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Original Research Paper

Anatomy

HISTOLOGICAL STUDY LEFT ATRIAL APPENDAGE(LAA) BY SPECIAL STAIN-PTAH

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ABSTRACT

BACKGROUND: The structure of the heart undergoes constant adaptation to physiologic changes in the organism .The left atrium possess a venous component , a vestibule and an appendage and shares the septum. 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. **AIMS:** Looking to the great clinical significance that is given to 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 muscular bundles of LAA histologically (with special stain-PTAH) and these findings were co-related with that described in the literature. **MATERIALS&METHODS:** The materials for 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 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 PTAH(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.F., L.A.A., PTAH

INTRODUCTION

It is axiomatic that understanding of the complex architecture of the atrial musculature should improve understanding of its activation and contraction. 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]

In the past LAA had been considered to be relatively insignificant portion of cardiac anatomy. It is now recognised that it is a structure with important pathological association.[2] A dual, secretory-contractile function has been postulated for atrial cardiocytes of the mammalian heart. These cells display morphological features common to both cardiac muscles and secretory cells.[2] The LAA shortens to a greater extent than the rest of the left atrium and has distinct pattern of contraction.[3]

The atrial appendages have a distinctive anatomy. While the atria are smooth-walled, the appendages contain numerous trabeculae (pectinate muscles), resembling the ventricles. The LAA is small, muscular extension of the left atrium arising near the left pulmonary veins. The LAA shortens to a greater extent than the rest of the left atrium and has distinct pattern of contraction. Saady et al (1999) have stated that LAA is the site most commonly associated with thrombus formation particularly of Non Valvular Fibrillation.[3]

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]

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.[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 muscular fibres in LAA histologically(with a special stain-PTAH) 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.

II) P.T.A.H.(Phospho-tungstic-acid-hamaematoxylin) :
Method for staining are-
1. Take sections to water.
2. Treat with iodine and sodium thiosulphate.
3. Place in 0.25% aqueous potassium permanganate for 5 minutes.
4. Wash in water for 2 minutes.
5. Place in 5% oxalic acid for 10 minutes.
6. Wash in water for 5 minutes and rinse in distill water.
7. Stain in P.T.A.H. for 4-6 hours.
8. Dehydrate in 95% alcohol.
9. Dehydrate in absolute alcohol, clear in xylene and mount in D.P.X

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 of thin layer of epicardium where deep to it greater amount of adipose tissue was present and the epicardium was thick if adipose tissue was scarce or absent (fig.2). These features were seen in all the three sites studied namely atrio-auricular junction, inferior margin and apex.
- Lining cells of epicardium in (Atrio-Auricular Junction) AAJ showed the presence of flattened cells (fig.2) which at places were cuboidal to columnar , in sections of apex and inferior margin
- At places in sections taken from apex, in sub-epicardial region there was large amount of fat and it was covered by flattened cells (fig.2).

B) Myocardium-
- Myocardium of AAJ in the auricular wall showed variable thickness. Myocardium was very vascular (fig.3). Very prominent transverse striations were seen in longitudinal sections of muscle fibers (fig.1). Similar findings were seen in sections taken from apex and inferior margin.
- Size of the fibers was variable. Fibers were oriented in different directions: where the atrial wall was thin the fibers were mostly circularly arranged. At places, longitudinal fibers were sandwiched between transversely oriented fibers.
- There was presence of large number of blood vessels of various sizes and types seen between the muscle fibers (fig.3). They were present in sections of all the three sites studied.
- Musculi pectinati were seen as projections in the auricular cavity being lined by endocardium. At some places, fat cells were seen permeating between muscle bundles along with veins and arteries. Thick muscle bundles were found with presence of longitudinally running capillaries between them. (fig.3)

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.
- Some special cells were present in the endocardium, below the endothelial lining. These cells were present in groups and at distances, were darkly eosinophilic having small and centrally placed nucleus. Similar finding was seen in sections taken from apex and inferior margin.
- The atrial wall endocardium was smooth with flattened endothelial cells. Whereas, auricular wall showed projections.

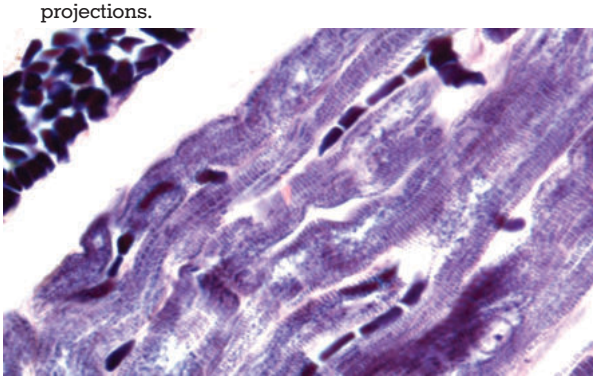

Fig. 1: Photomicrograph of section of Atrio-auricular junction showing transverse striations in longitudinal muscle fibers. (PTAH; 40x)

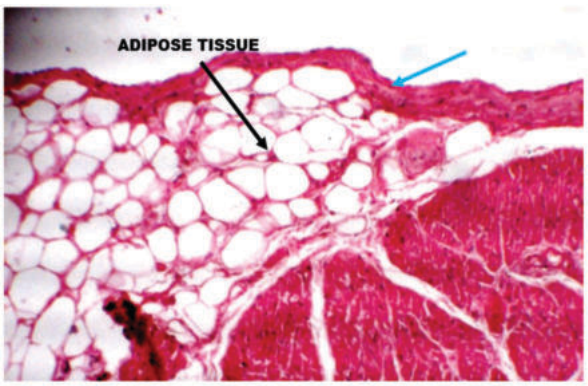

Fig. 2: Photomicrograph of section of Apex showing presence of flattened cells (blue arrow) in the epicardium. (H&E;10x)
(H & E; 4x)

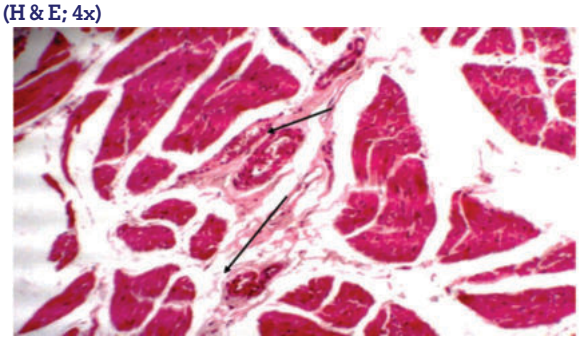

Fig. 3: Photomicrograph of section of Atrio-auricular junction showing blood vessels (black arrow) in the myocardium. (H & E; 10x)

Summary and Conclusion

The observations of the myocardium in the present article have shown that the myocardium has variable thickness. Fibers were oriented in different directions: where the atrial wall was thin the fibers were mostly circularly arranged.

Siew et al. (1995) have reported that smooth walls of LAA are composed of one to three, or more, overlapping layers of differently aligned myocardial fibers, with marked regional variations in thickness. Our present findings are in agreement with their findings.

This article is an attempt to study its histological features so that they can be correlated to establish the cause of various pathologies related to arrythmias.

The histological features of all the three regions of LAA studied conform to the classical description of all these layers of the atrial wall, with a few exceptions: i) some special category of cells were seen within the connective tissue of the endocardium;ii) at places the epicardium was lined by cuboidal to columnar cells.

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

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