AGE AND SEX VARIATIONS IN STRUCTURE OF HUMAN ATRIOVENTRICULAR ANNULI

Dissertation submitted in partial fulfilment of the requirements of intercalated Bachelor of Science degree in Anatomy, University of Nairobi

By

EL- BUSAIDY HEMED MOHAMED

Third year medical student

September, 2010

Department of Human Anatomy,

University of Nairobi,

P.O Box 30197

Nairobi.

S of

DECLARATION

I hereby confirm that this dissertation is my original work and has not been presented elsewhere for examination:

Sign: .... Date: 25/09/10

El- busaidy Hemed Mohamed (candidate)

This dissertation is being submitted with our approval as University supervisors:

Sign: .... Date: 25/09/10

Dr. Paul Odula, BSc. Anat (Hons), MBChB, MMed, FCS

Sign: .... Date: 25/09/10

Prof. Hassan Saidi, BSc. Anat (Hons), MBChB, MMed, FACS

DEDICATION

To my father and mother,

Mr and Mrs. Mohamed Swaleh El- busaidy,

To my sisters and brothers,

Aisha, Samya, Swaleh and Zubeir,

And

To my grandfather,

Mr. Swaleh Hemed El- busaidy.

ACKNOWLEDGEMENTS

I owe many thanks to my supervisors, Dr. Paul Odula and Prof. Hassan Saidi who always guided me on how to go about all the findings I made from beginning to end. I would like to express my gratitude to Dr. Julius Ogeng'o and Prof. Adel Malek for providing encouragement and scientific critique to the manuscript in its different stages. Furthermore, Drs. Kirsteen Awori, Wycliffe Kaisha, James Munene, Pamela Mandela and Gichambira Gikenye cannot go without mention for their most appreciated critique. My sincere thanks to Prof. Jameela Hassanali, Dr. Bernard Ndung'u, Dr. Moses Obimbo and Dr. Kevin Ongeti for their invaluable input. This work would not have reached its final stage without the technical assistance of Mr. James Macharia, Mr. Christopher Kamwaro, Mr. Murunga, Mr. Gimongo, Mr. Martin, Mr. Kimotho, Mr. Mbaka, Ms Margaret, Mrs. Esther and Ms. Judith who were readily available when needed. I would also like to thank other BSc anatomists for their invaluable advice. Lastly, I thank my classmates Fundi, Munguti, Gugu, Magoma and Anisha for their unforgettable support, encouragement and peer review of the written work.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AC	-	Atrial cavity
AVA	-	Atrioventricular annuli
cm	-	Centimeters
Fig	-	Figure
MA	-	Mitral annulus
mm	-	Millimeters
SPSS	-	Statistical package for social sciences
TA	-	Tricuspid annulus
VC	-	Ventricular cavity

LIST OF FIGURES

LIST OF TABLES

SUMMARY

Background: Atrioventricular annuli form part of the fibrous skeleton of the heart that support tricuspid and mitral valves, and form a firm framework for attachment and support of myocardium. Atrioventricular valve failures have been attributed to alterations in the structural integrity of their annuli. It is unclear why these failures increase with age, are more common in females, and involve mitral annulus more than tricuspid.

Objective: To determine age and sex differences in the structure of atrioventicular annuli

Study design: a descriptive cross- sectional comparative study

Materials and Methods: One hundred and one hearts (48 male, 53 female) from autopsy subjects ranging from 1 to 70 years old were studied in three age groups ($\leq$ 21 yrs, >21- 45 yrs and 46- 70 yrs). Eighty hearts were used to study annular circumferences from which respective surface areas were calculated. Sections from the 21 remaining hearts were processed for paraffin- embedding, sectioned and stained with Hematoxylin and Eosin to demonstrate general structure of the annuli, Masson's trichrome to demonstrate collagen and annular muscle fibres, and Weigert's stain with Van Gieson counterstaining to demonstrate elastic fibres.

Results: The mean circumference of tricuspid annulus (TA) was 9.1 ± 1.9 cm in males and 8.4 ± 1.8 cm in females, although this was statistically insignificant ($p = 0.12$). Mitral annular (MA) circumference was 7.1 ± 1.3 cm in males and 6.9 ± 1.5 cm in females ($p = 0.57$). In both males and females, the circumferences increased with increasing age. Myocardium was present in atrioventricular annuli in males. Females had thin strands of cardiac muscle fibres but only in mitral annulus. Myocardium was predominant in TA while collagen fibre discontinuity was extensive in MA. The annuli were more elastic in males especially at central and annulo-myocardial zones. Characteristic age changes included increased collagen fibre density and irregularity, and decreased cellularity, elasticity and myocardial content.

Conclusion: There is trend towards larger annuli in males which may be attributed to larger heart sizes. The age specific values of annular circumferences provide baseline values for Kenyans useful for annular reconstruction and prosthesis design. The annular myocardium, elasticity and cellularity, may confer additional flexibility, contractility and support to male annuli. The predominance of cardiac muscle cells in tricuspid annulus, and extensive collagen fibre discontinuity in mitral annulus, may explain the higher prevalence of mitral failures. The structural basis for age- related valve failures may include decreased cellularity, elasticity and myocardial composition in the annuli, as well as increased collagen fibre discontinuity and irregularity.

INTRODUCTION

Atrioventricular (AV) annuli are fibrous rings that surround tricuspid and mitral valve orifices. They form part of cardiac skeleton that support and prevent overdistension of AV valves and maintain patency of their orifices (Moore, 2007). The annuli confer hemodynamic stability to valves (Ormniston, 1981), and form a firm base for attachment of myocardium and placement of sutures during valve repair (Istvan, 2008). They include Tricuspid annulus (TA) with septal, anterior and posterior parts, and mitral annulus (MA) with anterior and posterior parts. The extents of these parts are demarcated by attachment of corresponding valve leaflets (Silver, 1971).

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). The myocardium plays an important role in conferring hemodynamic flexibility to the annuli, and this is critical in normal valve function during cardiac cycle (Yacoub and Cohn, 2004). Alterations in structural integrity of AV annuli such as organisation of connective tissue fibres, cellular density and myocardial composition, are features implicated in AV valve failures (Puff, 1972; Angellini, 1988). The failures are more common in mitral than tricuspid (Rahko, 1989), their incidence increases with age (Jar, 2009), and are more prevalent in females (Mutlak, 2007, Carpenter, 2004; Glower, 2009). These disparities may be attributed to age and sex differences in adaptive capabilities of the annuli to hemodynamic stresses, however they remain largely undetermined.

The circumference and surface areas of the rings supporting the valves have also been reported to determine normal annular and valvular function. An increase in these parameters is an independent risk factor for annular incompetence and valvular failures (Farry, 1975; Ormniston, 1982). The parameters are population specific and show age and sex variations (Skwarek, 2008; Singh and Mohan, 1994). However, they are undetermined for the Kenyan population.

S of

The present study therefore aimed to determine morphometric and morphologic features of AV annuli in males and females across different age groups.

NULL HYPOTHESIS

There are NO differences in morphometry and structural organisation of AV annuli with age or sex

STUDY QUESTION

What features in morphometry and structural organization of AV annuli show age and sex- related differences?

OBJECTIVES

Broad objective

To describe age and sex- related differences in the structure of AV annuli

Specific objectives: To determine age and sex- related differences in:

1) Circumference and surface area of AV annuli
2) structural organisation of AV annuli

STUDY DESIGN: A descriptive cross- sectional comparative study

MATERIALS

One hundred and one hearts (48 males and 53 females) were studied. The hearts were from subjects ranging from 1 year to 70 years. Three age groups were included: $\leq$ 21 yrs, >21- 45 yrs and 46- 70 yrs. Eighty hearts were obtained from the department of Human Anatomy, University of Nairobi, and used to study gross morphometry, while 21, used for histological study, were obtained during autopsies conducted at the Nairobi city and Chiromo mortuaries.

Ethical approval for use of autopsy specimens was granted by the Kenyatta National Hospital ethics and research committee before commencement of the study. Specimens were obtained after seeking informed written consent from relatives of the deceased.

