ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 04 | April - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ORCEIN- SPECIAL STAIN TO HISTOLOGICALLY STUDY LEFT ATRIAL APPENDAGE(LAA)

Anatomy

Pardhi Garima Demonstrator in Dept. of Anatomy, (A.B.V.G.M.C.) Atal Behari Vajpayee Government Medical College, VIDISHA.

Gadekar Savita* Professor in Dept. of Anatomy, R.K.D.F.Medical College, Bhopal. *Corresponding Author

ABSTRACT

Background: The left atrium possess a venous component , a vestibule and an appendage and shares the septum. The structure of the heart undergoes constant adaptation to physiologic changes in the organism. LAA is a major endocrine organ and is the main producer of ANP (atrial natriuretic peptide) in the human heart. Atrial cardiocytes of the mammalian heart contain granules which are morphologically similar to those in peptide secreting endocrine cells. The cardiocytes of LAA contain the greatest density of ANF granules found in Left Atrium and it is therefore of interest to carry out further studies on these granules.[3]

AIMS: Looking to the great clinical significance that is given to the morphology of LAA & its predilection as a site for thrombus formation, we've conducted a detailed study histologically of LAA. To investigate LAA and study the arrangement of elastic bundles of LAA histologically (with a special stain-Orcein) and these findings were co-related with that described in the literature.

Materials&methods: The materials for the present study were collected from the Department of Anatomy & Forensic medicine of Sri Aurobindo Medical College and Post Graduate Institute, Indore. Randomly five hearts were selected for histological purposes of Left Atrial Appendage (LAA) which included the following:

slides of LAA from different sites (apex, inferior margin and atrio-auricular junction) were prepared by routine Histological Techniques and stained. By Haematoxylin&eosin and Orcein(special stain)

CONCLUSION: This study of its histological features is an attempt to establish the cause of various pathologies related to arrythmias.

KEYWORDS

A.N.P., L.A.A., Orcein

INTRODUCTION

It is axiomatic that an understanding of the complex architecture of the atrial musculature should improve understanding of its activation and contraction. Describing Harvey as the 'patron saint' of anatomists, Arthur Keith, in his Harveian Lecture given in 1918, referred to the architecture of the musculature of the heart as a 'well-worn theme' of the 17th century. In his earlier treatise written in 1907 on the jugular pulse, Keith had opined that each atrium and ventricle contained two sets of muscular fibres—circular and longitudinal. He suggested that the circular fibres in the right atrium were for compressing the chamber and expelling the blood, while the longitudinal fibres were antagonists of those to be found in the right ventricle.[9]

The left atrium possess a venous component , a vestibule and an appendage and shares the septum. The Left Atrial Appendage (LAA) is characteristically a small finger like extension in human hearts and has crenations over all lobes that are potential sites for disposition of thrombus.[1]

For decades anatomy text books tended to focus on the ventricles because of their important role as pumping chambers. The two atria have long been looked at as collecting chambers, hence attracted less interest for studying them.

Actually, the LAA is a remnant of the original embryonic left atrium formed during the third week of gestation. The LAA lies within the pericardium in close contact with the free wall of the left ventricle. It is therefore likely that blood flow, in and out of the LAA, depends to a significant degree on a properly functioning left ventricle. [5]

The trabecular LAA is a remnant of the original embryonic left atrium that develops during the third week of gestation. The main smooth-walled left atrial cavity develops later and is formed from an outgrowth of the pulmonary veins. The appendages passively fill during ventricular systole and then passively empty during ventricular diastole.[2]

Atrial cardiocytes of the mammalian heart contain granules which are morphologically similar to those in peptide secreting endocrine cells. These secretory granules were discovered by Kisch (1956). These granules are known as Atrial Natriuretic Factor (ANF).[6]

LAA is a major endocrine organ and is the main producer of ANP (atrial natriuretic peptide) in the human heart. The ANP concentration is 40 times higher in the LAA walls than in the rest of the atrial free wall and in the ventricles. A study of patients having undergone the maze procedure and associated LAA removal found a significantly lower ANP secretion and a commensurate increase in salt and water retention. Whether this could eventually lead to hypertension is not known.[5]

So, analysis of ANF has shown that LAA contain about 30% of all cardiac ANF. The cardiocytes of LAA contain the greatest density of ANF granules found in Left Atrium and it is therefore of interest to carry out further studies on these granules.[3]

AIMS and OBJECTIVES

Looking to the great clinical significance that is given to the morphology of LAA & its predilection as a site for thrombus formation, we've conducted a detailed study histologically of LAA.

