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Coronary perivascular adipose tissue inflammation – An autopsy-based histomorphological study from a tertiary care hospital in South India

Chaithra Jadhav[1], Fremingston Marak[2], Bharathi Pradeep[3]

[1,3]Associate Professor, Department of Pathology, [2]Professor and Head, Department of Forensic Medicine, Indira Gandhi Medical College and Research Centre, Kathirkammam, Puducherry, India

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ABSTRACT

Background: The connective tissue around the blood vessels in the body (except cerebral vessels) is called perivascular adipose tissue (PVAT). Whenever there is a vascular pathology, PVAT increases in volume and becomes dysfunctional. This change promotes dedifferentiation of adipocytes. PVAT inflammation (PVATi) starts secreting anti-vasodilation and pro-inflammatory substance, leading to infiltration of inflammatory immune cells, endothelial dysfunction which initiates atherosclerosis followed by stenosis. It is now recognized that PVATi plays an important role in many vascular pathologies. **Aims and Objectives:** The objectives of the study were (1) to estimate the prevalence of coronary PVATi in an autopsy case, (2) to determine the coronary atherosclerotic changes and cardiac changes associated with PVATi, and (3) to correlate the association of PVATi with age and sex. **Materials and Methods:** Autopsy-based retrospective study done for a period of 1 year. From the autopsy requisition form, details such as age, gender, cause of death (COD), and gross findings of the organs were noted. The left coronary artery, right coronary artery, left anterior descending artery, left circumflex artery, and myocardium slides were reviewed microscopically to note the presence of PVATi, type of inflammatory infiltrate, atherosclerotic changes, percentage of stenosis, and associated cardiac changes. Range, frequencies, percentage, mean, standard deviation, and P-value were calculated. $P<0.05$ was taken as statistically significant. **Results:** Fifty-two cases showed PVATi among 400 autopsy cases performed during the period of 1 year. Forty-two cases were from males and 10 cases were from female. The most common COD noted was sudden death following chest pain. Ten cases showed PVATi involving major branches of the coronary artery. Thirty-six cases showed hypertrophic changes in myocardium. Acute myocardial infarction (MI) and old healed MI were noted in 7 and 23 cases, respectively. **Conclusion:** PVATi of coronary arteries has significant association with atherosclerosis and sudden death cardiac death.

Key words: Inflammation; Pathology; Coronary artery disease; Myocardial infarction; Atherosclerosis

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INTRODUCTION

Atherosclerosis is the most common coronary artery disease (CAD).[1] Hypertension, diabetes, hyperlipidemia, and smoking are the known risk factors for atherosclerosis. These factors, however, have generalized effect on vasculature. Local factors that could have an implicatory role in CAD are an interesting and clinically relevant element that requires further study. This could include local conditions such as non-laminar hemodynamic flow and local inflammation.[2] Several studies have established a causal role between inflammation and CAD.[3]

Address for Correspondence:
Dr. Chaithra R Jadhav, Associate Professor, Department of Pathology, Indira Gandhi Medical College and Research Institute, Kathirkamam - 605 009, Puducherry, India. **Mobile:** +91-7667435999. **E-mail:** jadhavchaithra@gmail.com

The focus of the early studies on the role of immune mechanism in pathogenesis of atherosclerosis has chiefly been on the plaque itself. Recent evidences have highlighted the role of perivascular inflammation as a key player in the development of atherosclerosis. It's seen that perivascular inflammation precedes endothelial dysfunction, its associated oxidative stress, and formation of an atherosclerotic plaque.[4]

Perivascular adipose tissue (PVAT) has been gaining increasing focus of late and subject of many studies. It is a key player and may have a role in a multitude of vascular pathologies.[5] The constituents of PVAT are connective tissue elements that include adipocytes, preadipocytes, mesenchymal stem cells, fibroblast, and inflammatory infiltrates. It surrounds most blood vessels of the body except the cerebral blood vessels.[6] It not only provides mechanical support but is also involved in maintenance of vascular homeostasis and acts as a local modulator of vascular function. The role of PVAT in physiological and pathological states differ.[7]

