A Study of atherosclerotic lesions of aorta with CD4 immunostaining

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Abstract
Indian population is affected with atherosclerosis at younger age when compared to other ethnic groups. In this study histopathological grading of atherosclerotic lesions of the ascending aorta in archival specimens were performed with immunohistochemical marker for CD4+ cells in each grade of atherosclerosis. Mean ages of males and females in our study were 52.11±15.79 and 40.18±17.73 years respectively. The prevalence of various grades of atherosclerosis revealed, Grade 1 atherosclerosis in 4.90%, Grade 2 in 17.65%, Grade 3 in 32.35%, grade 4 in 28.43%, grade 5 in 13.73% cases. Grade 6 was the least common grade of atherosclerosis (1.96%). Immunohistochemistry for CD4+ was performed in 18 subjects with three cases each of grade 1 to 6. CD4+ was quantified higher in grade 1 and grade 2 lesions while progressive reduction in CD4+ positivity was seen in the advanced lesions.

Keywords: Aorta, Archival specimens, Atherosclerosis, CD4, Histopathology.

Introduction
Atherosclerosis is a complex inflammatory disease leading to accumulation of fat and cholesterol within arterial wall. It is a disease of the large elastic and muscular arteries. The aorta is more frequently involved than any other part of the vascular tree. The reasons are its arched-shape and the very high blood pressure that it is exposed to during cardiac output. The aetiology of atherosclerosis comprises of genetic factors, alterations in local haemodynamics, vascular injury, age related changes, elevated homocysteine, endothelial inflammation and autoimmune because auto antibodies affect vascular remodeling T-cells were first described in human atherosclerotic plaques by Jonasson et al, in 1985.[1] It was later observed that CD4+ cells were prominent in atherosclerotic plaques.[2] Different subsets of T cells have different effects in this lesion. Native T helper cells can differentiate into Th1 cells which secretes INF –γ, Th2 which secretes IL-4, IL-5 and IL-13 and Th17 which secretes IL-17 and IL-22. Activated T lymphocytes of subtype Th1 has a major role in inflammation in atherosclerosis by proliferating in situ.[3]These CD4 T cells are capable of clonal proliferation and can be identified by immunohistochemistry. These CD4 T cells have pro atherogenic effect. The presence of active inflammation with T lymphocytes makes a plaque vulnerable to rupture. Indian population is affected with atherosclerosis at a younger age when compared to other ethnic groups.[4] The grading of atherosclerosis has undergone many changes over the last seven decades. Fatty streaks and fibrous plaques were the initial lesions that were recognized during the early twentieth century.[5] By the end of the twentieth century, an American Heart Association (AHA) classification was in place for the histopathological grading of atherosclerosis. Cells participating in the atherosclerotic process include macrophages, lymphocytes, dendritic cells, neutrophils, mast cells and smooth muscle cells.[6] The best-studied cells are T cells, B cells, dendritic cells [DCs] and macrophages.[6] T cells include both CD4+and CD8+ cells, although the former dominates the picture.[6] In this study an attempt has been made to histopathologically grade atherosclerotic lesions of the ascending aorta in archival specimens and perform and study immunohistochemical marker for CD4+ cells in atherosclerotic lesions of all grades.

Materials and Methods
This study was done in the department of pathology, Tirunelveli Medical College between May 2010 and June 2013. Archival specimens of ascending aortas, harvested from all autopsies performed for 3 years were included in our study. Corresponding heart specimens were available. Aorta specimens without heart specimens were excluded. We screened specimens of ascending aortas and hearts of all autopsies and histopathology studies of the ascending aorta were performed. At least three bits from the thoracic aorta in the region of fatty streaks were taken and graded from one to eight using American Heart Association diagnostic criteria:

Grade 1 - isolated intimal foamy cells

Grade 2 - numerous intimal foamy cells often in layers (fatty streaks)

Grade 3 - pools of extra cellular lipid without a well-defined core

Grade 4 - well defined lipid core with luminal surface covered by normal intima
(atheroma or fibro plaque);

Grade 5- lipid core with a fibrous cap with or without calcification (fibro- atheroma);

Grade 6- fibro-atheroma with cap defect such as haemorrhage and thrombosis;

Grade 7- calcification prominent; and
Grade 8- fibrous tissue change prominent.

Three specimens of each grade of atherosclerosis [n=18], were chosen for immunohistochemistry. Formalin-fixed, paraffin–embedded aortic autopsy specimens were used in the study. Sections were stained for CD4 + cells using the anti-CD4 antibody, clone 4B12 [Mouse, IgG1] obtained from BioGenex. The immunogen for this antibody was the prokaryotic recombinant protein corresponding to the external domain of the CD4 molecule. Statistical analysis was performed using IBM SSPS for Windows 21. Descriptive variables were expressed using the mean (SD). Comparison of categorical variables was performed using the X2 test. Levels of significance were calculated, with p value of<0.05 being considered as statistically significant.

