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Original Research Article

A Morphometric Study of Pulmonary Veins and Their Openings in the Left Atrium in Cadavers of Eastern Indian Population

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ABSTRACT

Background: Knowledge about the variations in number and drainage pattern is helpful as pulmonary veins are an important source of ectopic atrial electrical activity, frequently initiating paroxysms of atrial fibrillation.
Aims and objectives: This study will show the variation of number, drainage pattern of pulmonary veins draining at the left atrium and their variations with respect to age and gender, in heart samples obtained from cadavers.
Methods: 100 specimen of human heart with stumps of great vessels were collected from Anatomy and Forensic departments of IPGME&R and SSKM hospital. The number of right and left pulmonary veins draining into the left atrium was observed in the formalin preserved heart samples from the external aspect. The left atrium was opened by an incision along the left side of the left pulmonary veins of the heart to see the drainage pattern of the pulmonary veins. The distances between the approximate mid-point of limbus fossa ovalis on inter-atrial septum and each pulmonary venous opening were measured.
Result: During describing the drainage pattern the coefficient of variation for the distance of openings of male left superior pulmonary vein (LSPV) is 0.92%, and that of female LSPV is 0.87%. Unpaired t-test shows the P value <0.05 and therefore the means are significantly different.
Conclusion: The results obtained from data recorded showed that 10% specimens had single left pulmonary venous opening, and 10% had single right pulmonary venous opening. Rest of the specimens had normal four pulmonary venous openings.

Key words: Pulmonary venous opening, atrial fibrillation, radiofrequency catheter ablation.

INTRODUCTION

The pulmonary veins (PV) have been demonstrated often to play an important role in generating atrial fibrillation (AF). Hemodynamic compromise and formation of thrombo-emboli are two main dreaded complications of atrial fibrillation. It has been reported that ectopic foci responsible for the initiation of atrial fibrillation were in the walls of the superior vena cava, both the atria, the crista terminalis, ostium of the coronary sinus, inter-atrial septum and the muscular sleeves of the distal pulmonary veins. So the pulmonary vein is an important source of ectopic atrial electrical activity, frequently initiating paroxysms of atrial fibrillation. Increasingly, selective radiofrequency ablation of these arrhythmogenic foci is performed to treat patients with refractory

atrial fibrillation. The effectiveness of this invasive procedure relies on precise mapping and complete disconnection of the electrical initiators from the atrial tissue. Thus, detailed knowledge of pulmonary venous anatomy and relationships between the pulmonary veins and the left atrium is necessary during mapping and ablation.

Normal anatomy of the Left Atrium:

The left atrium has smaller volume and thicker walls than the right atrium. Its cavity and walls are formed largely by the proximal parts of the pulmonary veins, which are incorporated into the atrium during development. The only derivative of the left part of the embryonic atrium is the auricle and the vestibule of the mitral valve. The right atrium is in front and antero-lateral to the right part of the left atrium. Antero-inferiorly, and to the left, it adjoins the base of the left ventricle at the orifice of the mitral valve. Its posterior aspect is approximately quadrangular, receiving the terminations of the two pulmonary veins usually, from each lung. Interiorly, the four pulmonary veins open into the upper postero-lateral surfaces of the left atrium, two on each side. Their orifices are smooth and oval, the left pair frequently opening via a common channel. The left atrial aspect of the septum has a characteristically rough appearance, bounded by a crescentic ridge, concave upwards, which marks the site of the foramen ovale.

Normal anatomy of the Pulmonary Veins:

There are usually four pulmonary veins, two from each lung. They return oxygenated blood to the left atrium. The right and left pulmonary veins perforate the fibrous pericardium and open separately in the postero-superior aspect of the left atrium. The right superior pulmonary vein passes posterior to the superior vena cava and the right inferior behind the right atrium. Both superior and inferior left pulmonary veins pass anterior to the descending thoracic aorta. Sometimes the two left pulmonary veins form a single trunk. Or they may be augmented by a third left pulmonary vein. The pulmonary veins are devoid of valves. [1]

MATERIALS AND METHODS

Study area

- Department of Anatomy, IPGME&R, Kolkata.
- Department of Forensic and State Medicine, IPGME&R, Kolkata.

