SEX VARIATION IN STRUCTURE OF HUMAN ATRIOVENTRICULAR ANNULI

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Running Title: Sex variation in Atrioventricular annuli

Background: Atrioventricular annuli are important in hemodynamic flexibility, competence and support to tricuspid and mitral valves. Anatomical features of the annuli such as circumference, organization of connective tissue fibers, myocardium and cellularity may predispose to annular insufficiency and valvular incompetence. These pathologies occur more commonly in females, although the anatomical basis for this disparity remains unclear. Sex variations in structure of the annuli may provide a probable morphological basis for the patterns of these diseases.

Objective: To investigate sex variations in structure of human atrioventricular annuli

Materials and Methods: One hundred and one hearts (48males, 53 females) obtained from the department of Human Anatomy, University of Nairobi were studied after ethical approval. Annular circumferences were measured using flexible metric ruler and standardized for heart sizes. Results were analyzed using SPSS version 16.0 and sex differences were determined using student's t- test. A p- value of less than 0.05 was considered significant. For light microscopy, specimens were harvested within 48 hours post-mortem, processed, sectioned and stained with Masson's trichrome and Weigert's elastic stain with van Gieson counterstaining.

Results: Females had significantly larger annular circumference than males after standardizing for heart sizes. Histologically, myocardium was present in AV annuli in males, while females had strands of cardiac muscle but only in mitral annulus. The annuli were more elastic and cellular in males especially at annulo- myocardial and annulo- valvular zones respectively.

Conclusion: The standardized larger annular circumferences in females may limit heart valve co-aptation during cardiac cycle and can be a risk factor for valvular insufficiency. The predominance of myocardium, annular cellularity and elasticity, may be protective against heart valve incompetence in males than females.

Key words: Atrioventricular annuli; Sex variation; Valve incompetence.

INTRODUCTION

Atrioventricular (AV) annuli are part of cardiac skeleton that support and prevent overdistension of tricuspid and mitral valves, and maintain patency of their orifices (Moore, 2006). They also confer hemodynamic stability to valves (Ormniston, 1981), and form a firm framework for myocardial attachment, and placement of sutures during valve repair (Istvan, 2008).

The circumference of the annulus is an important parameter which determines normal annular and valvular function. An increase in this parameter is an independent risk factor for annular incompetence and valvular insufficiency (Farry, 1975; Ormniston, 1981). This parameter is population specific, shows sex variation, and depends on the heart size (Skwarek, 2008; Singh and Mohan, 1994). However, it is undetermined for the Kenyan population.

Histologically, the annuli are endothelial- lined fibrous structures containing collagen, elastic fibres and fibroblasts (Williams, 1995). Variable extension of myocardium from atria to the corresponding annuli forms the annular myocardium (Puff, 1978). This structural organization plays an important role in valvular support and annular adaption to hemodynamic forces during cardiac cycle (Yacoub and Cohn, 2004). Alteration in this structural integrity has been associated with heart valve incompetence (Puff, 1972; Angellini, 1988), which occurs more commonly in females (Carpenter, 2004; Glower et al, 2009). The structural basis for this sex predisposition may therefore be attributed to sex differences in adaptations of the annuli to hemodynamic stresses, however they remain largely undetermined. This study therefore aimed to determine sex variations in structure of human AV annuli.

MATERIALS AND METHODS

One hundred and one hearts (48 males and 53 females) were studied. These were from subjects ranging from 1 year to 70 years (mean age 33 years). Eighty (80) hearts were used to study gross morphometry, while 21, used for histological study, were obtained within 48 hours of autopsy at the Nairobi city mortuary. Ethical approval was granted before commencement of the study, and specimens were obtained after seeking informed written consent from relatives of the deceased. Hearts with abnormality of the annuli or valves were excluded. The mediastinum was opened by cutting bilaterally through costal cartilages and removing the sternum. The pericardium was incised to expose the heart and harvesting was done by dividing great vessels 2cm from superior extent of its base. The hearts were weighed using a digital weighing balance, ABC Japan (accurate to 0.01g) and external heart length was measured from its apex to the base using a tape measure. Harvesting of the annuli was done by making a circular incision around bases of corresponding valve cusps, and the entire annuli with attached cusps were harvested intact. Annular circumferences were measured (in cm) using a flexible metric ruler and then standardized for heart weights. For light microscopy, 5mm thin sections were harvested from anterior and posterior parts of both annuli. The specimens were harvested *en bloc* with attached valve cusps on one side, and myocardium on opposite side, in order to delineate extents of the annulus ie from annulo- valvular junction (AVJ) to annulo- myocardial junction (AMJ). Routine histological processing was done and 7 micron thick sections were obtained using a Lenz Wezlar sledge microtome. Masson's trichrome stain was used to study organization of connective tissue and myocardium in the annuli, while Weigert's elastic with van Gieson counterstaining was used to demonstrate elastic fibres. Mounting of cover slips was done using D.P.X mountant while slide observation was done using a bright- field light microscope.