Specimens included in the present study did not show any obvious pathology or abnormality of the heart, AV annuli, or valves. For microscopic studies, specimens were harvested within 72 hours post- mortem. The cause of death for autopsy specimens was enquired from the pathologist before examination of the heart. All individuals had died suddenly either from gunshots, strangulation, or road traffic accidents.

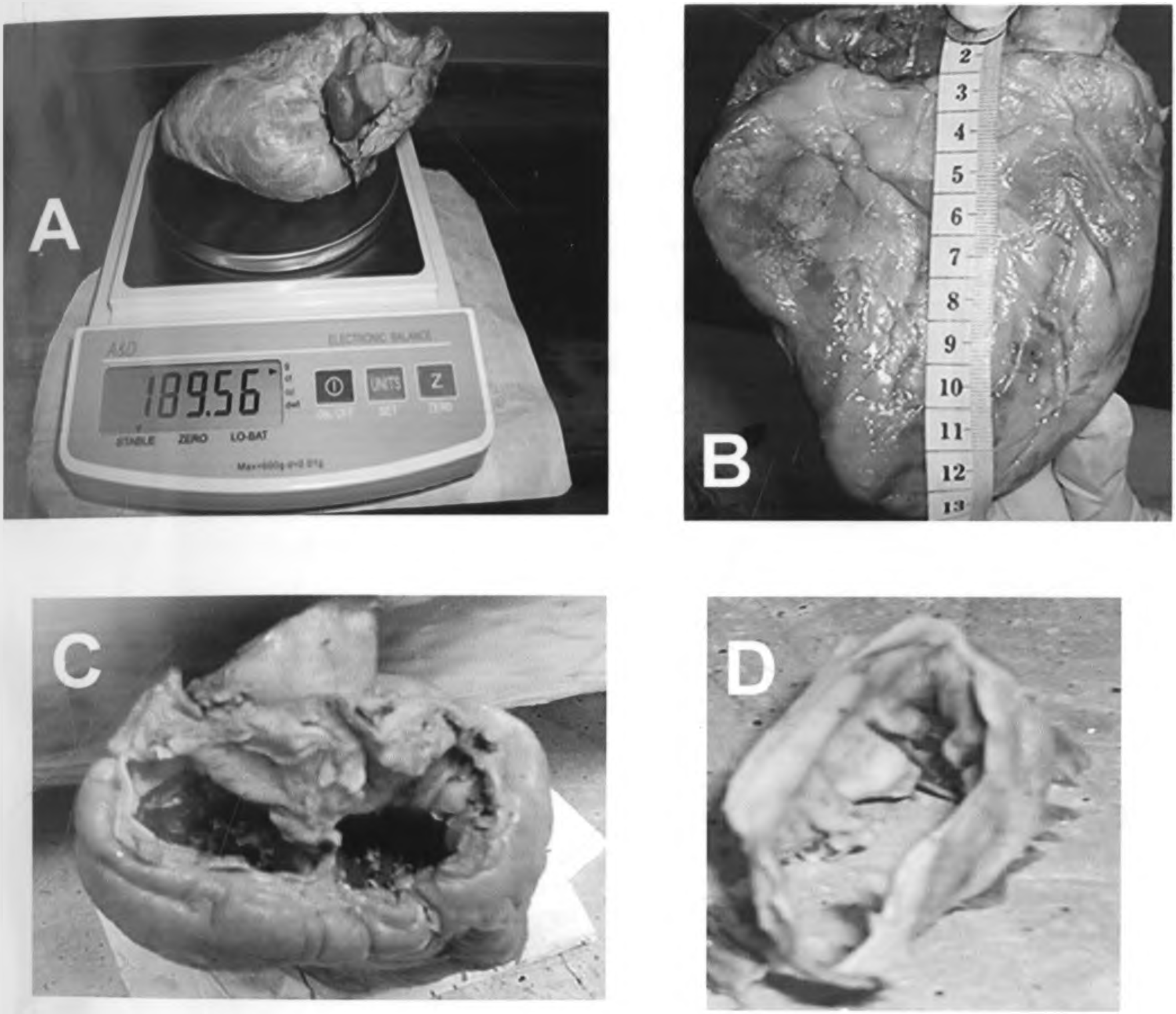
METHODS (1): Exposure and harvesting

Mediastinum was opened by cutting longitudinally and bilaterally through the costal cartilages and removing the sternum, then the pericardium was incised longitudinally to expose the heart. Harvesting of the heart was done by dividing the great vessels 2cm from the superior extent of its base. The hearts were weighed to the nearest gram using a digital weighing balance, ABC Japan (accurate to 0.01g) **Fig 1A**, and findings were recorded on data sheets.

The external length from the apex to the base of the heart, (midpoint between entry of left superior and right superior pulmonary veins) was measured in cm using a tape measure (**Fig 1B**). Entry into right and left atria were exposed. Harvesting of tricuspid and mitral annulus was done by making a circular incision around the bases of corresponding valve cusps (**Fig 1C**). The entire annuli with attached cusps were harvested intact (**Fig 1D**). This was followed by measurement of annular circumference (in cm) using a flexible metric ruler.

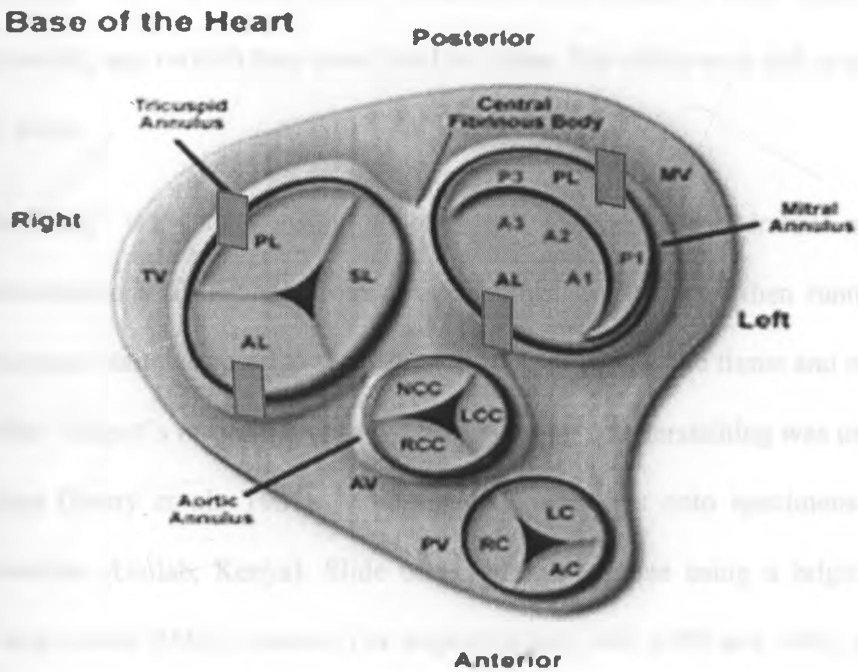
The surface areas of the annuli were calculated from measurements of circumference, as shown in this formula: $\mathbf{C^2 \div \pi}$ (where C is circumference of the annulus and $\pi = 3.142$).

Fig 1: Macrographs showing the materials and methods used in the study



METHODS 2: Light microscopy

Twenty one specimens (seven in each age group: 3M, 4F), were used for histological study. Hearts from male and female subjects were examined separately. From each specimen, anterior and posterior parts of tricuspid and mitral annulus were excised *en bloc* with attached valve cusps on one side, and myocardium on opposite side. This was done in order to allow examination of the extents of the annulus ie from annulo- valvular junction (AVJ) to annulo- myocardial junction (AMJ). Four millimetre thin sections were then obtained from the centres of anterior and posterior parts of the annuli as shown in **Fig 2**. These were processed for light microscopy



AL: Anterior leaflet

PL: Posterior leaflet

SL: Septal leaflet

TV: Tricuspid valve

MV: Mitral valve

Fig 2: **Sectioning protocol employed on AV annuli**. The centres of anterior and posterior parts of the annuli are denoted by shaded boxes. Longitudinal sections of the annuli were taken at the shaded boxes.

