RESEARCH ARTICLE

Comparative Anatomical Features of the Bicuspid Valve in Sheep and Goat

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Abstract

The bicuspid valve in sheep and goat showed similar anatomical features. The left atrio ventricular opening was circular and possessed valves. Both these species contained an anterior and a posterior leaflet. The anterior leaflet was large and semicircular with two zones while the posterior leaflet had three zones. The latter was divided into 2-3 scallops by clefts. The free edges of the cusps were attached to the papillary muscles by 6-9 chordae tendinae. Morphometric observations of these leaflets didn't show any significant variations in both species. The free-edge mean length, mean depth of these valves and the mean height of the aterio-posterior commissure and the posterior-anterior commissure were with insignificant differences. Histological features also revealed a similarity in both species. The leaflets were found attached to the annulus made of densely arranged collagen bundles. Lamina radialis formed the core of the leaflet and was composed of dense collagen fibers, few elastic and reticular fibers with fibrocytes and histiocytes. The atrial and ventricular surfaces were lined by a layer of endothelium. Beneath the endothelial lining was lamina spongiosa towards the atrial side and lamina fibrosa towards the ventricular side. The mean thickness of both these valves was with insignificant differences between these two species. These valves showed a mild to weak reaction to PAS in the lamina spongiosa, whereas a moderate activity for Alcian blue was observed in the endothelial linings that confirmed the presence of neutral and acid mucopolysaccharides. The reaction to acid and alkaline phosphatase activity was strong in fibrosa, moderate in spongiosa and weak in lamina radialis. The valves were negative for Oil red O and cholesterol activity. Moderately positive succinic dehydrogenase activity was observed at the endothelial surfaces of the leaflet in both species.

Key words: Bicuspid valves, Histochemistry, Lamina fibrosa, Leaflets, Sheep-Goat, Spongiosa.

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Introduction

The cardiac valve that guards the left atrio-ventricular orifice is the mitral valve or bicuspid valve. It is formed of two cusps from forming the circular orifice between the left atrium and left ventricle with chordae tendinae and the papillary muscles (Sisson and Grossman, 1975). They form part of the fibrous skeleton of the heart that also supports tricuspid, aortic and pulmonary valves (Dyce *et al.,* 2010). This valve regulates the unidirectional blood flow from the left atrium to the left ventricle. Auricular contraction during left auricular systole with left ventricular diastole opens these valves, while ventricular contraction during left ventricular systole closes them to prevent backflow or regurgitation. They are passive regulators influenced by the myocardial movements. Derangements in development, growth, plasticity and inflammation due to pathological reasons compromise their function (Wallby, 2008). This pathology is always associated with alteration in its structural components (Roy and Saha, 2016). Some of the common pathologies include mitral valve regurgitation; the second most common valvular pathology in humans, mitral valve prolapse and mitral valve stenosis etc.

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Research on valvular and pericardial morphology, microanatomy and histochemistry are already in progress in bovine, porcine and equine species. Their anatomical similarities and biocompatibility are being assessed and are now utilized as xenografts to treat various valvular pathologies in humans (Singhal *et al.,* 2013). These tissue valves prostheses are most preferred and are better than mechanical valves due to their biocompatibility, physiological haemodynamics, and least systemic anticoagulation therapy (Ciubotaru *et al.,* 2013). Nevertheless, ovines are medium in size, easily available, affordable, (Ali *et al.,* 1996; Honge *et al.,* 2011) comparatively resistant to infection and can recover faster from anaesthesia, and major surgery are now preferred as a best animal model. Further, valvular anatomy of sheep is similar to humans. It is considered as the animal of choice for preclinical testing of cardiac devices (Alexis *et al.,* 2023), ischemic mitral valve regurgitation (Kim *et al.,* 2018), and long term heart failure (HF) (Byrne *et al.,* 2002). Given, the clinical significance and the advantages of the small ruminants in valvular research, the current study was aimed to report in detail the gross morphological, histological and histochemical components of the bicuspid valves in sheep and to compare it with goat.