- To investigate LAA and study the arrangement of elastic fibres in LAA histologically(with a special stain-Orcein) and these findings were co-related with that described in the literature.
- The specimens were photographed and photomicrography of appropriate histological sections was carried out.
- These findings were correlated with those cited in the literature. An attempt was made to draw a correlation of these findings with the functions of LAA.
- These in turn help in understanding on its clinical significance and would be of help manage these patients.

MATERIALS & METHODS

The materials for the present study were collected from the Department of Anatomy & Forensic medicine of Sri Aurobindo Medical College and Post Graduate Institute, Indore. Randomly five hearts were selected for histological purposes of Left Atrial Appendage (LAA) which included the following:

For histology: slides of LAA from different sites (apex, inferior margin and atrio-auricular junction) were prepared by routine Histological Techniques and stained.

II. HISTOLOGY:

Small pieces (2.0cm X 0.5cm) of LAA were taken from different sites (apex, inferior margin and atrio-auricular junction).The tissues were fixed in 10% formalin, and were processed for routine histological procedures – like dehydration, clearing and after wax embedding, blocks were prepared. Sections 5-6micron thick were cut and stained with the following stains: [7,8]

I) H & E: Method for staining are- 1. Sections were dewaxed with xylene and the sections were taken to water (with descending grades of alcohol).
2. Stain in haematoxylin, in a jar for 5-10minutes.
3. Wash well in running tap water for 2-3mnutes.
4. Remove excess stain by decolorizing (differentiating) in 0.5-1% hydrochloric acid in 70% alcohol for few seconds.
5. Wash in alkaline running tap water for 5minutes.
6. Stain in 1% aqueous eosin for 1minutes.
7. Wash surplus stain in water.
8. Dehydrate in alcohol, clear in xylene.
9. Mount in D.P.X. or Canada balsam.

iv) Orcein: Method for staining are-
1. Dewax the sections.
2. Bring sections to 70% alcohol.
3. Stain in the orcein solution for 30minutes -2hour.
4. Wash thoroughly in 70% alcohol.
5. Differentiate in 1% acid alcohol for destaining of collagen fibers.
6. Wash thoroughly in tap water.
7. Counterstain nuclei with methylene blue.
8. Dehydrate in alcohol.
9. Clear in xylene.
10. Mount in D.P.X.[7]

OBSERVATIONS

The Left Atrial Appendage (LAA) is a very unique structure within the pericardial cavity, although small in size and having a variegated appearance, it is notorious for arrhythmias and thrombo-embolic phenomenon. This study was undertaken with the objective that certain features of histological anatomy may help in elucidating certain special features of LAA which can be correlated with its tendency for malfunctioning.

HISTOLOGY

Histological features of the three regions (atrio-auricular junction, inferior margin and apex) studied showed almost identical features with only very slight variations that have been included in the observations described below. Due to the same reason, only selected photomicrographs have been included. Following are the microscopic findings:

Epicardium-

- The outer layer of the heart is termed epicardium. There was a thin layer of epicardium where deep to it greater amount of adipose tissue was present. These features were seen in all the three sites studied namely atrio-auricular junction, inferior margin and apex.
- Deep to the lining epithelium were layers of fibrous tissue intermixed with elastic fibers. (fig1.). Similar findings were seen in sections taken from apex and inferior margin.
- Where the wall of the left auricle was very thin sub-epithelial tissue was continuous with sub-endocardial tissue with only thin connective tissue being present between them. Epicardium showed a thin layer of elastic fibers in sections taken from inferior margin (fig.1).

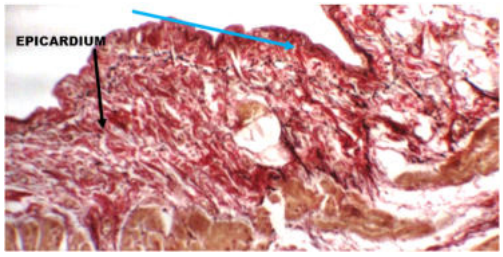
b) Myocardium-

- Myocardium of AAJ in the auricular wall showed variable thickness.
- There was presence of large number of blood vessels of various sizes and types seen between the muscle fibers. They were present in sections of all the three sites studied.