Annular tissue processing and microscopic analysis

Annular tissue sections were fixed for a period of 72 hours by immersion in 10% formal saline. They were then dehydrated in ethyl alcohol of increasing concentrations starting with 70% alcohol to absolute alcohol, each change lasting for one hour.

Clearing was done in Toluine (S.G. 0.866) for 2 hours, followed by wax impregnation for 12 hours at 58^0 C. Embedding was done in fresh molten paraffin wax (Paraplast, McCormick Scientific LLC, USA) using metal moulds. Tissues were then fixed on wooden blocks to facilitate cutting on a sledge microtome. A Lenz Wezlar (Germany) microtome was used for this purpose to produce 7- micron thick sections. These were then floated in warm water bath at 45^0 C to enhance spreading upon which they were fixed on slides. The slides were left to dry in an oven at 38^0 C for 12 hours.

Dewaxing was done using xylene, and segments rehydrated using xylol, descending concentrations of alcohol, from absolute down to 70%, and then running tap water. Masson's trichrome stain was used to study organization of connective tissue and myocardium in the annuli, while Weigert's resorcin fuchsin with van Gieson counterstaining was used to demonstrate elastic fibres (Drury et al., 1967). Mounting of cover slips onto specimens was done using D.P.X mountant (Unilab, Kenya). Slide observation was done using a bright- field light microscope (Leica model BME, Germany) at mignifications x40, x100 and x400, and features recorded on data sheets. Where notable histological features requiring transverse sections were observed, orientation of the tissue blocks was adjusted accordingly, and processing for light microscopy repeated.

Data analysis and presentation

Data for gross morphometry were entered into SPSS software (Version 16.0, Chicago, Illinois) for statistical analysis. Measurements were expressed in means ± standard deviations. Student's t- test was used for analysis of morphometric sex differences. Comparison of means between the age groups was performed using the analysis of variance (ANOVA) test. A p- value of ≤ 0.05 was considered significant at a confidence interval of 95%. Representative photographs, tables and graphs were used in data presentation.

RESULTS

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). With respect to age groups, the weights and external lengths of the hearts increased with increasing age. (**Table1**).

Table 1: Age group differences in heart weight and external heart length

	AGE GROUPS		
Parameter	**≤ 21 (n= 22)**	**22- 45 (n= 33)**	**46- 70 (n= 25)**
Heart weight, g (Mean ± SD)	164 ± 60	273 ± 54	303 ± 59
External length, cm (Mean ± SD)	10.8 ± 2.2	12.9 ± 1.8	13.9 ± 1.7

Table 2: Sex differences in heart weight and external length in specific age groups

AGE GROUP	**Heart weight** (g)	p- value	**External length** (cm)	p- value
≤ 21	M= 199.1 ± 70.0 F= 143.9 ± 65.1	**0.150**	M= 11.9 ± 2.2 F= 10.2 ± 2.0	**0.080**
22- 45	M= 296.0 ± 51.2 F= 245.6 ± 44.2	**0.005**	M= 12.8 ± 1.7 F= 13.0 ± 2.0	**0.790**
46- 70	M= 330.4 ± 67.3 F= 277.1 ± 35.4	**0.200**	M= 14.1 ± 1.6 F= 13.7 ± 1.9	**0.530**

M= male   F= female

Gross Morphometry

The circumferences of tricuspid (TA) and mitral annulus (MA) were greater in males than females by a difference of 0.7cm and 0.2cm respectively. This difference was however not statistically significant (p= 0.12 for TA and 0.57 for MA). Surface areas of corresponding annuli were also greater in males than females although the difference was also statistically insignificant (p= 0.11 for TA and 0.68 for MA) **(Table 3).** There was a significant positive correlation between heart weights and the annular circumferences (r= 0.6 for TA and 0.56 for MA).

Table 3: Sex differences in Gross Morphometry

Parameter	Sex	Mean ± SD	p value
Tricuspid Circumference (cm)	M F	9.1 ± 1.9 8.4 ± 1.8	0.12
Tricuspid Area (cm^2)	M F	6.8 ± 2.7 5.9 ± 2.3	0.11
Mitral Circumference (cm)	M F	7.1 ± 1.3 6.9 ± 1.5	0.57
Mitral Area (cm^2)	M F	4.1 ± 1.4 3.9 ± 1.5	0.68

M= male F= female

After standardising corresponding annular circumferences with the weights of the hearts, it was actually females who had larger circumference than males, and in the 22- 45 year age group, this difference was statistically significant (**Table 4**). The standardised values were obtained using the formula: Circumference (cm) ÷ heart weights (g)

Table 4: Standardised sex differences in Gross Morphometry

PARAMETER	**≤ 21 yrs**	p value	**22- 45 yrs**	p value	**46- 70 yrs**	p value
Tricuspid circumference	M= 0.48 F= 0.64	**0.17**	M= 0.30 F= 0.37	**0.01**	M= 0.33 F= 0.31	**0.40**
Mitral circumference	M= 0.38 F= 0.49	**0.19**	M= 0.25 F= 0.29	**0.02**	M= 0.24 F= 0.28	**0.07**

M= male **F**= female

Age differences in gross morphometry

The circumference and area of both tricuspid and mitral annulus increased with increasing age. This difference was significant between the ≤ 21 year and 22- 45 age groups (**Table 5**)

Table 5: Age group differences in Gross Morphometry

	Age groups			Significance between
Parameter	**≤ 21**	**22- 45**	**46- 70**	**age groups**
Tricuspid circumference, cm	7.6 ± 2.0	8.9 ± 1.5	9.5 ± 1.6	<0.01*, 0.21**
Tricuspid area, cm^2	4.9 ± 1.9	6.5 ± 2.2	7.3 ± 2.5	<0.01*, 0.17**
Mitral circumference, cm	6.0 ± 1.6	7.1 ± 1.1	7.6 ± 1.1	<0.01*, 0.14**
Mitral area, cm^2	3.0 ± 1.3	4.1 ± 1.2	4.7 ± 1.3	<0.01*, 0.11**