Materials and Methods

This study was done at the post-graduate research lab of the Department of Veterinary Anatomy, College of Veterinary Science, Rajendranagar, Hyderabad (India) during 2020. The hearts of sheep and goat were collected during slaughter from healthy animals at the slaughter houses near Hyderabad. The specimens were thoroughly washed with normal saline after making slits on the chambers to make them totally blood free. The bicuspid valve at the left atrio-ventricular orifice was exposed carefully with an incision in the left atrium around mitral valve annulus. The natural contour of the valves guarding this orifice was preserved with the help of cotton wool. The topology and gross morphological features of these valves were recorded. Morphometric measurements such as annular diameter, length of the valves at their free edge, depth and thickness of valves and commissural height were done in both sheep and goat to appreciate variations, if any. All measurements were taken with thread, graduated transparent ruler and digital Vernier caliper and were recorded as mean ± standard error. Interspecies comparisons were made using analysis of variance (ANOVA) with a level of significance set at $p<0.01$ and $p<0.05$.

The bicuspid valves were fixed in 10% neutral buffered formalin and were processed by routine paraffin technique (Singh and Sulochana, 1997) to cut microsections of 5-7 µm thickness for histological examination and histometric measurements. Sections from all specimens were stained by ordinary haematoxylin and eosin method and by special stains, *viz.,* Van Gieson's method, Wilder's method (Singh and Sulochana, 1997) Verhoeff's method (Culling, 1974) and by Masson's trichrome method (Luna, 1968) to identify the histological components of these valves.

Histochemical analysis for glycogen by PAS reaction and acid and neutral muco- polysaccharides by PAS-Alcian Blue method (Singh and Sulochana, 1997) were done using paraffin sections. While for enzymatic analysis like acid phosphatase by Gomori's method, succinic dehydrogenase by Tris buffer method, Fats Lipids by Oil red O in propylene glycol method and Cholesterol by Schutz's method (Singh and Sulochana, 1997), cryosections of 10-15 µm thickness were used.

Results and Discussion

Gross Morphology

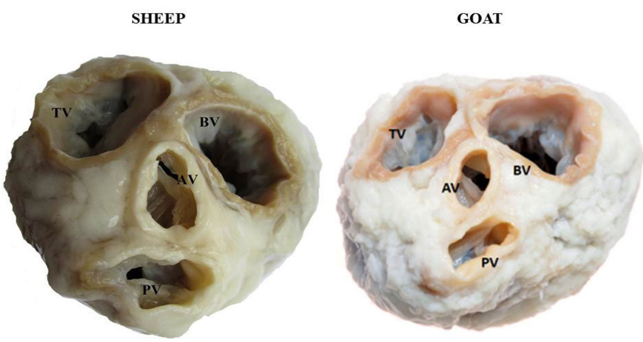
The left atrio-ventricular orifice was located behind the pulmonary and aortic orifice and was on the left of the right atrio-ventricular orifice in sheep and goat. It was guarded by the mitral or bicuspid or left atrio-ventricular valve (Fig. 1) as reported by Getty (1975), Sisson and Grossman (1975) in domestic animals. These are dual-flap valve in the heart situated between the left atrium and the left ventricle. Together this apparatus comprised the left atrio-ventricular myocardium with the fibrous annulus, two leaflets, chordae tendinae and papillary muscles as in humans.

Both the valves were supported by the surrounding left atrio-ventricular myocardium. The mitral annulus was a fibrous ring and was roughly circular in sheep and goat. It encircled the left atrio-ventricular orifice and anchored the bicuspid valve leaflets (Fig. 1). This annular attachment helped the leaflets to withstand complex changes in their shape in both horizontal and vertical planes during cardiac cycle and facilitated valvular function (Salgo *et al.,* 2002). The two leaflets were an anterior and a posterior. The anterior or aortic or septal leaflet was anchored and located posteriorly to the aortic root. It was large and semicircular in shape and had a free edge with few or no clefts (Fig. 2). It possessed rough and clear zones that were separated by a prominent ridge on the atrial surface of the leaflet, which in turn was the line of its closure. The rough zone was thick, opaque and had insertion for the chordae tendineae on its ventricular surface, whereas the clear zone was defined prominently without chordae tendineae. It was located between the rough zone and annulus. These observations were similar to the report of Walmsley (1978) in humans. However, it contradicted with his findings that the oppositional rough zones do not come in to contact during valvular closure.

