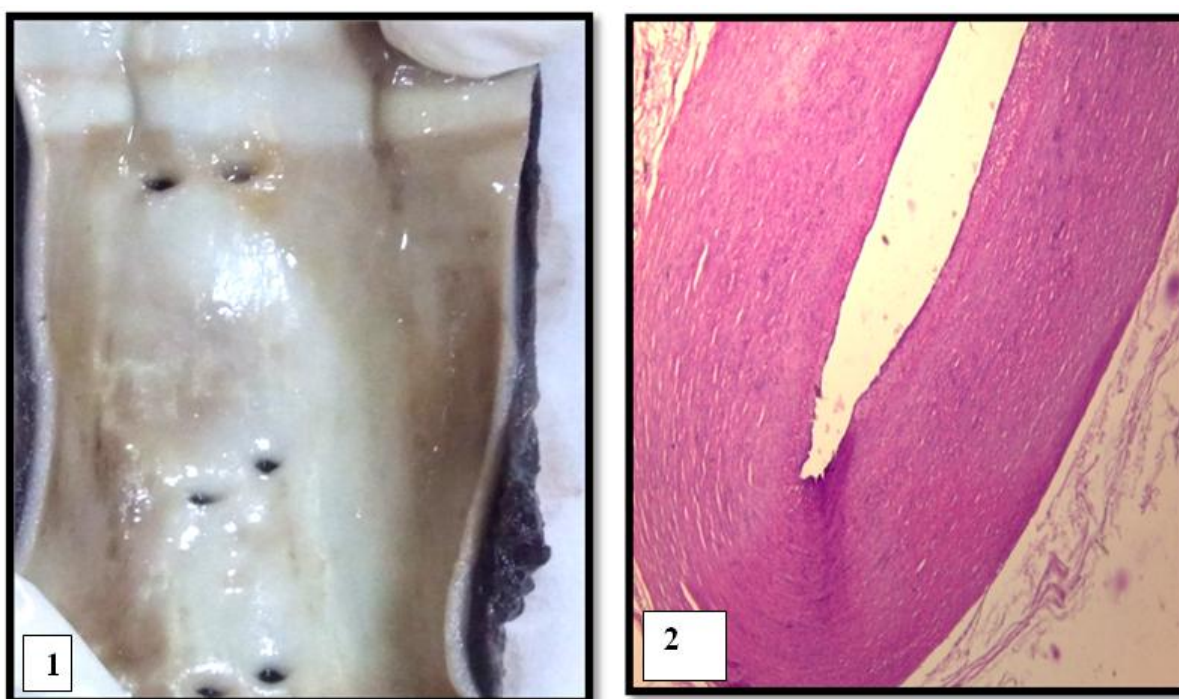
This study is in concordance with the present study where mean mast cell count is increased with the increases of the type / grade of lesion in both thoracic and abdominal aorta.

In a study done by Laine et al.<sup>[18]</sup>, 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%),



( $79 \pm 12\%$ ) and ( $104 \pm 5\%$ ) in types 0-I, II, III, IV lesions respectively. It is stated that as the lesion complication increases the mast cell numbers also increase.

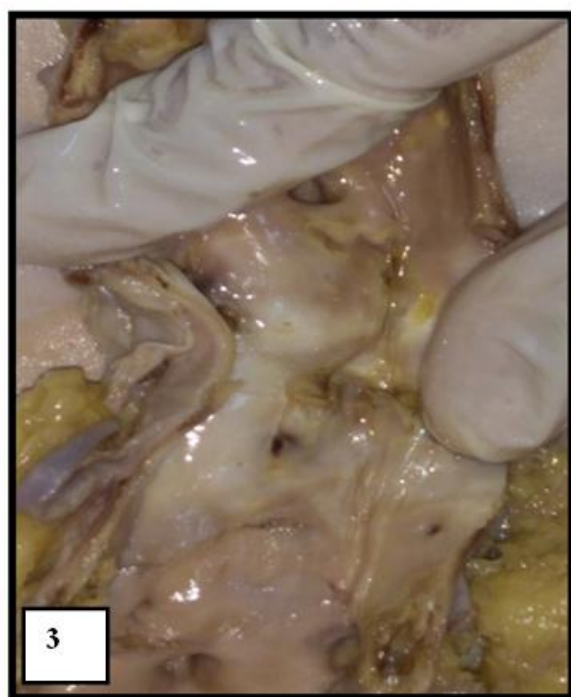
In a study done by Kaartinen *et al.*<sup>[10]</sup>, the coronary arterial segments with the atherosclerotic lesion and mast cell percentage were studied. IHC was used and percentage of mast cells along with T cells and macrophages were studied. It was observed that in normal Intima, the mean mast cell 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 was also increased.