***significance between age group 1 and 2** ****significance between age group 2 and 3**

Figure 3: Line graph showing age differences in annular circumference

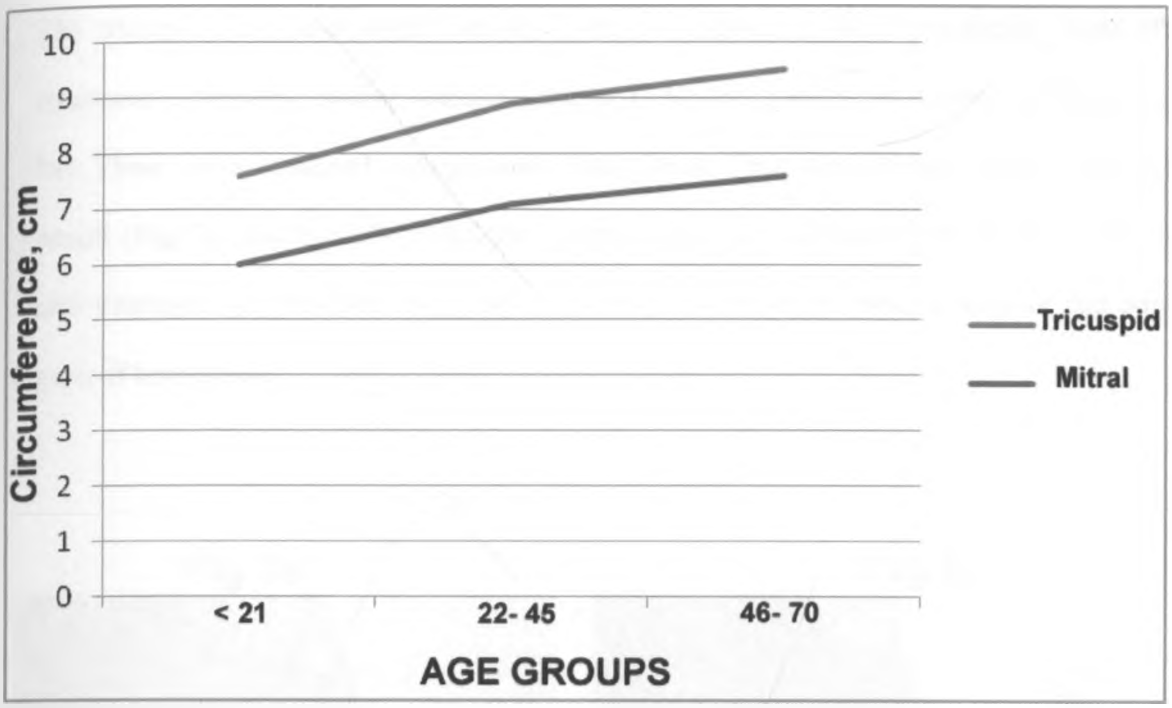
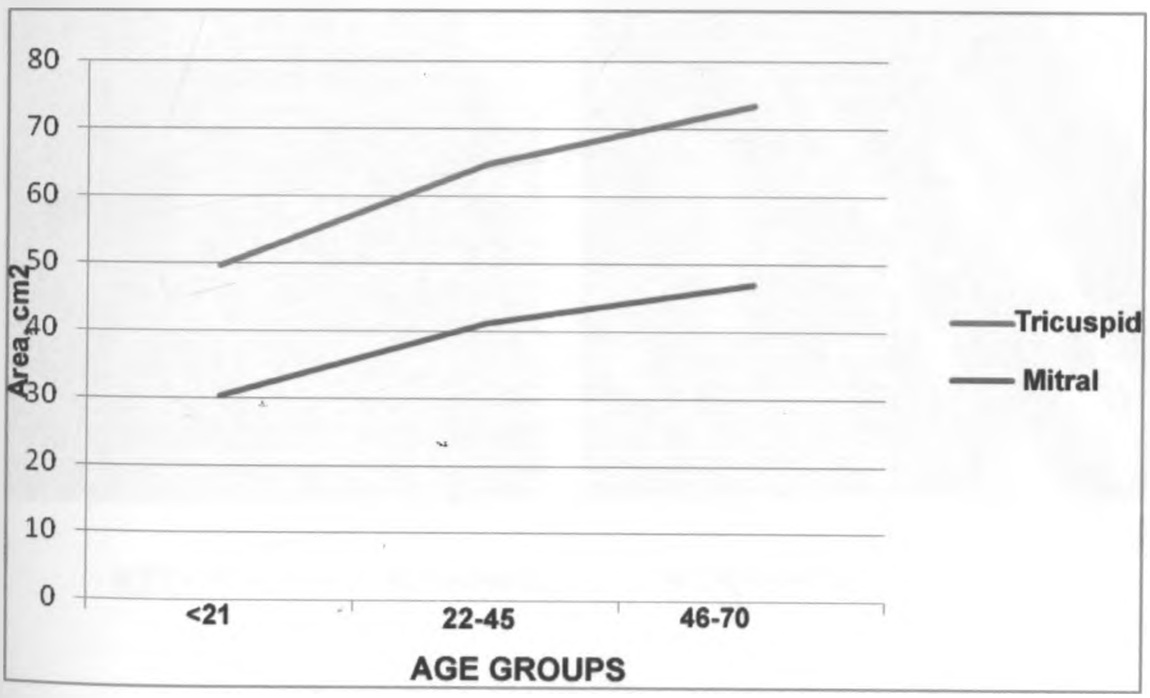


Figure 4: Line graph showing age differences in annular area



Histomorphology of AV annuli (1): Sex differences

The tricuspid (TA) and mitral annulus (MA) in both sexes were endothelial- lined fibrous structures comprising central, annulo- valvular (AV) and annulo- myocardial (AM) zones (**Fig 5a**). These zones contained collagen and elastic fibres. There were cardiac muscle cells in male annuli (**Fig 5b**). Additionally, cellularity, elastic content and collagen fibre density in the annuli also displayed sex variation. No notable differences were found between anterior and posterior parts of both annuli.

Fig 5a

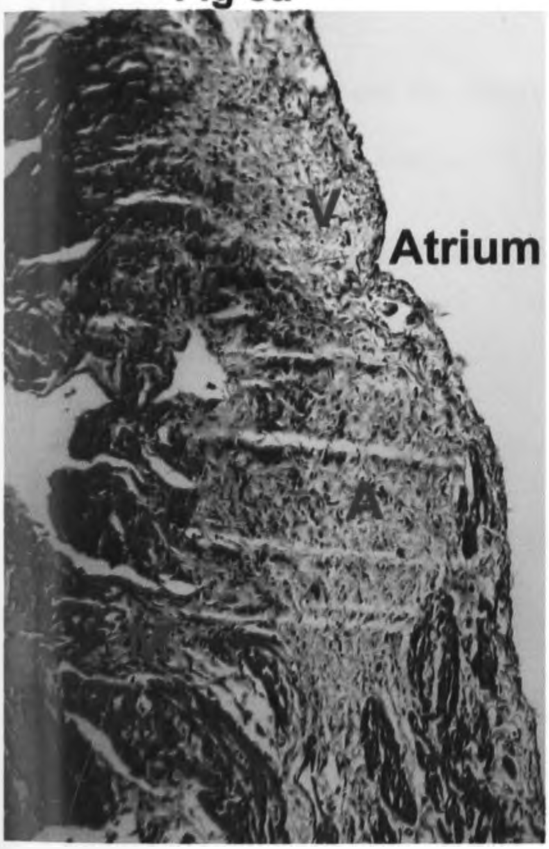
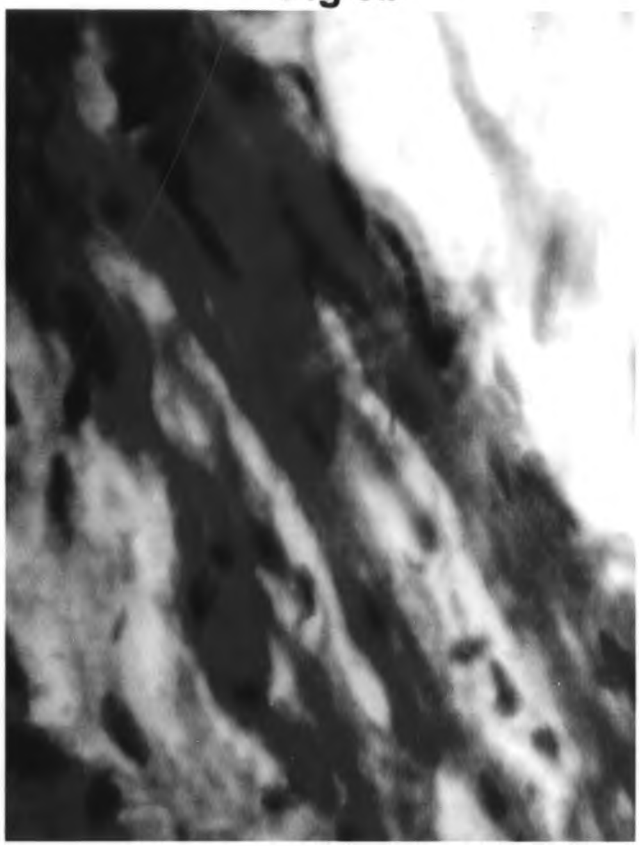


Fig 5b



<u>KEY</u>: **V**: Valve **A**: Annulus **M**: Myocardium

Annulo- myocardial zone (AMZ)

This zone was separated from the myocardium by an annulo- myocardial junction (AMJ). It contained collagen and elastic fibres in both sexes, and myocardium but only in male annuli.

In males, myocardium was present in both TA and MA (**Fig 6a and 7a**). The myocardium was organised into distinct bundles in TA (**Fig 6a**) and strips in MA (**Fig 7a**). In both annuli, this myocardium was interposed between collagen fibres.

There was no muscle in this zone in female annuli, (**Fig 6b**), with exception of MA where only thin strands of cardiac muscle fibres were demonstrable (**Fig 7b**). Collagen fibres were densely packed in this sex for both TA and MA (**Fig 6b and 7b**).

Elastic fibres were denser and un- interrupted in this zone in male annuli (**Fig 8a**). Their orientation was predominantly longitudinal, and they were interposed between collagen fibres.