The posterior or ventricular or mural leaflet was wide and located posterior to the mitral annulus as reported by Kunzelman *et al*. (1993) in humans. The posterior cusp attachment was almost two-thirds of the annular circumference and it occupied larger area than the anterior cusp. It was divided into 2-3 scallops/ supernumerary/ accessory/ commissural cusps by clefts (Fig. 2). It possessed three zones, *viz*., rough, clear and basal zone. The former was distal to the ridge of line of leaflet closure, whereas the clear zone was free of insertions from chordate tendineae and was located in the middle between the rough and the basal zones. The basal zone was between the clear zone and the mitral valve annulus and had insertions from basal chordae tendineae. This zone was seen only in posterior leaflet and was best visualized on the middle scallop because most of the basal chordae are inserted into it. The free edge of the leaflets had attachments for papillary muscles by 6-9 no's of chordae tendinae (Fig. 2) similar to the observations of Millington-Sanders *et al.* (1998) and Yacoub and Cohn (2004), who also reported that these tendinous insertions reduced the stress on valves and also preserved their durability. The shape, size, orientation and attachment of the leaflets and chordae tendinae decided the valvular functional efficiency in cardiac cycles.

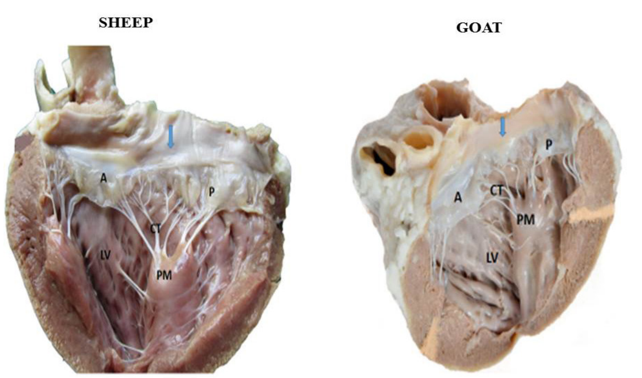
Morphometry

The annular mean diameter in sheep and goat was 1.9 ± 0.04 cm and 2.0 ± 0.03 cm, respectively, and it was closer to 2.2 ± 0.5 cm as reported by Gunnel *et al.* (2012) in humans. The free edge mean length of the anterior leaflet in sheep and goat was 3.0 ± 0.07 cm and 3.1 ± 0.04 cm, respectively, while the same for the posterior leaflet measured 3.4 ± 0.08 cm and 3.4 ± 0.05 cm, respectively. No significant difference was found between the anterior and posterior leaflet free edges between the two species.



TV- Tricuspid Valve, BV – Bicuspid valve, AV – Aortic Valve, PV – Pulmonary Valve

Fig. 1: Photograph of cross sectional view at A-V junction of heart showing the cardiac openings with valves in sheep and goat



LV – Left Ventricle, PM – Papillary muscle, CT- Chordae Tendinae, AL- Anterior leaflet, PL – Posterior leaflet, Arrow points the Annulus

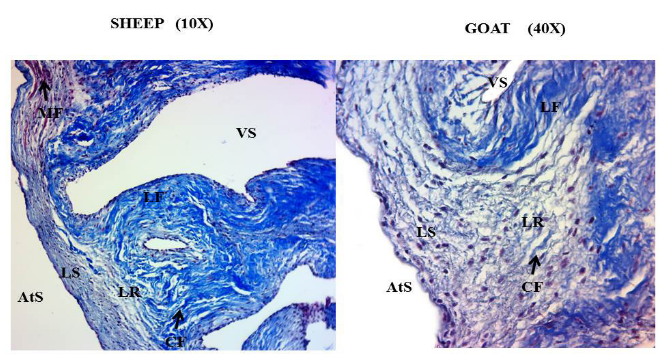
Fig. 2: Photograph of sagital section of heart showing the position and attachments of bicuspid valves in sheep and goat

The mean depth of the anterior leaflet in sheep and goat was 2.0 ± 0.03 cm and 1.9 ± 0.05 cm, while for the posterior leaflet it was 1.5 ± 0.05 cm and 1.7 ± 0.06 cm. The mean height of the antero-posterior commissure in sheep and goat was 0.6 ± 0.04 cm and 0.6 ± 0.06 cm, respectively, while the same for the posterio-anterior commissure was 0.7 ± 0.04 cm in both without any significant differences between these two species.