Study population

- Cadaveric samples from the bodies donated at the Department of Anatomy, IPGME&R, voluntarily for academic purposes.
- Fresh corpses undergoing medico-legal autopsy at the Department of Forensic AND State Medicine, IPGME&R.

Study period

March 2013 to February 2014.

Sample size

As this is a purely observational and descriptive study so a convenient sample size was taken, which were 100.

Study design

This study is an observational study.

Parameters to be studied

1. Total number of pulmonary venous openings in the left atrium.
2. Number of right pulmonary venous openings in the left atrium.
3. Number of left pulmonary venous openings in the left atrium.
4. Distances of left and right superior pulmonary venous openings from the inter-atrial septum in heart specimens of different age groups between 20 to 80 years.
5. Distances of left superior pulmonary venous openings from the inter-atrial septum in male and female heart specimens.
6. Distances of right superior pulmonary venous openings from the inter-atrial septum in male and female heart specimens.

Study tools

1. Measuring metric scale.
2. Slide callipers.

3. Scalpel blade with handle.
4. Non-toothed forceps.
5. Straight scissors.

Study techniques

The specimens of human heart with stumps of great vessels were collected from respective departments as mentioned before. During dissection of thoracic region the heart were taken out, washed in water and preserved in formalin. The dissection in the Department of Forensic and State Medicine was carried out in collaboration with the faculty of the said Department. The numbers of right and left pulmonary veins draining into the left atrium were observed in the preserved heart samples from the external aspect. The left atrium was opened by an incision along the left side of the left pulmonary veins of the heart to see the drainage pattern of the pulmonary veins. The distances between the approximate mid-point of limbus fossa ovalis on the inter-atrial septum and each of the right superior and left superior pulmonary venous opening also were measured with the help of slide callipers.

The specimen after collection and dissection was taken up for measurements, quantification and tabulation. The measurements as required for the study were taken by metric scale and slide callipers.

Photographic records of the samples and methods employed were kept for further reference.

The epidemiological data like the age; gender etc were collected from the relevant papers supplied by the police authorities to the Head of the Department, Forensic and State Medicine, as necessary for conduction of medico-legal examination. The same data from the Department of Anatomy were collected from departmental records.

All the data were quantified and tabulated in the proposed proforma of the study provided by the investigator. Statistical analysis was made accordingly.

Informed Consent for The Study

As the study was carried out on cadavers, corpses brought to the Department of Forensic and State Medicine for medico legal autopsy and from voluntarily donated bodies in the Department of Anatomy, examination of any organ or, structure for academic purpose is permitted, according to the prevailing laws of the land. Hence no further consent is necessary for the preview of the study.

The investigator will use the data generated only for the study for further reviews on medical, educational & statistical purposes. The records obtained from cadavers will be a confidential matter and would not be published outside without the permission of the nearest kith and keens of the deceased. The records will be allowed to be viewed by the requesting party upon clearance from the investigator and respective authorities.

Statistical Methods:

Data would be summarized by descriptive statistics, namely mean and standard deviation for numerical variables in counts and percentages for categorical variables. Numerical data would be compared between subgroups by students unpaired ‘t’ test if normally distributed or, by Mann-Whitney U test if otherwise. Chi-square test or, Fisher’s exact test would be used for intergroup comparison of numerical variables. All analysis will be two tailed and $p<0.05$ would be considered statistically significant.

RESULTS

TABLE : 1 Distribution of Pulmonary veins among Male and Female Heart Specimens

Number of Pulmonary Venous Openings	Male	Female
RSPV	52	33
RIPV	52	33
RPV	12	3
LSPV	52	31
LIPV	52	31
LPV	12	5

CHART: 1

Comparative representation of number of Right and Left Superior and Inferior

pulmonary veins in male and female heart specimens in clustered columns

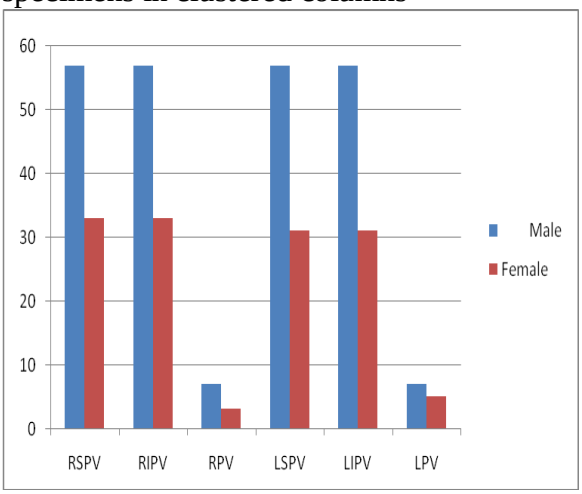

TABLE : 2 Variation of Distance of pulmonary venous opening among male and female heart specimens