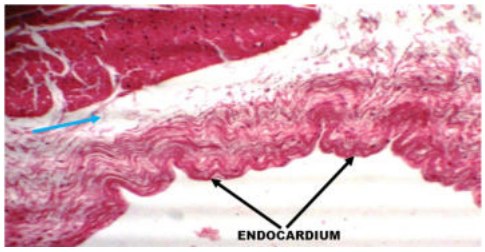
c) Endocardium –

- The endocardium is the innermost layer that lines the chambers of the heart. Endocardium of AAJ was not uniform; it was wavy in character where it was thin. This was also, seen in sections taken from apex and inferior margin. Elastic fibers, which were non-cellular and eosinophilic, were seen (fig.3). Well-defined zonation could be observed in sub-endothelial connective tissue (fig.3), which showed wavy elastic fibers also. Elastic fibers were present along with variable amount of connective tissue. There was stratification of elastic lamella (fig.3). These features were also seen in sections taken from apex and inferior margin.

- The atrial wall endocardium was smooth with flattened endothelial cells. At places sub-endocardial space with loose connective tissue was observed (fig.2).



- **Fig.1: Photomicrograph of section of Inferior margin showing elastic band (blue arrow) in the epicardium. Thin epicardium (blue arrow) and thick epicardium (black arrow) are seen. (Orcein;10x)**



- **Fig.2: Photomicrograph of section of Inferior margin showing thick endocardium ith elastic fibers (black arrows) and loose connective tissue in sub-endocardial space(blue arrow). (H&E;10x)**

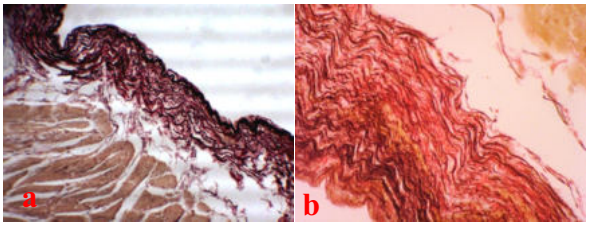


Fig. 3: Photomicrograph of section of Atrio-auricular junction showing thick elastic fibers in the endocardium. (Orcein; a-10x, b-40x)

Summary and conclusion

This study is an attempt to establish various dimensions of LAA and study its histological features so that they can be correlated to establish the cause of various pathologies related to arrythmias. The shapes of shapes and depths that can become the potential sources for thrombus formation leading to embolic phenomenon.

The histological features of all the three regions of LAA studied confirm to the classical description of all these layers of the atrial wall, with a few exceptions: i) sub-endocardial tissue showed stratification along with a number of elastic lamellae;

The present findings will be of great help in the interpretation of Trans-Esophageal-Echocardiography (TEE) and the understanding of the thrombo-embolic phenomenon.

REFERENCES

1. Wang K, Ho SY, Gibson DG, Anderson RH. Architecture of atrial musculature in Humans. Br. Heart J 1995; 73:559-565.
2. Bold AJD, Raymond JJ, Bencosme SA. Atrial specific granule of the rat Heart Light Microscopic staining and Histochemical Reaction. J of Histochemistry and Cytochemistry 1978; 26:1094-1102.
3. Saady NMA, Obel OA, Camm AJ. Left Atrial Appendage: Structure, Function and role in thrombo-embolism.Heart 1999; 82:547-555.
4. Cardiac histology (I). e--heart.org. Cited on 02/08/2013; Available fromURL:http://www.eheart.org/ pages/01 cardiac_structure/01 Cardiac_Structure Cardiac Histology 001.htm.
5. Larsen HR. Left Atrial Appendage: Useless or Priceless? The Afib Report. Available fromURL:http://www.afibbers.org.
6. Lindop GBM, Mallon EA, Maclntyre G. Atrial natriuretic peptide in the heart and pancreas. Histol Histopath 1986. 1:147-154.
7. Carleton HM, Drury RAB. Histological technique for normal and pathological tissues and the identification of parasites. London Oxford University Press New York Toronto 3rd Edition 1957; 100-105.
8. Culling CFA. Handbook of Histopathological and Histochemical Techniques. Butterworths 3rd Edition 1975; 211-219.
9. Ho SY, Anderson RH, Sanchez-Quintana D. Atrial structure and fibres: Morphologic Basis of Atrial conduction. Cardiovascular Research 6th Edition 2002; 54:325-336.