Histomorphology and Micrometry

The histological features of the leaflets of bicuspid valve in sheep and goat were almost similar. The annulus gave attachment to the leaflets and was comprised of densely arranged collagen bundles and few muscle cells (Fig. 3) as seen in humans. The leaflets were formed by an endothelial lining with a core of dense collagen fibers. Atrial and ventricular surfaces revealed a lamina spongiosa and fibrosa, respectively, which were lined by endothelium (Fig. 4). These observations concurred with the findings of Misfeld and Sievers (2007) in humans. Lamina radialis was composed of collagen bundles, fibrocytes and histiocytes. The former formed the core of the leaflet. This stratified extra cellular matrix in valves determined their biomechanical properties (Sacks *et al.,* 2009). Defects in remodeling of extra cellular matrix by the valvular interstitial cells resulted in valvular pathologies (Hinton and Yutzey, 2011). Muscle fibers were present only at the regions of valvular attachments and were absent at its core (Fig. 5), whereas Borgarelli *et al.* (2011) observed muscle fibers in all the three regions of mitral valves, *i.e.,* at the proximal, middle and distal segments of the bicuspid valves in dogs. However, it was significantly diffuse only at the base. The ventricular surface of the valves presented folds (Fig. 6).

The mean thickness of the anterior leaflet in sheep and goat was 477.9 ± 5.6 µm and 482.1 ± 6.4 µm, respectively, whereas it was 462.9 ± 4.2 µm and 367.8 ± 5.5 µm, respectively, for the posterior leaflet. No significant difference was present between two species.



Collagen fibers– CF & Muscle fibers – MF, in Ventricular Surface – VS, Atrial Surface – AtS, Lamina Spongiosa- LS, Lamina Radialis – LR & Lamina Fibrosa - LF

Fig. 3: Photomicrograph of the bicuspid valve showing distribution of collagen fibers (Masson's trichrome stain)

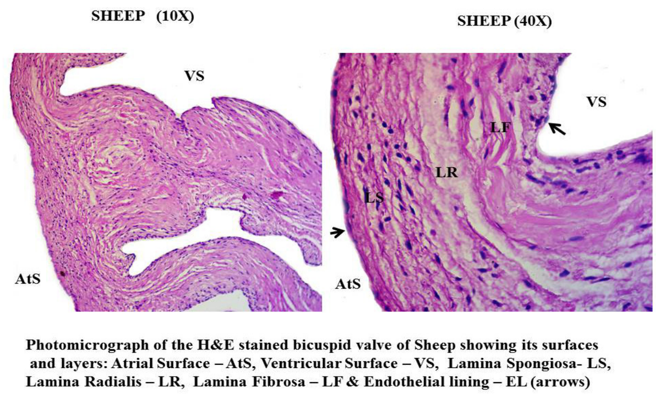


Fig. 4: Photomicrograph of the bicuspid valve of sheep showing its surfaces and layers (H & E stain)

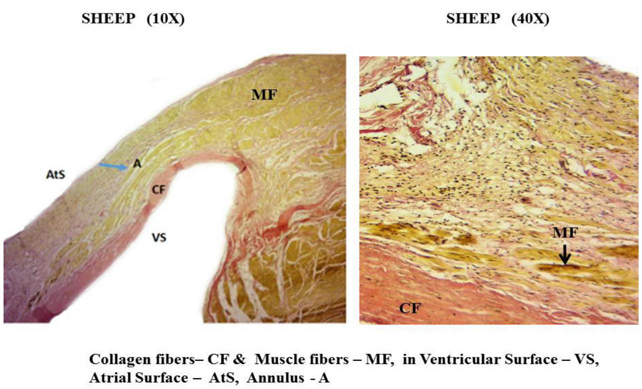


Fig. 5: Photomicrograph of the bicuspid valve in sheep showing distribution of collagen and muscle fibers (Van Gieson's stain)

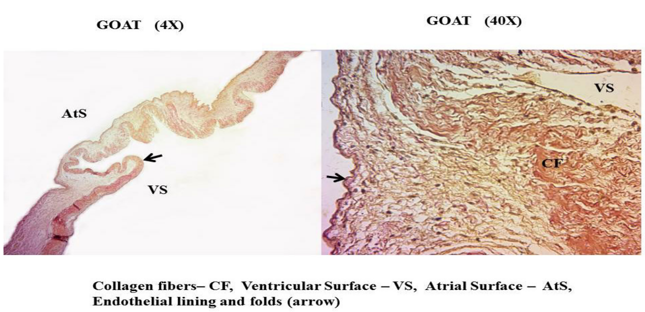


Fig. 6: Photomicrograph of the bicuspid valve in goat showing endothelial lining and folds (Van Gieson's stain)