Distance of Opening (cm)		Male	Female
RSPV (Right Superior Pulmonary Vein)	Mean	0.96	0.96
	SD	0.03	0.03
	CI	0.96-0.97	0.95-0.97
RPV (Right Pulmonary Vein)	Mean	0.96	0.97
	SD	0.03	0.01
	CI	0.93-0.99	0.95-0.99
LSPV (Left Superior Pulmonary Vein)	Mean	3.99	3.98
	SD	0.03	0.04
	CI	3.98-4	3.96-3.99
LPV (Left Pulmonary Vein)	Mean	3.99	3.99
	SD	0.03	0.03
	CI	3.96-4.02	3.96-4.04

Mean distance of opening of the pulmonary veins in male is 2.48 cm, with a Standard Error of 0.13 and Coefficient of Variation 61.35% .

Mean distance of opening of the pulmonary veins in female is 2.47 cm, with a Standard Error of 0.18 and Coefficient of Variation 61.50% .

Unpaired t-test shows that P value >0.05 and therefore the means are not significantly different.

TABLE : 3 Distribution of distance of openings of right pulmonary veins in male and female heart specimens of different age groups

Distance of Opening (cm)		MRSPV	FRSPV
20-40 years	Mean	0.96	0.97
	SD	0.04	0.03
	CI	0.95-0.98	0.95-0.99
41-60 years	Mean	0.97	0.97
	SD	0.03	0.03
	CI	0.96-0.98	0.95-0.99
61-80 years	Mean	0.96	0.95
	SD	0.02	0.02
	CI	0.95-0.98	0.93-0.96

The Standard error of the Distance of opening of male Right superior pulmonary vein (MRSPV) is 0.004 and of female RSPV 0.005.

The Coefficient of variation for the above parameter of male RSPV is 3.04%, and that of female RSPV is 2.98% .

Unpaired t-test shows the P value >0.05 and therefore the means are not significantly different.

TABLE : 4 Distribution of distance of openings of left pulmonary veins in male and female heart specimens of different age groups

Distance of Opening (cm)		MLSPV	FLSPV
20-40 years	Mean	3.99	3.98
	SD	0.04	0.04
	CI	3.97-4.01	3.95-4.01
40-60 years	Mean	3.99	3.97
	SD	0.04	0.03
	CI	3.98-4.01	3.94-3.99
60-80 years	Mean	3.99	3.98
	SD	0.03	0.03
	CI	3.98-4.01	3.96-4.00

The Standard error of the Distance of opening male Left superior pulmonary vein (LSPV) is 0.007 and of female LSPV 0.005.

The Coefficient of variation for the above parameter of male LSPV is 0.92%, and that of female LSPV is 0.87%.

Unpaired t-test shows the P value <0.05 and therefore the means are significantly different.

TABLE : 5 Comparison of distance of openings of right and left pulmonary veins according to gender

Gender	Distance of opening (cm)	RSPV	LSPV
Male	Mean	0.96	3.99
	SD	0.03	0.03
	CI	0.96-0.97	3.98-4
Female	Mean	0.96	3.98
	SD	0.03	0.04
	CI	0.95-0.97	3.96-3.99

The SD for RSPV is 0.03 and for LSPV 0.59 .

The coefficient of variation for RSPV is 3.01% and LSPV is 15.19% .

Unpaired t-test shows the P value is <0.05 and therefore the means are significantly different.