Data obtained was analysed using statistical package for social sciences version 16.0, and sex differences were determined using student's t- test. A p- value of less than 0.05 was taken as significant.

RESULTS

Gross measurements

The mean age for males and females was 35 years and 32 years respectively. The average heart weight was 286 ± 84g in males and 222 ± 76g in females (p= 0.001). The external heart length was 13.0 ± 1.9 cm in males and 12.3 ± 2.4 cm in females (p= 0.13). The unstandardized tricuspid (**TA**) and mitral (**MA**) circumferences were greater in males than females, although the differences were statistically insignificant (p= 0.12 and 0.57 for tricuspid and mitral respectively) **Table 1**. There was however, a significant positive correlation between heart weights and annular circumferences (r= 0.6 for **TA** and 0.56 for **MA**).

Table 1: **Unstandardized sex differences in annular circumferences**

Parameter	**Sex**	**Mean ± SD**	**p value**
Tricuspid Circumference (**cm**)	M F	9.1 ± 1.9 8.4 ± 1.8	**0.12**
Mitral Circumference (**cm**)	M F	7.1 ± 1.3 6.9 ± 1.5	**0.57**

M= male F= female

After standardizing annular circumferences with corresponding heart weights, females had significantly larger circumferences than males **(Table 2).**

Table 2: **Standardized sex differences in annular circumference**

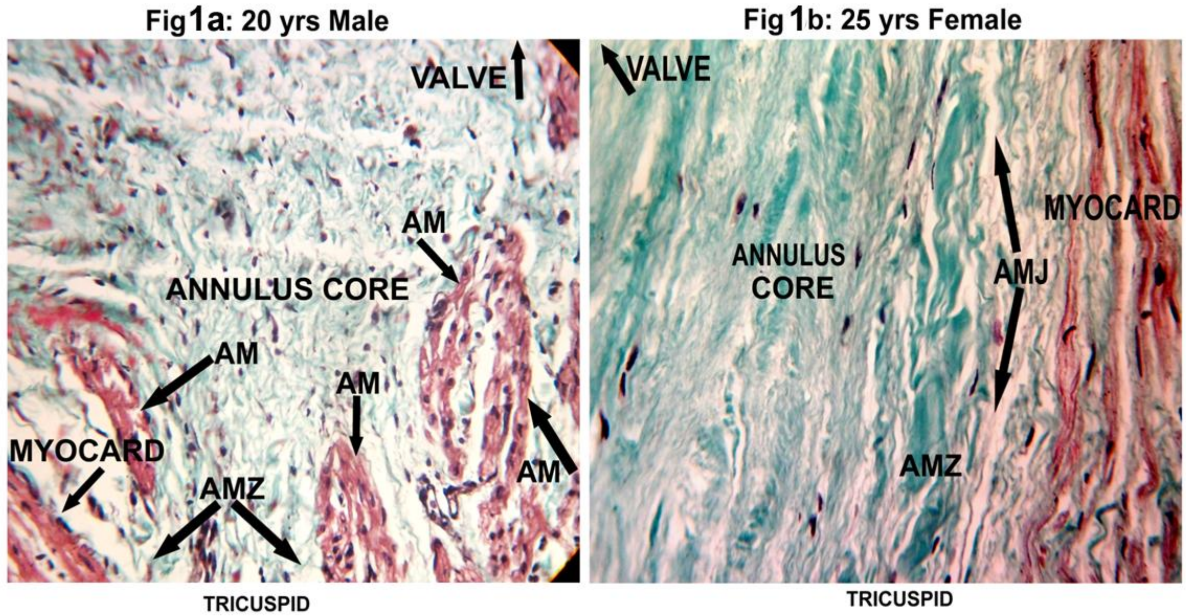
Parameter	**Sex**	**Standardised mean values**	**p value**
Tricuspid Circumference (**cm**)	M F	0.35 0.44	**0.025**
Mitral Circumference (**cm**)	M F	0.27 0.36	**0.007**