In the physiological state, PVAT has been identified to have anti-inflammatory effects and has a role in free fatty acid metabolism. It is a reservoir for adiponectin and omentin thereby has a role in regulation of vasodilatation. PVAT has been noted to increase in volume and exhibit dysfunctionality when it is associated with vascular pathology. These changes studied in PVAT of such cases include adipocyte dedifferentiation, whereby it stops being only lipid storage cells. Anti-vasodilation factors are secreted along with pro-inflammatory factors resulting in infiltration of PVAT by inflammatory cells.[8] Many studies on humans and animals now support an "outside-in" inflammatory process.[9,10]

PVAT inflammation (PVATi) starts secreting pro-atherogenic factors and causes endothelial dysfunction which initiates atherosclerosi.[11] It has been recognized that a dysfunctional PVAT has an important role in the pathogenesis of cardiovascular disease.[12] It is a key player in the development of various vascular pathologies, especially atherosclerosis in all its phase.[13] In humans, coronary artery is the most atherosclerosis-prone artery with an abundance of PVAT. Mice do not have PVAT around the coronary arteries.[14] In humans, the part of the left anterior descending artery (LAD) which lies intramyocardial lacks PVAT. An interesting point to note is that both experimental mice and the intramyocardial LAD artery are resistant to atherosclerosis development.[15] This suggests an association between PVAT and atherosclerosis development.

The present study is undertaken to estimate the prevalence of PVATi involving left coronary artery (LCA), right coronary artery (RCA), LAD, and left circumflex artery (LCX) which constitute the major coronary arteries.

Aims and objectives

The objectives of the study were as follows:

1. To estimate the prevalence of coronary PVATi in an autopsy case
2. To determine the coronary atherosclerotic changes and cardiac changes associated with PVATi
3. To correlate the association of PVATi with age and sex.

MATERIALS AND METHODS

This is a retrospective observational study carried out in a tertiary care hospital. Material used was archived hematoxylin and eosin (H and E) slides in the department of pathology. All autopsy forms received for histopathology examination were reviewed for a period of 1 year from January 1, 2021, to December 31, 2021. The study was approved by the Institutional Ethics Committee (IEC) with approval number (No. 392/IEC-33/IGMC&RI/PP-26/2022). After obtaining the approval, the study was conducted. The clinical information provided on the autopsy form; morphological findings as recorded during grossing of specimen in pathology department were used for the study purpose. All the autopsy cases received for a period of 1 year, all age groups and all genders were included in the study. Autolyzed hearts noted while grossing, and cases where heart was autolyzed/not received with other organ were excluded from the study. LCA, RCA, LAD, LCX, and myocardial sections from the left ventricle, right ventricle, interventricular septum, and apex were reassessed for the purpose of the study. Following points were noted: PVATi present/absent, predominant inflammatory cell type, degree of involvement of vessel wall if any, and associated vascular adventitial fibrosis. Microscopically coronary atherosclerotic lesions were examined and categorized into six groups, according to the American Heart Association (AHA) classification 1995.[16] Types of lesions according to the AHA are: Type I – initial lesion with isolated macrophages, type II – mainly intracellular lipid and fatty streak, type III – small extracellular lipid pools, type IV – core of extracellular lipid, type V – large lipid core and calcification, and type VI – hemorrhage and other complications. White, Edwards and Dry method was used to record the degree of stenosis, to grade the amount of luminal block in arteries and found to be a good and reproducible method with degree of interobserver concordance. Percentage of the lumen block up to 25%: Stenosis was considered Grade 1, 26–50% block was Grade 2, 51–75% was Grade 3, and 76–100% stenosis was Grade 4.[17] Myocardial changes such as ischemia, fibrosis, and hypertrophic changes were noted.