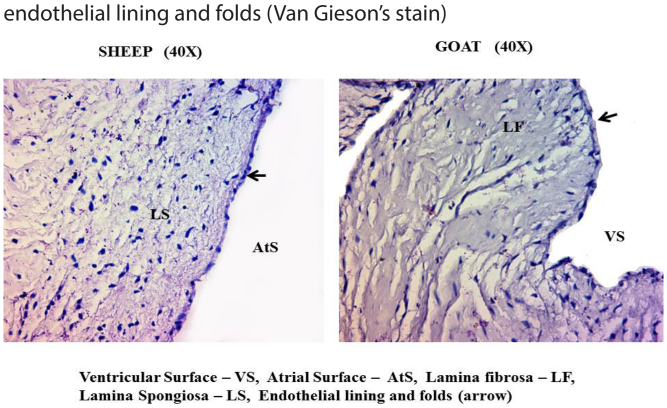


Fig. 7: Photomicrograph of the bicuspid valve in sheep and goat showing PAS activity (Alcian Blue PAS stain, X 400)

Histochemical Features

The lamina spongiosa on the atrial surface of bicuspid valve was weakly positive for PAS activity (Fig. 7), while, a moderate Alcian blue activity was observed on both the endothelial surfaces for the presence of acid mucopolysaccharides (Fig. 8). Endothelial and subendothelial regions on the ventricular surface were strongly positive for AKP, which was moderate in lamina radialis and weak in the regions of lamina spongiosa. A weak ACP activity was observed in the lamina radialis, while a moderate to strong reaction was seen in lamina spongiosa and fibrosa respectively (Fig. 8). The leaflets were negative for Oil red O and cholesterol activity in both the species. However in dogs, Borgarelli *et al.* (2011) reported that adipose tissue was profusely present in the proximal and middle segment of the mitral valves and speculated that it helped to protect these valves against natural stress encountered during their mechanics. The endothelial surfaces of the leaflets were moderately positive for SDH activity.

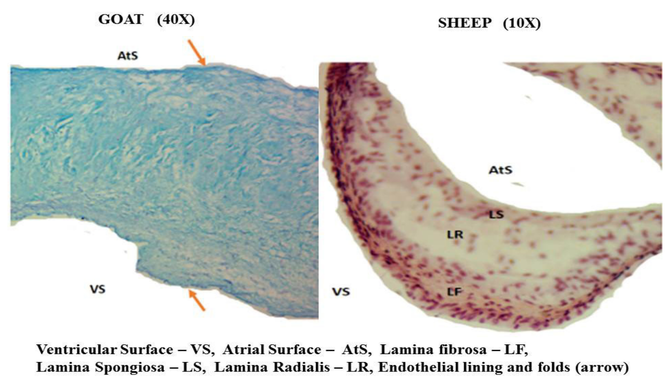


Fig. 8: Photomicrograph of the bicuspid valve in goat and sheep showing PAS, Alcian Blue and ACP activity (Alcian Blue PAS stain)

Conclusion

The valvular morphology, morphometry and histochemisty of the bicuspid valves in sheep and goat are found similar. These similarities in goat may favour researchers to consider it as one of the experimental animal models for bicuspid valvular research in the near future.

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References

Alexis, M., Laurence, F., Marie-Aude, G., Christian, L., & Nicolas, B. (2023). Comparative anatomy of the mitral valve in four species (human, ovine, porcine and canine): A pre-clinical perspective. *Anatomia, Histologia, Embryologia, 2023*, 1-9.

Ali, M.L., Kumar, S.P., Bjornstad, K., & Duran, C.M.G. (1996). The sheep as an animal model for heart valve research. *CradioVascular Surgery, 4*(4), 543-549.

Borgarelli, M., Tursi, M., Rosa, G.L., Savarino, P., & Galloni, M. (2011). Anatomic, histologic, and two-dimensional–echocardiographic evaluation of mitral valve anatomy in dogs. *American Journal of Veterinary Research, 72* (9), 1186-1192.