M= male F= female

Microscopic structure

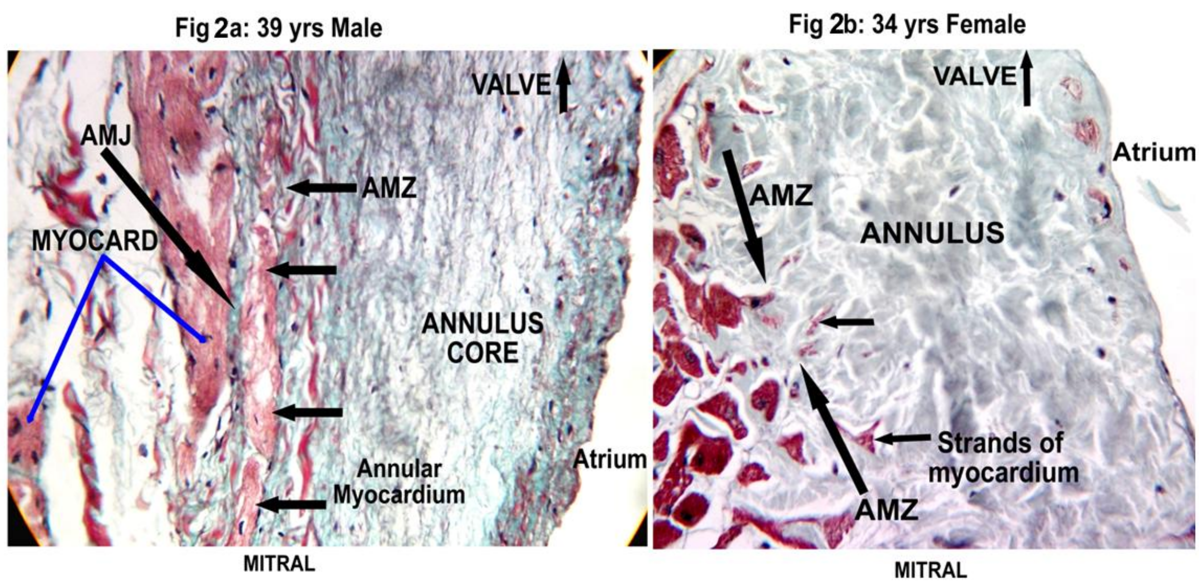
Histologically, myocardium was present in annulo- myocardial zone (**AMZ**) in both tricuspid and mitral annulus in males (**Fig 1a and Fig 2a**). Females had strands of cardiac muscle but only in mitral annulus (**Fig 2b**). The tricuspid annulus in females was devoid of myocardium (**Fig 1b**), and instead dense regular collagen fibers characterized this zone. The annuli in males were also characterized by two layers of dense elastic fibers enclosing the annular myocardium in between (**Fig 3a**). In females, elastic fibers were less dense and regularly interrupted (**Fig 3b**). The annuli in males were characteristically cellular especially at annulo- valvular zone (AVZ), **Fig 4a**, and the cells had elongated nuclei resembling fibroblasts. In females, there was scarcity of cells in this zone, and instead dense regular collagen fibers dominated the zone (**Fig 4b**).

Photomicrograph showing sex differences in presence of myocardium in Tricuspid annulus



AM: Annular myocardium; **AMZ**: Annulo- myocardial zone; **AMJ**: Annulo- myocardial junction (Masson's Trichrome stain; Magnification x400).

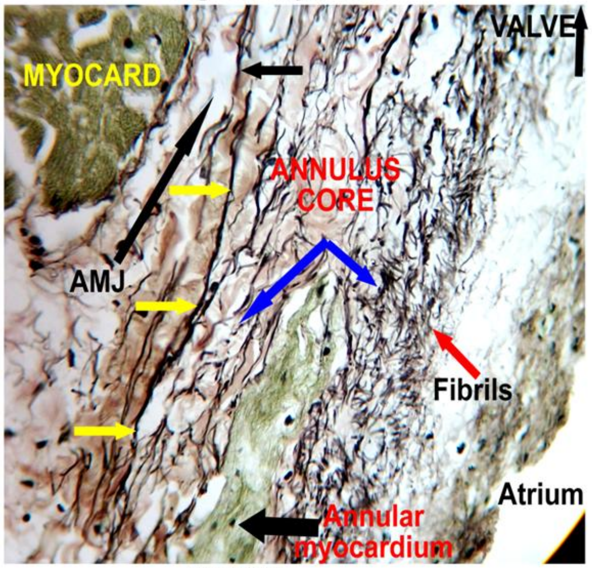
Photomicrograph showing sex differences in presence of myocardium in Mitral annulus