Statistical analysis

Statistical analysis was carried out for 52 cases. The data collected were statistically analyzed using SPSS (Statistical Package for the Social Sciences) version 16.0. Variables were summarized using frequency and percentage. Chi-square test was performed to analyze the categorical variables. Fisher's exact test was utilized for checking the association between categorical variables. P=0.05 was considered statistically significant.

RESULTS

Four hundred heart tissues obtained from autopsy were studied to see for the presence of PVATi. Fifty-two cases showed PVATi in one of the larger branches of the coronary artery. Forty-two cases were of males and 10 cases were females. Age group ranged from 28 to 75 years (mean age of 48 years) in males. In females, the age ranged from 38 to 85 years with mean age of 62 years. Overall age ranged from 28 to 85 years with mean age of 51 years and standard deviation 12.9.

As per requisition form filled by forensic department, cause of death (COD) was sudden death in 40 cases (80.8%), five cases of poisoning, one case each of road traffic accident, snakebite, post-vaccination, burns, and alcohol overdose, and two cases of chronic kidney disease patients on dialysis. Please note that the most common COD in each gender was sudden death (seven cases in women and 29 cases in men) (Table 1).

In 10 (19%) cases, PVATi was noted in all the major branches of coronary arteries studied, that is, LCA, RCA, LAD, and LCX. In rest of the cases, there was single/double/triple artery involvement in 14, 15, and 13 cases, respectively.

Table 1: Baseline characteristic of study population

Variables	Frequency	Percentage
Gender		
Male	42	80.8
Female	10	19.2
Age (in years)		
<30	4	7.69
31–40	12	23
41–50	7	13
51–60	20	38.4
61–70	5	9.6
71–80	2	3.8
>80	2	3.8
COD		
Sudden death	40	76.9
Poisoning	5	9.6
Chronic kidney disease	2	3.8
Post-vaccination death	1	1.9
RTA	1	1.9
Burns	1	1.9
Snakebite	1	1.9
Alcohol over dose	1	1.9

COD: Cause of death, RTA: Road traffic accident

Among the studied coronary arteries, the RCA showed maximum involvement (67%) followed by LAD and LCA of 61% and 60%, respectively. Artery least involved was LCX (48%) (RCA>LAD>LCA>LCX).

PVATi was predominantly composed of lymphocytes with a few macrophages in all cases except one case which showed eosinophils in addition. The PVATi was either circumferential or focal (Figure 1). The PVATi involved the outer part of tunica adventitia to varying degree with associated tunica adventitia fibrosis also of varying degrees. Denser and deeper tunica adventitia inflammation, greater is the extent of fibrosis. In 29 cases, lymphocytes were seen in aggregates forming dense clusters.

Atherosclerosis

Advanced lesions were more common in all branches of coronary artery except RCA. The RCA was the least involved by advanced lesions (Figure 2). There was significant association between atherosclerosis staging in LCA (P<0.05), LAD (P<0.05), and LCX (P<0.05) (Table 2).

Stenosis

LAD showed maximum number of cases involving Grade 4 stenosis. Out of the 32 cases of LAD with PVATi, 15 of them showed Grade 4 stenosis. There was significant association between the grade of stenosis in LCA and PVATi (P=0.001) (Table 3).

Thirty-six cases showed hypertrophic changes in myocardium. Old myocardial infarction (MI) and acute MI were noted in 30 cases. In seven cases of acute left ventricular MI, there was an involvement of LAD with PVATi.