In females, elastic fibres were less dense and regularly interrupted along their course (**Fig 8b**)

Central Zone (Annular core)

In both sexes, the predominant CT fibre in this zone was collagen. Elastic fibres were also present. Organisation and density of these fibres showed sex variation.

In males, collagen fibres were loosely packed in this zone. Interspersed between the loose fibres were numerous cells with elongated nuclei characteristically seen in TA (**Fig 6a**). Myocardium was present in this zone in both annuli (**Fig 6a and 8a**). The myocardium was surrounded by two layers of dense elastic fibres on both its inner and outer aspects (**Fig 8a**). One layer predominantly consisted of elastic fibrils whose orientation was irregular, while the other layer comprised longitudinal elastic fibres. Collagen fibres intervened between the elastic fibre layers surrounding the annular myocardium.

In females, there was paucity of elongated cells in the annular core and instead densely packed collagen fibres characterised this zone (**Fig 6b**). No muscle fibres were observed in this zone in either TA or MA (**Fig 6b and 7b**). Elastic fibres were scarce and discontinuous (**Fig 8b**).

Legends: Fig 6a-b, 7a-b and 8a- b

Fig 6a: Photomicrograph of tricuspid anulus in a **male** showing presence of annular myocardium (**AM**) in the central (annulus core) and annulo- myocardial zones (**AMZ**). Note the prominent muscle bundles (**arrows**) and the high cellular density. **Masson trichrome stain**; Magnification x400

Fig 6b: Photomicrograph of tricuspid annulus in a **female** showing central (annulus core) and annulo- myocardial zones (**AMZ**). Note the dense regular collagen fibre arrangement in these zones with no muscle content and low cellular density. **AMJ-** Annulo- myocardial junction. ***Masson trichrome stain***, Magnification x400

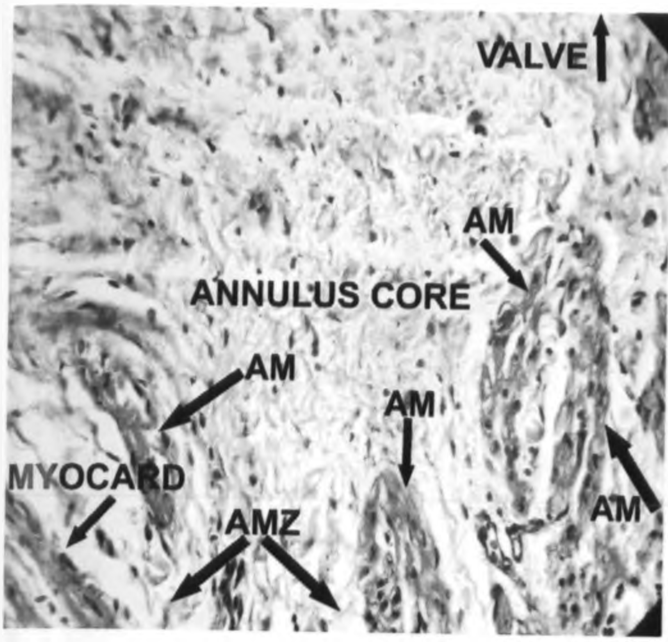
Fig 7a: Photomicrograph of mitral annulus in a **male** showing strips of annular myocardium in the annulo- myocardial zone (**AMZ**). **AMJ-** Annulo- myocardial junction. ***Masson trichrome stain***; Magnification x400

Fig 7b: Photomicrograph of mitral annulus in a **female** showing thin strands of myocardium in the annulo- myocardial zone (**AMZ**). Note the dense collagen fibre arrangement in this zone. ***Masson trichrome stain***; Magnification x400

Fig 8a: Photomicrograph of mitral annulus in a **male** showing dense elastic fibres at the annulo- myocardial zone (**yellow arrows**). Note the two layers of elastic fibres (**blue arrows**) enclosing the annular myocardium in between at the cental zone (annulus core). **AMJ-** Annulo- myocardial junction. ***Weigerts elastic with van Gieson counterstain***; Magnification x400

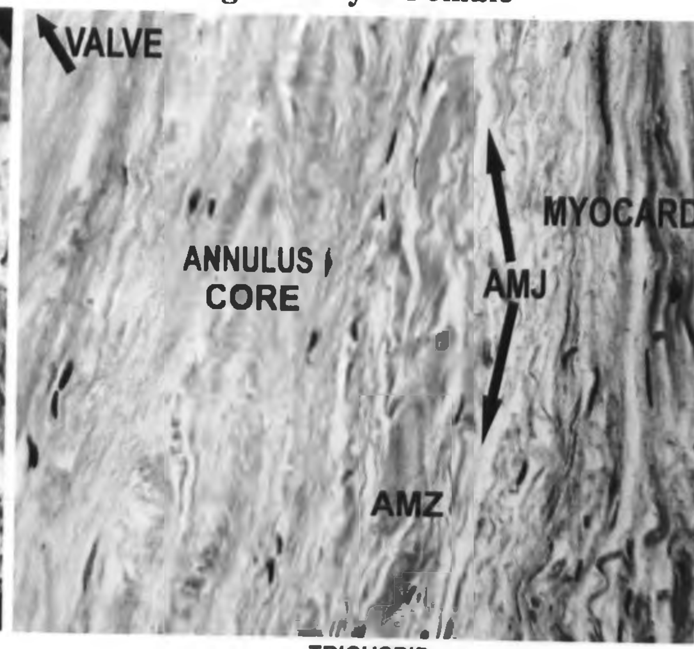
Fig 8b: Photomicrograph of mitral annulus in a **female** showing less dense and discontinuous elastic fibres (**big arrows**) in the annulo- myocardial zone (**AMZ**). Note the interrupted elastic fibres (**small arrows**) in the annular core. ***Weigerts elastic with van Gieson counterstain***; Magnification x400