AMZ: Annulo- myocardial zone; **AMJ**: Annulo- myocardial junction (Masson's Trichrome stain; Magnification x400)

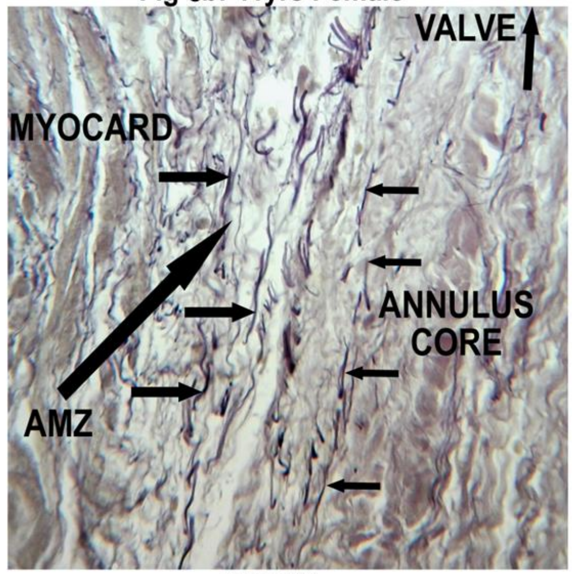
Photomicrograph showing sex differences in distribution of elastic fibers

Fig 3a: 37yrs Male



MITRAL

Fig 3b: 41yrs Female

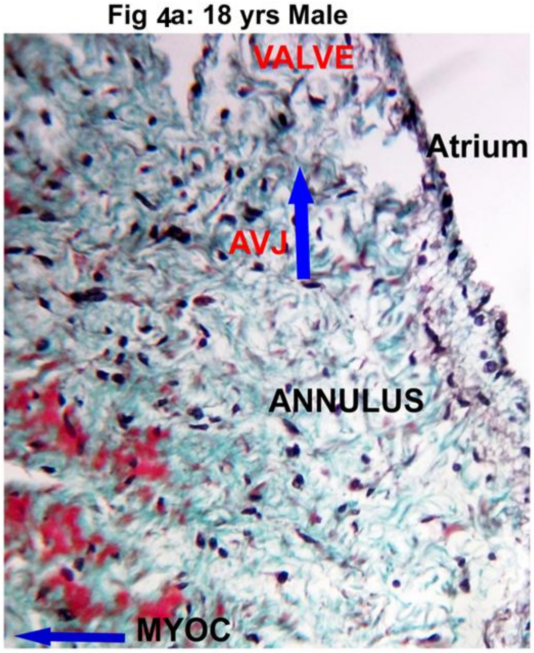


MITRAL

AMJ: Annulo- myocardial junction; **AMZ**: Annulo- myocardial zone (Weigert's elastic with van Gieson counterstaining; Magnification x400)

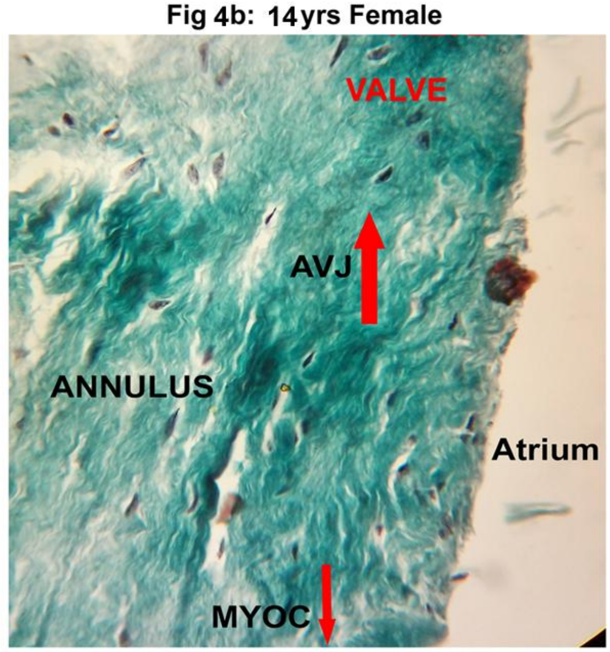
Photomicrograph showing sex differences in annular cellularity