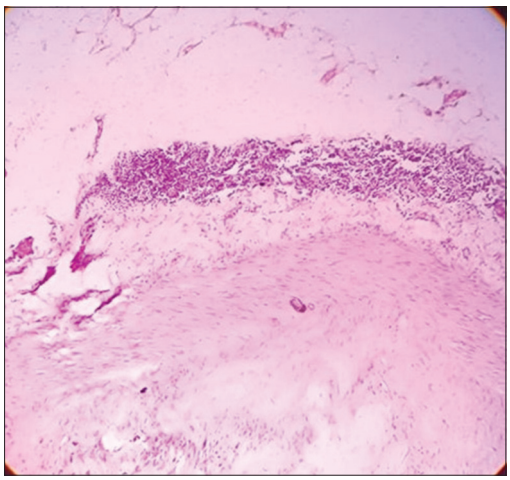

Figure 1: Circumferential PVATi composed of lymphocytes (×40)

DISCUSSION

PVAT is an element of great focus due to its direct causal role for PVATi in the pathogenesis of atherosclerosis. Studies in apolipoprotein E(apoE) knockout mice, demonstrated that pathogenic fat with PVATi was transplanted on to the carotid vessels resulting in atherosclerosis. another study in which transposition of aortic PVAT from obese nice to non-obese mice accelerated neointimal hyperplasia and adventitial macrophage infiltration in coronary artery.[18] Human observational studies also report that PVATi localizes with atherosclerotic plaques and associates with advanced lesions. The later was an important finding in our present study as well.[19]

Several reports from literature focusing on epicardial adipose tissue in patients with CAD describe a more pro-inflammatory profile compared with subcutaneous fat in the same patient and higher secretion of adipocytokines and monocyte chemotaxis near stenotic coronary segment.[20] Thickness of PVAT measured on computed tomography images was higher in those patients with atherosclerotic plaques in coronary arteries as compared to patients without plaques in the coronary arteries.[21] Postmortem studies done earlier demonstrated that atherosclerotic plaque size and complex lipid core composition were positively associated with PVATi volume and macrophage infiltration of adventitia.[13]

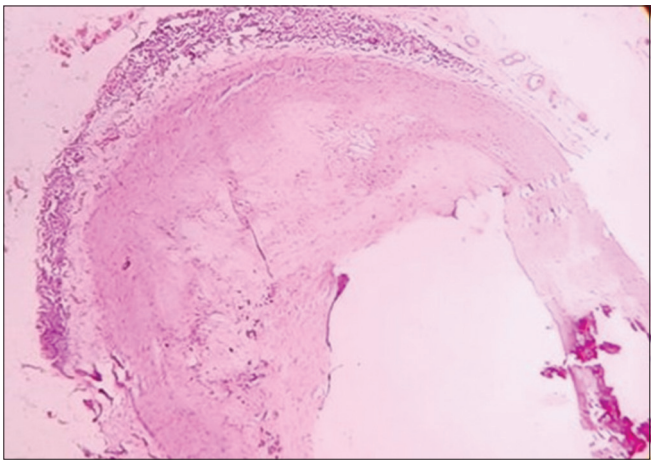

Figure 2: PVATi accompanying calcified atherosclerotic plaque (×40)

One study has shown high density of CD20+B lymphocytes and CD68+ macrophages in peri plaque tissue. Same studies also showed that PVATi was increased with greater plaque size and CD68+macrophages near unstable atherosclerotic plaques than near stable lesions. This inflammation was more intense in the peri plaque PVAT level than in the PVAT distal to the atherosclerotic plaques.[22]

Few autopsy studies have suggested that plaque/media ratio increased with increasing PVATi. PVATi density and macrophage infiltration are highly related to atherosclerotic plaque size and composition in patients with coronary atherosclerosis.[13] Inflammatory cell invasion in adventitia is associated with the extent of local atherosclerosis in patients with severe CAD.[23] Lymphocytic aggregates and an increase in adventitial macrophage content are well correlated with higher AHA grades of atherosclerosis and markers of plaque instability (ulceration, thrombosis, and intra-plaque hemorrhage).[24]