Fig 6a: 20 yrs Male



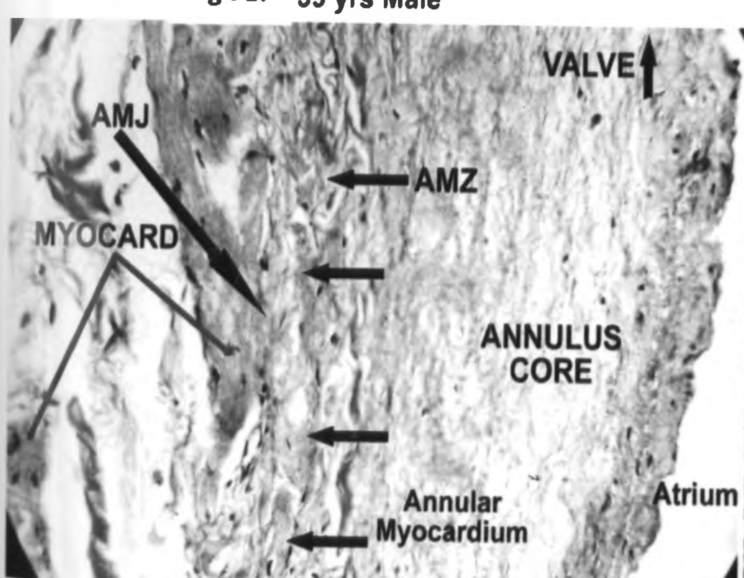
TRICUSPID

Fig 6b: 26 yrs Female



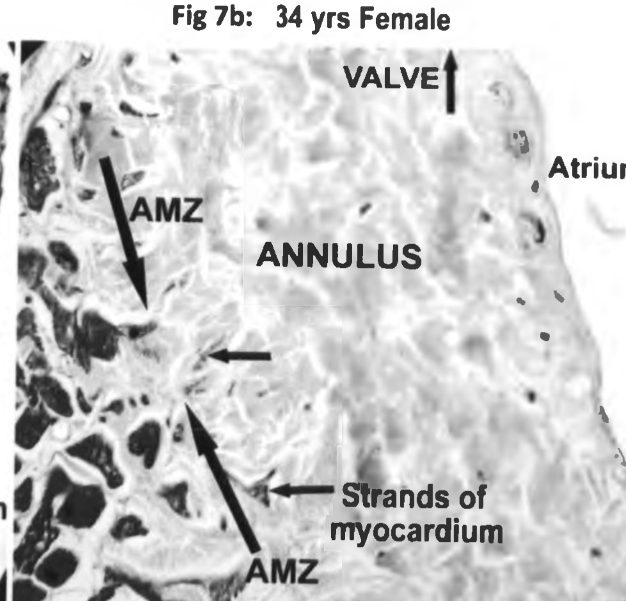
TRICUSPID

Fig 7a: 39 yrs Male



MITRAL

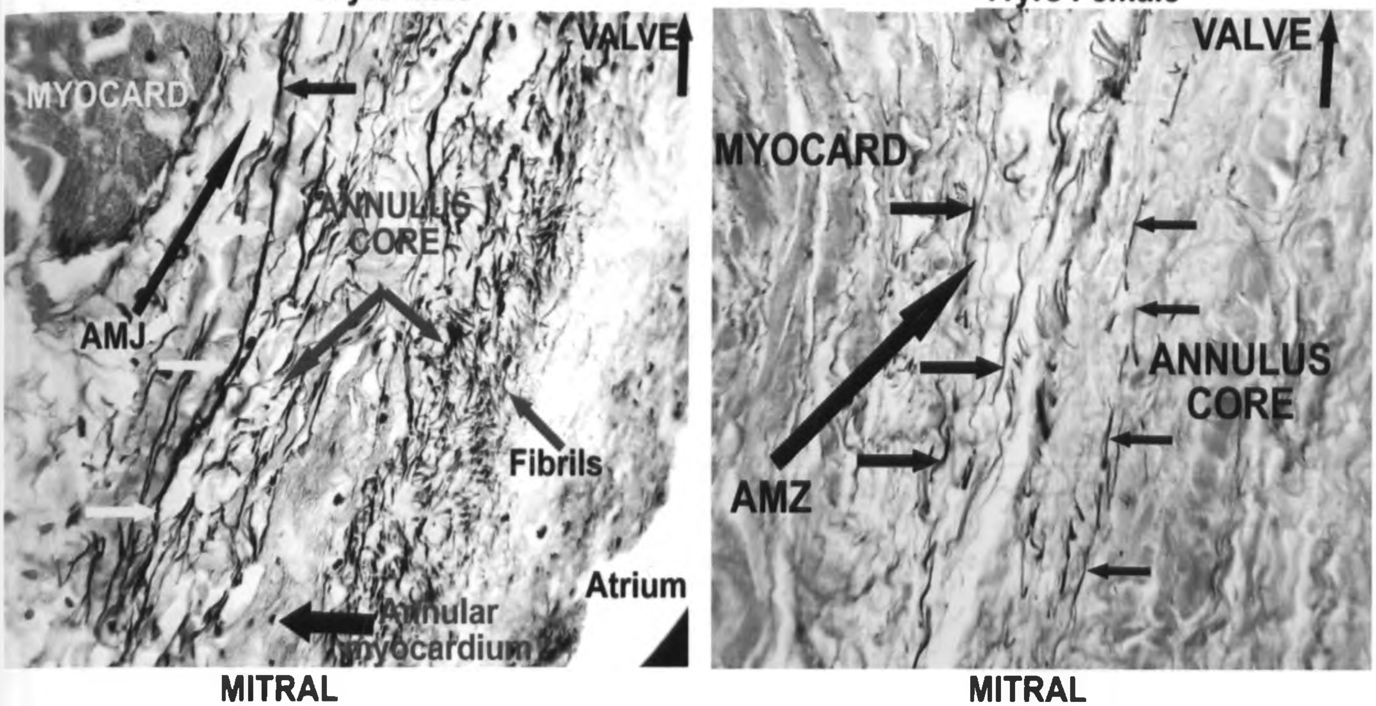
Fig 7b: 34 yrs Female



MITRAL

Fig 8a: 37yrs Male

Fig 8b: 41yrs Female



Annulo- valvular zone (AVZ)

This zone was separated from the valves by an annulo- valvular junction (AVJ). The predominant connective tissue fibre here was collagen for both males and females. Elongated cells were also present.

In males, this zone was characteristically cellular (**Fig 9a**). Numerous elongated cells were interspersed between loosely packed collagen fibres. The fibres did not show specific orientation.

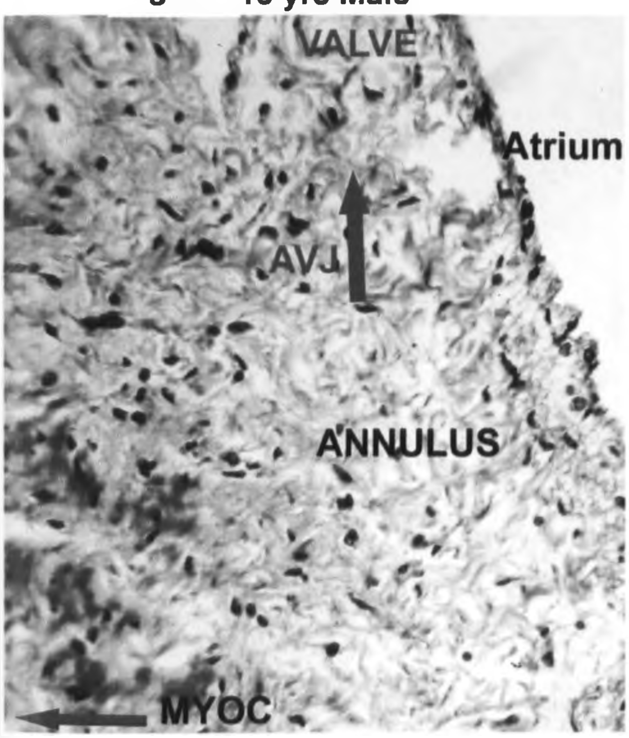
In females on the other hand, this zone was characterised by scarcity of cells and instead, dense and regular arrangement of collagen fibres dominated the zone (**Fig 9b**). Orientation of the fibres was predominantly longitudinal.

Legends: Fig 9a and 9b

Fig 9a: Photomicrograph of tricuspid annulus in a male showing highly cellular annulo- valvular junction (AVJ). Note the elongated nuclei in these cells. ***Masson trichrome stain***; Magnification x400

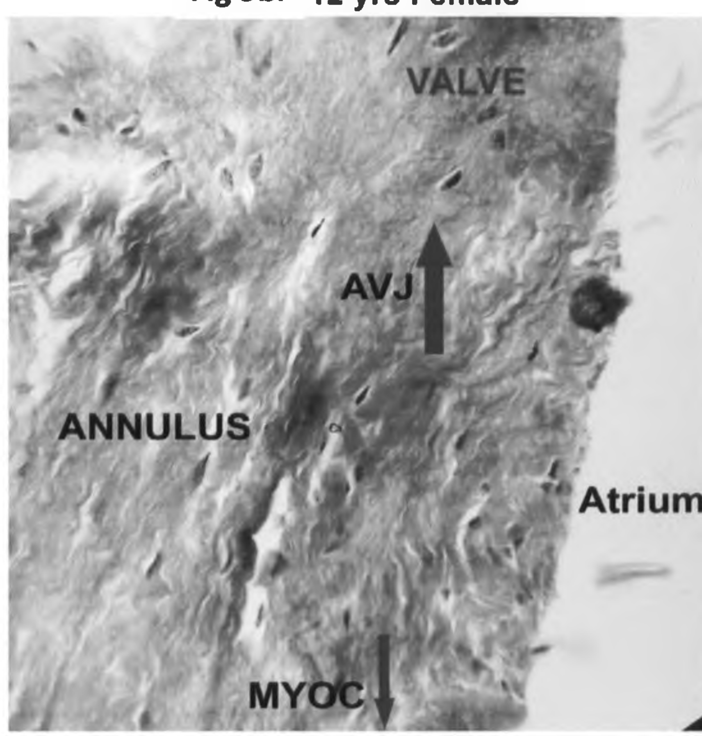
Fig 9b: Photomicrograph of tricuspid annulus in a female showing scarcity of cells at the annulo-valvular junction (AVJ). ***Masson trichrome stain***; Magnification x400

Fig 9a: 18 yrs Male



TRICUSPID

Fig 9b: 12 yrs Female



TRICUSPID

Histomorphology of AV annuli (2): Age- related changes

There were age- related changes in cellularity, organisation and density of collagen and elastic fibres, and thickness of annular muscle fibres. Except for annular myocardium, these changes were similar for males and females. However, age- related changes in the organisation of collagen fibres were different for TA and MA.