DISCUSSION

Atrial fibrillation is one of the most common cardiac rhythm disorders. It remains a cause of substantial morbidity and mortality, despite development of numerous treatments and antiarrhythmic drugs. Although initial experience with catheter ablation procedures that created linear lesions in the atria was disappointing, it led to the key observation that electrical initiators in the pulmonary veins were frequently responsible for atrial fibrillation. Thus, the pulmonary veins themselves became the targets of catheter-directed radiofrequency ablation. Focal ablation of specific arrhythmogenic foci has proved to be the less popular technique for several reasons. There may be several arrhythmogenic foci in one vein. Unless all foci are identified and ablated, treatment may not be successful. Arrhythmogenic foci may be present in more than one pulmonary vein. This may account for the high recurrence rates reported with focal ablation. Ectopic electrical activity in the pulmonary vein may be infrequent or absent during the electro-physiologic study, even when provocative pharmacologic or pacing procedures are performed. The catheters used are fixed in size and shape. This can lead to difficulties with catheter manipulation and may account for the significantly longer fluoroscopic times reported with this technique, which leads to increased radiation dose to both the patient and the operator. Complications like pulmonary vein stenosis occur more frequently with this technique. For these reasons, complete electrical isolation of all pulmonary veins by creating circumferential lesions at their ostia has become the more popular and widely performed procedure. Thus, it would seem that mapping the pulmonary veins and identifying anomalous veins prior to the procedure could be beneficial.

In the year 1994, Cavalcanti JS [2] and others published a study on the "Functional anatomy of the junction of the left atrium and the pulmonary veins" at Centro de Ciências Biológicas da Universidade Federal de Pernambuco, Recife. The aim was to study the spatial arrangement of the bundles of myocardial fibers presents in the left atrial-venous junctions and in the wall of the pulmonary veins. It was made on 24 human adult hearts, together with pulmonary vessels, fixed in 10% formaldehyde solution. They concluded that the extremities of the pulmonary veins and their junctions with the atrium had a morphological substract which might be of physiological importance in the control of the pulmonary venous pressure and blood flow.

Haïssaguerre, M et al [3] in 1998 published their work on "Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins" at France. Atrial fibrillation is the most common sustained cardiac arrhythmia and a major cause of stroke, resulting from simultaneous reentrant wavelets. They studied 45 patients with frequent episodes of atrial fibrillation (mean [±SD] duration, 344±326 minutes per 24 hours) refractory to drug therapy. They came to the conclusions that the pulmonary veins are an important source of ectopic beats, initiating frequent paroxysms of atrial fibrillation. These foci responded to treatment with radio-frequency ablation.

The article on "Anatomy of the left atrium: implications for radiofrequency ablation of atrial fibrillation" by Ho SY et al [4] in 1999 described the feasibility of treating atrial fibrillation with radiofrequency ablation which revived interest in the structure of the left atrium, a chamber that had been neglected in many textbooks of anatomy. They reviewed the gross structure of the left atrium by examining the septum, the appendage, and insertions of the pulmonary veins in normal hearts.

In the year 2001 Jennecy Sales Cavalcanti, Laura Patrícia Ferreira Santos [5] published their study on "Morphofunctional Study of the Junction Between the Left Atrium and the Pulmonary Veins in Patient

with Pulmonary Hypertension" in Brazil. They studied the arrangement of the myocardial fibre bundles at the pulmonary venous left atrial junction in patients with pulmonary hypertension, and to discuss the pathophysiological importance of this element in the etiology of acute pulmonary edema. For this they obtained 12 hearts and their pulmonary vein extremities from post mortem examinations of patients with the anatomico-pathological diagnosis of acute pulmonary edema. They concluded that anatomical changes that result in a reduction in the amount of myocardial fibre bundles in the pulmonary venous left atrial junction, isolated or associated with other factors, may be the cause of disorders in pulmonary circulation, leading to an increase in pulmonary venous pressure, and, consequently, to acute pulmonary oedema.

S Y Ho [6] and others in 2001 published a study on "Architecture of the pulmonary veins: relevance to radiofrequency ablation". Radiofrequency ablation of tissues in pulmonary veins could eliminate paroxysmal atrial fibrillation. They studied to explore the characteristics of normal pulmonary veins so as to provide more information relevant to radiofrequency ablation. In the study they used 20 structurally normal heart specimens were examined grossly. Histological sections were made from 65 pulmonary veins. They concluded that the myocardial architecture in normal pulmonary veins was highly variable. The complex arrangement, stretch, and increase in fibrosis might produce greater non-uniform anisotropic properties.