Results

Our study had a total of 102 patients with 101 of them having atherosclerosis. There were 91 males and 11 females in our study. (Fig.1).

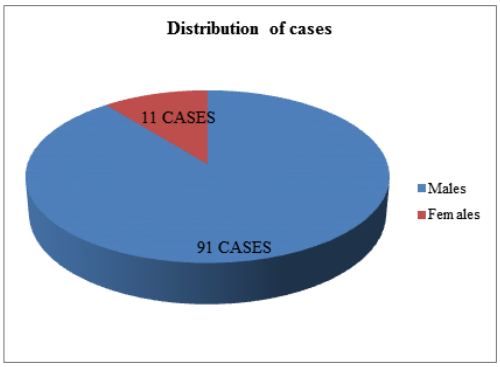

Fig. 1: Distribution of cases.

Mean ages of males and females in our study were 52.11±15.79 and 40.18±17.73 years respectively the youngest patient was seven months old, while the oldest among them was 82 years old. Most patients belonged to the fifth [20.6%] and sixth [22.5%] decades. Causes of death in our patients were not evaluated. Only one patient who was 7 months old did not have atherosclerosis, thereby returning figures of a 99% incidence of atherosclerosis based on study of autopsy specimens. Mean ages of cases with early lesions (Grade I & II) were 38.21±14.72 and that of advanced lesions were 57.37±14.18. (Table 1).

Table 1: Mean ages of patients with lesion

Type of Lesion	AGE ± SD
Early lesions	38.21±14.72
Advanced lesions	57.37±14.18

In the current study, the prevalence of various grades of atherosclerosis [Fig. 2] revealed, Grade 1 atherosclerosis (Fig. 3) in 4.90%, Grade 2 (Fig. 4) in 17.65%, Grade 3 (Fig. 5) in 32.35%, grade 4 in (Fig. 6) 28.43%, Grade 5 (Fig. 7) in 13.73% cases Grade 6 was the least common grade of atherosclerosis (1.96%).

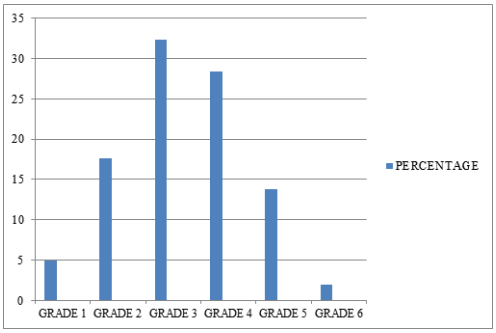

Fig. 2: Bar diagram showing prevalence of various grades of atherosclerosis

Immunohistochemistry for CD4+ (Fig. 8) was performed in 18 subjects with three cases each of grade 1 to 6. CD4+ was quantified higher in grade 1 and grade 2 lesions while progressive reduction in CD4+ positivity was seen in the advanced lesions.

Discussion

We conducted an analysis of 102 cases of autopsied thoracic aortas. Histopathological grading of atherosclerosis according to the American Heart Association was performed in all these aortic specimens. Though many autopsy studies on atherosclerosis have been performed in other parts of India and worldwide, no similar study has been performed in rural southern Tamil Nadu that could have given an idea regarding the prevalence of atherosclerosis in the local population. The prevalence of atherosclerosis is significantly increasing in both urban and rural populations of India.[4] Our study of autopsy specimens revealed that atherosclerosis is observed from the second decade itself. The youngest patient with atherosclerosis was 15 years old; we had only three patients in the second decade, all of whom had early atherosclerotic lesions. The mean age of our study cohort was 50.83±16.34 years which were higher than those seen in other studies.[4]

As in other studies,[4] atherosclerosis was more common in males when compared to females, with females contributing to 10.8% of the total number of cases in our study. The prevalence of atherosclerotic lesions in autopsy specimens was very high with a rate of > 99%. This finding is much higher than the figures quoted in studies from Haryana and Karnataka (73 to 86%).[7] Advanced lesions constituted 44.11% of atherosclerotic lesions, which is higher when compared to those reported in some Indian studies.[4] In our study, grade 2 lesions proportionally increased from the second decade onwards, while grade 3 and grade 4 lesions increased from fourth decade. Surprisingly grade 5 lesions began increasing from the third decade

itself, before either grade 3 or grade 4.Our patients had grade 1 lesions even in the 5th and 6th decades. The prevalence of intermediate lesions was more common in the middle and older age groups in our study which was in contrast to other studies.[4]