There was a general decline in the cellular content of both annuli with increasing age (**Fig 10 and 11**). Collagen fibre density increased with age, but discontinuity of the fibres was distinct in mitral than tricuspid annulus (**Fig 10b vs 11b**). The density of elastic fibres decreased with age as the fibres became more disrupted and irregular (**Fig 12a, b and c**). In males, thickness of annular myocardium declined with age (**Fig 13a, b and c**). There was also a general decrease in the number of annular muscle strips with increasing age.

Legends: Fig 10 and 11

Fig 10: Photomicrographs of tricuspid annulus in a young **(a)** and old **(b)** **male** showing an increase in collagen fibre density and irregularity with increasing age. Note the decrease in cellularity with increasing age. ***Masson trichrome stain***; Magnification x400

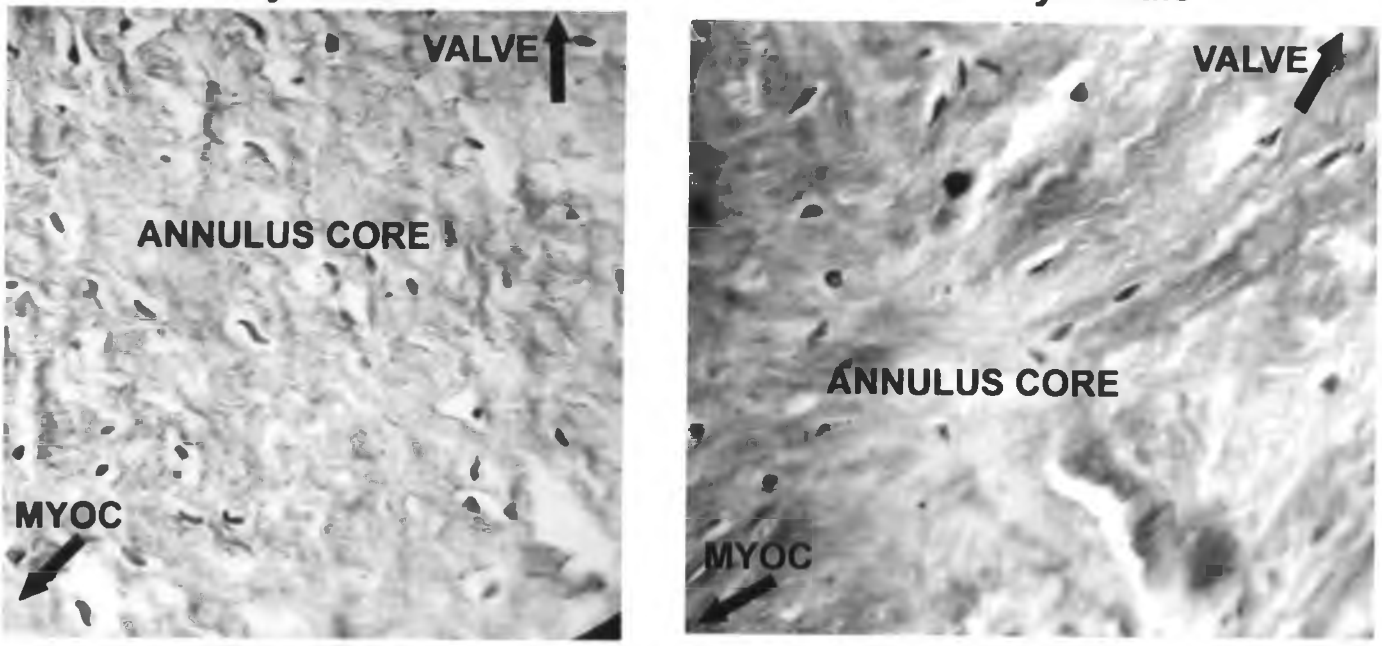
Fig 11: Photomicrographs of mitral annulus in a young **(a)** and old **(b)** **female** showing decrease in cellularity, and increase in collagen fibre density and irregularity with increasing age. ***Masson trichrome stain***; Magnification x400

NB: Note the extensive collagen fibre discontinuity with increasing age in mitral **(Fig 11b)** than tricuspid annulus **(Fig 10b).**

Figure 10

a. 22 yrs Male

b. 54 yrs Male



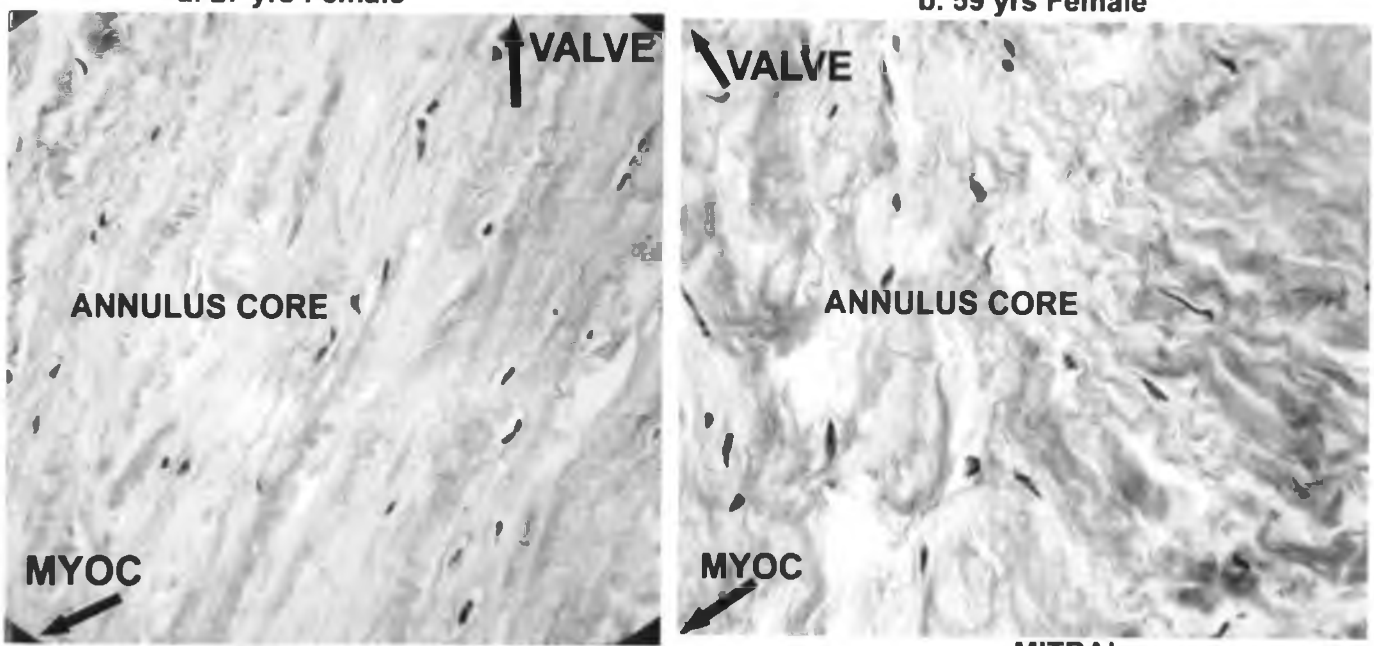
TRICUSPID

TRICUSPID

Figure 11

a. 27 yrs Female

b. 59 yrs Female



MITRAL

MITRAL

Legends: Fig 12 and 13

Fig 12: Photomicrograph showing a general decline in elastic fibre density (arrows) and regularity with increasing age **(a, b, c).** Note the increasing interruption of the fibres with increasing age. ***Weigerts elastic with van Gieson counterstain***; Magnification x400