In 2002 a paper on "Origin, Differentiation, and Maturation of Human Pulmonary Veins" was published by Susan M. Hall and her co-workers. [7] Recent studies on human embryonic and fetal lungs showed that the pulmonary arteries form by vasculogenesis. Little was known of the early development of the pulmonary veins. Using immunohistochemical techniques and serial reconstruction, they studied 18 fetal and neonatal lungs. They found, both pulmonary arteries and veins arose by vasculogenesis, but the origins of their smooth muscle cells and their cytoskeletal protein content were different.

Oral et al, [8] in 2002, published their study on "Pulmonary Vein Isolation for Paroxysmal and Persistent Atrial Fibrillation". As the pulmonary veins (PVs) had been demonstrated to often play an important role in generating atrial fibrillation (AF) so the purpose of their study was to determine the safety and efficacy of segmental PV isolation in patients with paroxysmal or persistent AF.

In the year 2003, to define the technique and results of magnetic resonance imaging (MRI) of pulmonary vein (PV) anatomy before and after catheter ablation of atrial fibrillation (AF), Ritsushi Kato et al [9] published a study on "Pulmonary Vein Anatomy in Patients Undergoing Catheter Ablation of Atrial Fibrillation Lessons Learned by Use of Magnetic Resonance Imaging" in the Johns Hopkins Hospital, Baltimore. In this study they took twenty eight patients with AF who underwent ablation. They concluded that this study demonstrated the AF patient had larger PVs than control subjects and demonstrated the value of MRI in facilitating AF ablation. The benefits of pre-procedural MRI of PVs include the ability to evaluate the number, size, and shape of the PVs. MRI also provides an assessment of the severity of PV stenosis.

In the year 2004, from the Departments of Radiology (E.M.M., Y.H.K., H.P.M.) and Biostatistics and Bioinformatics (J.E.H.), Duke University Medical Center, Durham, NC, Edith M. Marom and colleagues [10] did the study on "Variations in Pulmonary Venous Drainage to the Left Atrium: Implications for Radiofrequency Ablation" , to evaluate and classify the various drainage patterns of the pulmonary veins as depicted with thin-section chest computed tomography (CT). Finally they developed a classification system to succinctly describe pulmonary venous drainage patterns. Right-sided venous drainage was more variable than

left-sided venous drainage. One-quarter of patients had more than two venous ostia on the right side.

In the year 2005, Yun-Hyeon Kim et al [11] performed a study on "Pulmonary Vein Diameter, Cross-sectional Area, and Shape: CT Analysis" at Duke University Medical Center, Durham, NC, over a period of 6 months, on 104 patients, 68 women and 36 men (age range, 19–86 years; mean, 49 years) who underwent thin-section contrast material–enhanced chest CT. Diameter and cross-sectional area of the left superior pulmonary vein were significantly larger in men than in women ($P < 0.005$). They also observed the caliber of three of the four veins gradually increased as they approached the left atrium.

In 2005, Monique R. M. Jongbloed et al [12] published the study on "Atrial Fibrillation: Multi–Detector Row CT of Pulmonary Vein Anatomy prior to Radiofrequency Catheter Ablation-Initial Experience", to evaluate multi– detector row computed tomographic (CT) depiction of pulmonary veins to provide a road map for radiofrequency catheter ablation. As a conclusion they opined that, multi– detector row CT provides a valuable road map for pulmonary vein anatomy prior to radiofrequency catheter ablation. Variations in number and insertion of pulmonary veins were observed in a considerable number of patients and control subjects.

In 2006 M. C. Niculescu and his co-workers [13] published their original article on "Study of the diameter and number of the pulmonary veins orifices". The study was made in the anatomy laboratory on 100 heart specimens. It was studied the morphological parameters about diameter and number of the atrial orifices of the pulmonary veins. The number of the orifices and their diameter depends on the lungs weight. Generally (70% of the cases) the orifices number is four and rarely three or five. An increased number of orifices are more frequently in the right side and a decreased number especially in the left side. The orifices diameter was much larger at the male's veins than the female's ones, and much larger in the right than the left side and also much larger at the superiors than the inferior veins.

Shan F and others, [14] in April, 2007 published a study on "Variations of pulmonary venous drainage and venous ostium index detection in atrial fibrillation patients prior to radiofrequency catheter ablation by MDCT pulmonary venography" to evaluate variations of pulmonary venous drainage and venous ostium index (VOI) in patients with atrial fibrillation (AF) prior to radio-frequency catheter ablation (RFCA) by MDCT pulmonary venography. Multidetector row CT pulmonary venography (MDCT-PV) could provide valuable informations on pulmonary venous anatomy in AF patients referred to RFCA and should be used as a routine examination prior to the operation.