Immunostaining revealed quantitative reduction in CD4+ positivity as the grade of lesions increased. It was maximally observed in grade 1and grade 2 lesions and hardly detectable thereafter. This was similar to that reported in experimental studies by Sagan.[8] In a study by VSV Paul et al,[9] it was found that in advanced lesions CD8+ was the more quantitatively dominant subset as against CD4+. Most studies of immunohistochemistry of human atherosclerotic plaques have been studied using specimens of carotid arteries, obtained during end arterectomy or surgery. We found CD4 cells populating mostly early lesions. In a study by Erik A. L et al,[10] he concluded that there is progressive accumulation of CD4 cells up to the stage of plaque rupture, with a striking decline in the aftermath of rupture, especially in the intima. And over-representation of CD8 T cells in the earlier stages of disease, with more-balanced CD4-CD8 ratios later on. A study by Michiel et al,[11] investigated the hypothesis that the presence of circulating CD4+CD28null T cells establishes a risk factor for a novel atherosclerotic vascular event. Given the proinflammatory and plaque destabilization potential of these cells, their direct involvement in plaque rupture seems likely. Jie Li et al,[12] studied the essential role for the homing of CD4 T cells to the atherosclerotic aortic wall where they exacerbate atherosclerosis. We did not have atherosclerosis grading beyond type VI, although severe complications like thrombi, myocardial ischemia/necrosis had been seen in nearly 50% of patients.

Conclusion

Autopsy studies are useful means to gain information regarding pathogenesis, pathology, prevalence, mortality and morbidity of atherosclerosis. This study of archival specimens of the thoracic ascending aorta showed that the incidence of advanced atherosclerotic lesions were greater when compared to other studies, partly due to higher mean age of our subjects. Larger studies are needed (retrospective and prospective) to study this population with respect to premorbid conditions, occupation, dietary and lifestyle factors and corresponding aortic atherosclerosis. More autopsy studies are required to study patterns of CD4+ staining in archival aortic specimens. Presence of scattered CD4+ cells in early grades of atherosclerosis signifies that atherosclerosis is an inflammatory disorder, where immunity plays a major role in its pathogenesis. Moreover, it may foster new investigations into the immune-cell responses subsequent to plaque rupture and thrombus reorganization. Prevention and treatment of atherosclerosis can be modulated based on our understanding of T cell inflammation which plays a major role in atherosclerotic plaque.

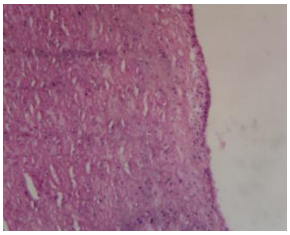

Fig. 3: Photomicrograph of Grade 1 lesion with isolated foamy cells [H&E, 10x].

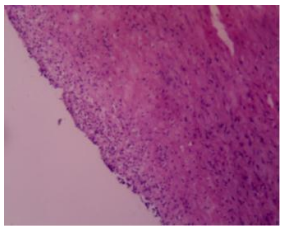

Fig. 4: Photomicrograph of Grade 2 lesion showing numerous foamy cells in layers H&E, 10x]

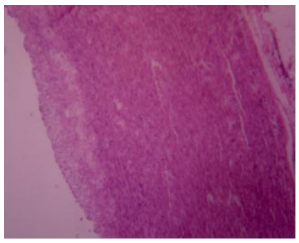

Fig.5: Photomicrograph of Grade 3 lesion with extracellular lipid pool [H&E,10x]

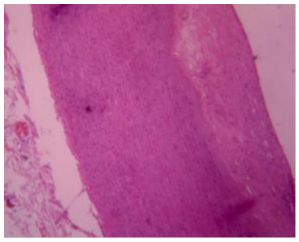

Fig. 6: Photomicrograph of Grade 4 lesion well defined lipid core [H&E, 10x]

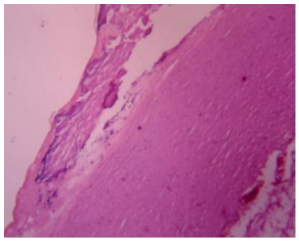

Fig. 7: Photomicrograph of Grade 5 atherosclerotic lesion with calcification [H&E, 10x]

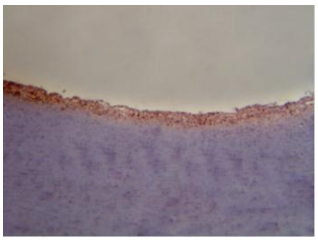

Fig. 8: Photomicrograph of type 1 atherosclerotic lesion showing strong CD4 positivity [immunostain, 10x]

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