Fig 4a: 18 yrs Male



TRICUSPID

Fig 4b: 14yrs Female



TRICUSPID

AVJ: Annulo- valvular junction; **MYOC**: Myocardium (Magnification x400)

DISCUSSION

Results of the present study have provided data on adaptations of atrioventricular annuli to hemodynamic stresses in different sexes, and probable morphological basis for the pattern of diseases affecting them. Previous studies on these annuli mainly described interspecies variations, with little mention on sex variations (Racker et al, 1991; De Biasi et al, 1984).

GROSS MEASUREMENTS

Annular circumferences

Overall, males had larger annular circumferences before standardization. This is not entirely surprising as males had significantly larger heart weights. Further, there was positive correlation between heart weights and annular circumference. Hence the larger circumference in males may be due to their larger heart sizes and probably body mass index, as previous reports have shown an association between these (Sairanen, 1992). Thus, when circumferences were standardized for corresponding heart weights, females had significantly larger circumferences than males. Clinically, larger circumferences may decrease valve co-aptation during cardiac cycle (Ormniston, 1981), and this may be an independent risk factor for valvular incompetence (Roberts et al, 1983; Mutlak et al, 2007). The present study therefore suggests a higher female predisposition to this valvular anomaly. However, this may not be fully concluded without considering microscopic structure of the annuli themselves.

MICROSCOPIC STRUCTURE

Annular myocardium

The presence of cardiac muscle in male annuli, and indistinct myocardium in female mitral annulus is unique to our study. Dudziak et al (2009) found no myocardium in the tricuspid annulus (TA). Puff et al (1978) provided the basis for sphincter mechanism of TA as composed of myofibres arising from atrial musculature in an electrophysiological study. Racker et al (1991), demonstrated myofibres in the TA of a canine model. Although we did not come across demonstration of mitral annular myocardium, the present findings provide evidence for presence of cardiac muscle in both tricuspid and mitral annulus (MA) in males, and indistinct myocardium in female mitral annulus. The earlier studies did not categorize their specimens into gender, and hence it is difficult to ascertain whether there was any pattern in terms of sex variations. Further, comparative animal and human studies have also demonstrated presence myocardium in some valvular apparatus (De Biasi et al, 1984 and Gatonga et al; 2009 respectively). Clearly, the annulus which was previously described as mere fibrous structure lined by endothelium contains distinct myocardium. What is the function of this myocardium?

AV annuli have been for a long time considered passive structures, moving only in response to hemodynamic forces generated by cardiac contractions (Guyton, 2000). Current findings indicate that both TA and MA are capable of independent contractions, and this may influence timing and effectiveness of AV valve closure (Yacoub and Cohn, 2004). Myocardium in the annuli may therefore serve to regulate annular contraction and relaxation during cardiac cycle. The myocardium may also play supportive role by conferring structural integrity and mechanical strength to the annuli and also to valves where they attach on the annulus. Contraction of this muscle may also aid in closure of corresponding valve orifices during ventricular systole (Yacoub and Cohn, 2004). Thus comparing myocardial composition in male and female annuli, one may suggest that the composition in males may serve to support and strengthen their annuli, making them less predisposed to annular and valvular insufficiency.

Annular elasticity

The interruption and density of elastic fibers in females observed in the present study may be functionally significant. Elastic fibers are usually found in areas subjected to stretch, and have unique feature of being extensible (Dobrin, 1978). Relating these facts to our current findings suggest that interruption of these fibers may make female annuli less adapted to withstand stretch and extensibility. Hence, when subjected to varying biomechanical forces, they may have a higher chance of insufficiency.

Annular cellularity

The dense cellular profile in males observed in this study could be fibroblastic in nature, due to their elongated nuclei (Dudziak, 2009). This author showed that fibroblasts are widely distributed in human annular tissue, and studies done on these cells in culture have shown that they are contractile, and may display smooth muscle properties (Fillip, 1986). This feature may therefore add pliability to male annuli, making them better adapted hemodynamically.

CONCLUSION

The standardized larger annular circumferences in females may limit heart valve co-aptation during cardiac cycle and can be a risk factor for valvular insufficiency. The predominance of myocardium, annular cellularity and elasticity, may be protective against heart valve incompetence in males than females.

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