In the present study, we have estimated the prevalence of PVATi in important branches of coronary arteries. Furthermore, we noted if PVATi involves single/double/triple/all branches of the coronary artery among the given cases. Most of these cases were brought to hospital with a history of collapse/sudden death. PVATi was noted in both genders and in all age groups ranging from 28 years to 85 years. Preponderance in male gender and among 50–60 years age group was noted. Predominant inflammatory infiltrate was lymphocytes along with few macrophages, the inflammatory infiltrate was found to be circumferential in some cases, while focal (multifocal) in others. Factors that were correlated with PVATi included grade of atherosclerosis (AHA), grade of coronary artery stenosis, and myocardial changes, namely, acute infarction, old infarction, and hypertrophic changes. Advanced lesions of atherosclerosis showing PVATi were most seen in RCA (67%), LAD (61%), and LCA (60%). Among all coronary arteries with subtotal to near-total occlusion, the most frequently involved was LCA in cases of PVATi. An important point to note is that all cases in our study who had acute MI showed PVATi involving LAD. Significant association was noted in between atherosclerosis staging

Table 2: Atherosclerosis AHA staging in coronary arteries with PVATi

AHA atherosclerosis staging	LCA (%)	RCA (%)	LAD (%)	LCX (%)
I	0 (0)	0 (0)	0 (3.1)	0 (0)
II	1 (3.2)	0 (0)	1 (6.3)	1 (4)
III	1 (3.2)	3 (8.6)	2 (15)	3 (12)
IV	10 (32.3)	19 (54.3)	11 (3)	6 (24)
V	15 (48.4)	10 (28.6)	15 (46.9)	8 (32)
VI	4 (12.9)	3 (8.6)	3 (9.4)	7 (13.5)
P-value	<0.05	0.064	<0.05	<0.05

AHA: American Heart Association, PVATi: PVAT inflammation, LCA: Left coronary artery RCA: Right coronary artery LAD: Left anterior descending, LCX: Left circumflex artery

Table 3: Stenosis grading in coronary arteries with PVATi

Stenosis grading	LCA	RCA	LAD	LCX
1–25% (Grade 1)	3	2	3	3
26–50% (Grade 2)	7	9	10	5
51–75% (Grade 3)	15	12	4	8
76–100% (Grade 4)	6	12	15	9
Total	31	35	32	25
P-value	0.001	0.83	0.112	0.008

LCA: Left coronary artery, RCA: Right coronary artery, LAD: Left anterior descending, LCX: Left circumflex artery

in LAD, LCA, LCX, and PVATi. There was significant association between the grading of stenosis in LCA and PVATi.

These results indicate potential involvement of PVATi in coronary atherosclerotic plaque development and further complications such as thrombosis and hemorrhage, leading to MI. Thus, targeting PVAT function is emerging as a novel therapeutic approach for the treatment of atherosclerosis that warrants validation in the future research. In summary, accumulating evidence indicate PVAT as a double-edged sword that possess both anti-atherogenic and pro-atherogenic effects under different conditions. Further studies are needed to evaluate whether targeting PVAT function can be used as a novel approach for the treatment of atherosclerosis and subsequent coronary vascular disease.

Limitations of the study

This is the first study to find the prevalence of PVATi using autopsy material. Most studies have studied PVATi in relation to LAD. In this study, we have looked for the presence of PVATi in all the coronary arteries along with LAD.

As it is a retrospective record-based study, we did not get the history of comorbid conditions and known risk factors such as diabetes, hypertension, family history, smoking, body fat distribution, and medications taken.

CONCLUSION

PVATi has a role in CAD. These results support the concept that PVATi may play a role in the development of coronary atherosclerosis and contribute to sudden death.

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Authors Contribution:
CRJ- Design of the study, prepared first draft of manuscript, statistical analysis, and results interpretation; **FM-** Performed autopsy, provided intra-autopsy findings, concept, and coordination; and **BKV-** Concept of the study, coordination, reviewed the literature, and revision of the manuscript.

Work attributed to:
Indira Gandhi Medical College and Research Institute, Kathirkamam - 605009, Puducherry, India.

Orcid ID:
Chaithra Jadhav - https://orcid.org/0000-0002-2950-3539
Fremingston Marak - https://orcid.org/0000-0002-9361-3126
Bharathi Pradeep - https://orcid.org/0000-0002-7365-756X

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