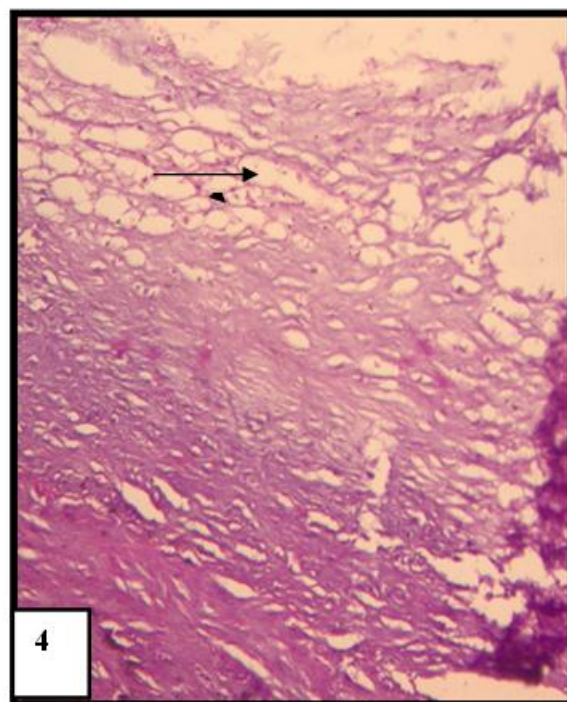
**Pic. 1. Gross photograph of a Non - atherosclerotic Aorta.**

**Pic. 2. Microphotograph showing Non - atherosclerotic aorta.(H and E, X4).**

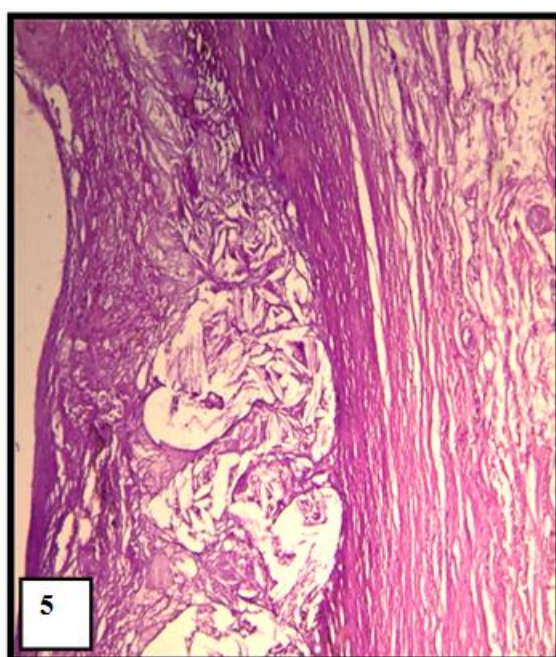




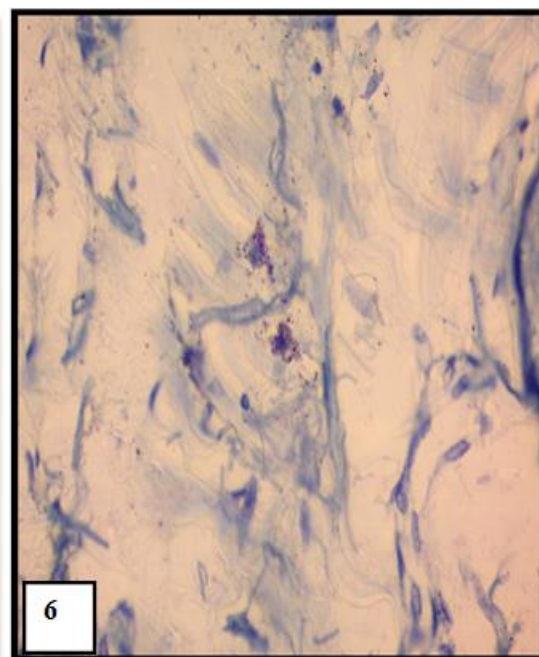
**Pic. 3.** Gross photograph showing Plaque Lesion within Aorta.



**Pic. 4.** Microphotograph showing Type IV atherosclerotic aorta.(H and E , X40). Arrow showing abundant foamy macrophages with inflammatory cells.



**Pic. 5.** Microphotograph showing Type V atherosclerotic aorta.(H and E, X10).



**Pic. 6.** Microphotograph showing Mast Cell within atherosclerotic aorta of Type V lesion. (Toluidine Blue Stain, X40).

## 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 a 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 the 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.

## ACKNOWLEDGEMENTS

The authors would like to thank Dr Kiran J, Professor and Head of the Department Forensic Medicine, for helping in getting the autopsy specimens, Dr Sunil, Assistant Professor in the Department of Family Medicine for statistical analysis, Dr Chanchal Kumar Manna, Retd. Prof., University of Kalyani, Kalyani, India, for his immense help in formatting the manuscript and Histopathology division for processing the samples for this study. Authors also acknowledge the immense help of the Lab Technicians for their continuous help received and from Scholars whose articles are cited and included in references of this manuscript.

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