Byrne, M.J., Raman, J.S., Alferness, C.A., Esler, M.D., Kaye, D.M., & Power, J.M. (2002). An ovine model of tachycardia-induced degenerative dilated cardiomyopathy and heart failure with prolonged onset. *Journal of Cardiac Failure, 8*(2), 108-115.

Ciubotaru, A., Cebotari, S., Tudorache, I., Beckmann, E., Hilfiker, A. & Haverich, A. (2013). Biological heart valves. *Biomedizinische Technik, 58*(5), 389-397.

Culling, C.F.A. (1974). *Handbook of Histopathological and Histochemical Techniques. Including Museum Techniques*. 3rd edition, Butterworth, London, UK.

Dyce, K.M., Sack, W.O. & Wensing, C.J.G. (2010). *Textbook of Veterinary Anatomy*, 4th edition, Philadelphia, Saunders publications, pp. 227-231.

Getty, R. (1975). *The Anatomy of the Domestic Animals*, Vol. 1, 5th edition. W.B Saunders Company, Philadelphia, USA.

Gunnel, S. A., Farooqui, M. S. & Wabale, R. N. (2012). Study of mitral valve in human cadaveric hearts. *Heart views*. 13, 132-135.

Hinton, R.B., & Yutzey, K.E. (2011). Heart valve structure and function in development and disease. *Annual Review of Physiology, 73,* 29-46.

Honge, J.L., Funder, J.A., Pedersen, T.B., Kronborg, M.B., & Hasenkam, J.M. (2011). Degenerative processes in bioprosthetic mitral valves in juvenile pigs. *Journal of Cardiothoracic Surgery, 6*(72), 1-7.

Kim, D.H., Morris, B., Guerrero, J.L., Sullivan, S.M., Hung, J., & Levine, R.A. (2018). Ovine model of ischemic mitral regurgitation. *Methods in Molecular Biology, 1816,* 295-308.

Kunzelman, K.S., Cochran, R.P., Murphree, S.S., Ring, W.S., Verrier, E.D., & Eberhart, R.C. (1993). Differential collagen distribution in the mitral valve and its influence on biomechanical behaviour. *The Journal of Heart Valve Disease, 2*(2), 236-44.

Luna, L.G. (1968). *Manual of Histological Staining Methods of the Armed Forces Institute of Pathology*. 3rd edition, McGraw Hill Book Co, New York.

Millington-Sanders, C., Meir, A., Lawrence, L. & Stolinski, C. (1998). Structure of chordate tendinae in the left ventricle of human heart. *Journal of Anatomy, 192,* 573-581.

Misfeld, M., & Sievers, H.H. (2007). Heart valve macro and microstructure. *Philosophical Transactions of the Royal Society, B, 362*, 1421-1436.

Roy, S. & Saha, A. (2016). Mitral valve beyond classical view. Journal *of the Anatomical Society of India, 65*(1), 20-23.

Sacks, M.S., Merryman, W.D., & Schmidt, D.E. (2009). On the biomechanics of heart valve function. *Journal of Biomechanics, 42,* 1804-1824.

Salgo, I. S., Gorman III, J. H., Gorman, R. C., Jackson, B. M., Bowen, F. W., Plappert, T., Sutton, M.G.S., & Edmunds Jr, L. H. (2002). Effect of annular shape and leaflet curvature in reducing leaflet stress. *Circulation, 106,* 711-717.

Singh, U. B. & Sulochana, S. (1997). *Handbook of Histological and Histochemical Techniques*, Premier Publishing House. Hyderabad, India.

Singhal, P., Adriana, L., & Butany, J. (2013). Bioprosthetic heart valves: Impact of implantation on biomaterials. *Biomaterials, 2013,* 1-14.

Sisson, S.S.B., & Grossmann, G.J.D. (1975). *The Anatomy of the Domestic Animals,* 4th ed. Saunders, Philadelphia, pp. 626-629.

Wallby, L. (2008). Signs of *inflammation in different types of heart valve disease,* Linkoping University Medical dissertation No. 1052, Sweden.

Walmsley, R. (1978). Anatomy of human mitral valve in adult cadaver and comparative anatomy of the valve. *British Heart Journal, 40,* 351-366.

Yacoub, M.H., & Cohn, L.H. (2004). Novel approaches to cardiac valve repair. Structure to function, Part-I. *Circulation, 109,* 942-950.