In the study on " Normative analysis of pulmonary vein drainage patterns on multidetector CT with measurements of pulmonary vein ostial diameter and distance to first bifurcation" in the Department of Radiology, Division of Thoracic Radiology, University of Michigan Medical Center, Cronin P et al [15] in 2007, documented the frequency of normal and anomalous drainage patterns of the pulmonary veins, and tried to establish normal values for pulmonary vein ostial diameters, and distance to first bifurcation using multi detector computed tomography, as pertinent to ablation procedures for atrial fibrillation. There was significant variability in pulmonary vein diameter and distance to first bifurcation.

Though fresh human heart specimen would probably give more correct result, but in this study formalin fixed human heart specimens were used.

The number of pulmonary venous openings in the left atrium of the male and female heart specimens are listed in Table :1 . It is shown that among 64 male heart specimens 52 had opening of all the four pulmonary veins, but in 12 specimens this normal distribution was not present. In 5

specimens there was a single left pulmonary venous opening with two right pulmonary venous openings, and in 7 specimens there was a single right pulmonary venous openings with two left pulmonary veins opening normally. Among 36 female heart specimens 5 had single opening for left pulmonary vein, and 3 had single opening for the right pulmonary vein. Therefore in this study 10% had single left pulmonary venous opening, and 10% had single right pulmonary venous opening. In a previous study in 2011 on "CT evaluation of pulmonary venous anatomy variation in patients undergoing catheter ablation for atrial fibrillation" Thorning C et al [16] described that the anatomical variability was greater for right PVs i.e 82% of patients had 2 ostia, 17% had 3 ostia, 0.5% had 4 ostia and 0.5% a common ostium. For left PVs, 91% of patients had 2 ostia, 8.5% a common ostium and 0.5% 3 ostia in their study. Lovesh Shukla and others in the year 2012 in another study on "Variation in number and drainage pattern of pulmonary veins draining into the left atrium" [17] showed that in 10.3% cases there were three veins with two ostia on the right side and in 17.2% cases one vein with a single ostium on the left side.

The pulmonary veins also vary in their position of opening in the left atrium. So previously to characterize pulmonary vein (PV) anatomy and the relative position of the PV ostia, Thorning C et al in 2011 recorded the position of the PV ostium relative to the nearest vertebral body edge was in their work on "CT evaluation of pulmonary venous anatomy variation in patients undergoing catheter ablation for atrial fibrillation". Mean ostial distances from vertebral margin were: right PVs 3.62±7.48 mm; left PVs 3.84±8.46 mm (p=.72). In the present anatomical study the limbus fossa ovalis has been chosen as an anatomical landmark on the inner aspect of the left side of the inter-atrial septum for positioning of the right and left superior pulmonary veins. The distance between the approximate mid-point of the limbus fossa ovalis and the openings of the above mentioned two veins were measured with slide calipers. No significant difference was found between the mean distance of openings among male and female heart specimens (Table:2). But the difference among male and female was significant for the left superior pulmonary venous opening (Table:4). The difference of means was also significant between right and left sides (Table:5). There was no significant difference among different age groups namely 20years-40 years, 40years-60years, & 60years-80years (Table:3).

CONCLUSION

Hundred cadaveric specimens of both genders were collected from Anatomy Department and post mortem room of Forensic and State Medicine Department of Institute of Post Graduate Medical Education & Research, SSKM Hospital, Kolkata during the period of March 2013 to February 2014, of which sixty four were male and thirty six were female. The specimens were subdivided into three subgroups according to the recorded age of cadavers. These were all formalinised heart specimen without any visible deformity.

Different parameters like age, gender, number of pulmonary venous openings in the left atrium and the distance of pulmonary venous ostia from the mid-point of the limbus fossa ovalis were recorded.

The results obtained from data recorded showed that 10% specimens had single left pulmonary venous opening, and 10% had single right pulmonary venous opening. Rest of the specimens had normal four pulmonary venous openings.

During describing the drainage pattern of pulmonary venous ostia into the left atrium it was revealed that the distance of opening of the left and right superior pulmonary veins were variable and the difference was statistically significant between male and female left superior pulmonary venous openings.

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