Fig 13: Photomicrographs showing a general decrease in the thickness and number of annular muscle strips **(arrows)** with increasing age (a, b, c). ***Masson trichrome stain***; Magnification x400

FIGURE 12

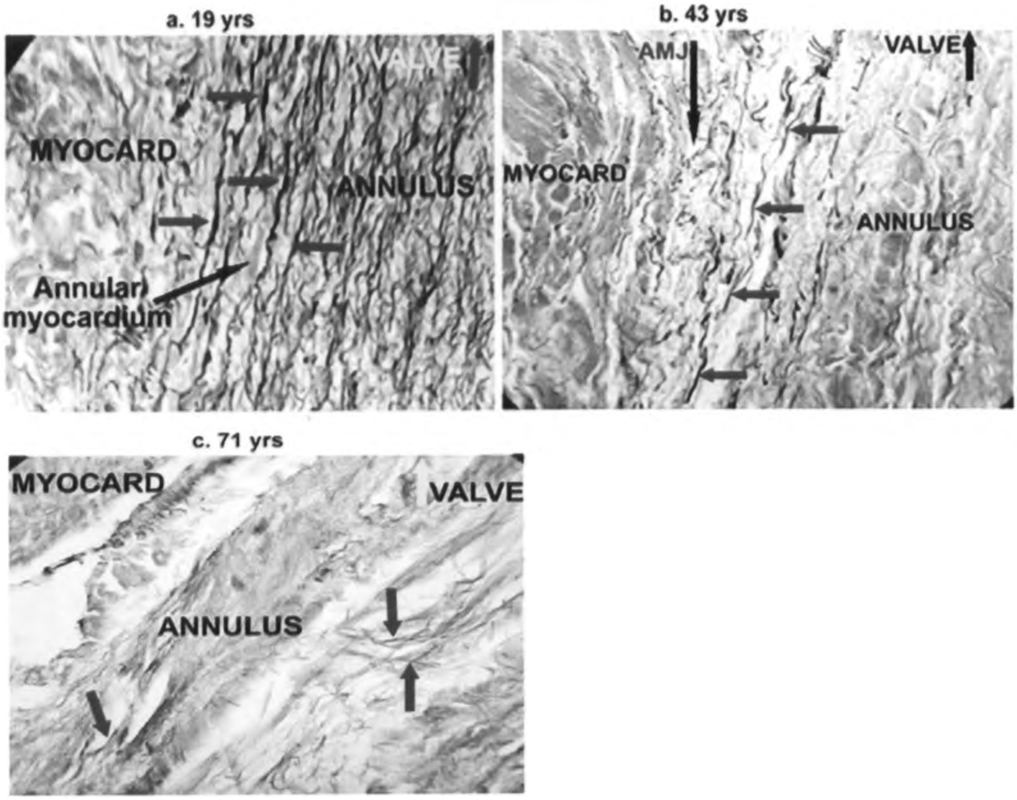
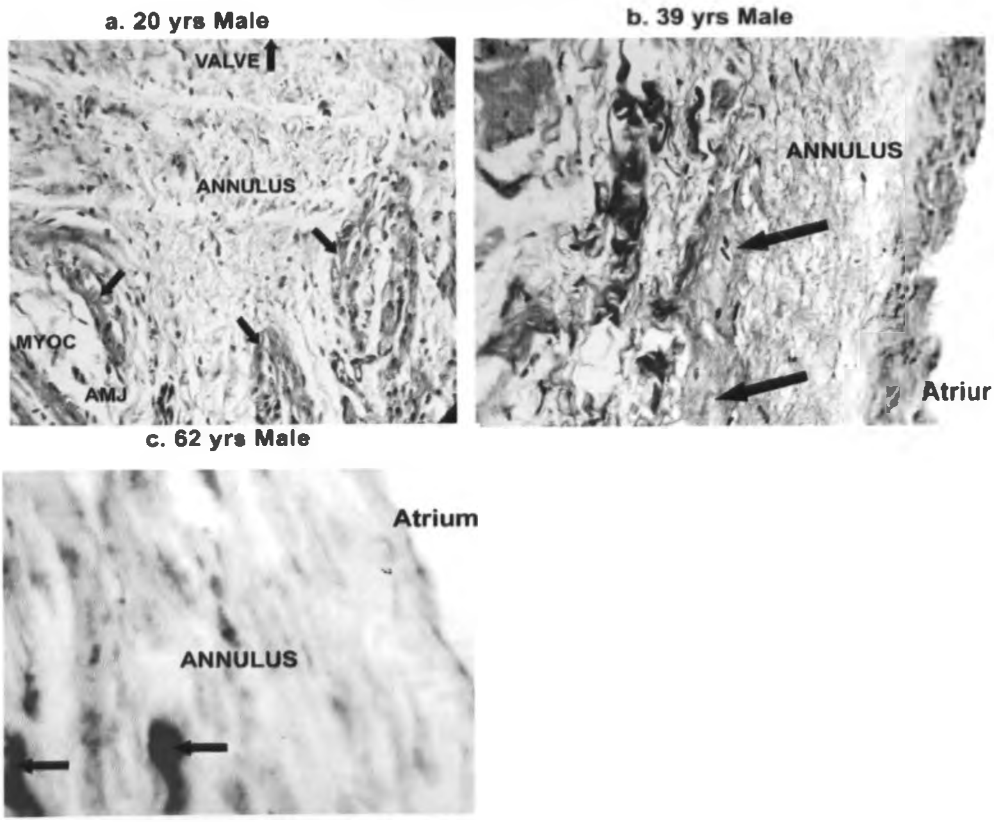


FIGURE 13



DISCUSSION

Results of the present study have elaborated age and sex differences in the morphometry and morphology of AV annuli in a sample of Kenyans. The results provide, probably for the first time, data on adaptive capabilities of the annuli to hemodynamic stresses in different sexes and age groups, and morphological basis for the patterns of pathology affecting them. The results also provide local age specific baseline values for annular morphometry. Previous studies on these annuli mainly described interspecies variations, with little mention on age and sex variations (Racker, 1991; De Biasi, 1984).

Gross morphometry

Overall, males had larger annular circumference and surface area. This is not entirely surprising as males had larger heart weights and external heart lengths in almost every age group. Further, there was positive correlation between heart weights, external heart lengths, and the measured circumferences, which may explain the increase in annular dimensions with increasing age as shown in the present study. The results on sex differences may therefore mean the larger circumferences 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).

The current results are also in agreement with previously reported sex differences in other heart dimensions. Skwarek et al (2008) found larger tricuspid orifice circumference in males, with no statistically significant sex differences. However, the average dimensions for male and female circumferences in their population (Polish) are larger than ours. This may be attributed to inter-population variations in body surface area of individuals, as Polish may have larger body mass indices than Kenyans.

The results on significant increase in annular dimensions between the < 21 year and 22- 45 year age groups could be functionally significant. It may suggest a significant difference in hemodynamic loads, which may explain the rapid increase in heart sizes and annular dimensions between these age groups. Increase in annular circumference may decrease valve co-aptation during cardiac cycle, and this may be clinically associated with valvular regurgitation (Ormniston, 1981). The present study showed that females had larger annular circumference than males, after standardising for the heart weights. This may therefore be an independent risk factor for heart valve incompetence in females since larger circumferences are clinically encountered in patients with prolapsed and regurgitant valves (Roberts, 1983; Mutlak, 2007). However, this cannot be fully concluded without considering the intrinsic structure of the annuli themselves to determine the sex predisposition to heart valve incompetence.

Histomorphology of AV annuli

Puff et al (1960) described the general histological structure of the fibrous rings of the heart as consisting of central and peripheral zones containing collagen and elastic fibres. Later, a similar architecture was reported by Martin (2007). The present study supports the findings of these two workers. The general histological structure of AV annuli may therefore be similar among different populations. An important addition in the present study however, is the existence of age and sex-related differences in the different zones of the annuli.

Annulo- myocardial zone (AMZ)

The presence of cardiac muscle in TA and MA in males, and myofibres in mitral annulus in females, is unique to our study. Dudziak et al (2009) found no myofibres in the TA. Puff et al (1978) provided the basis for the 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. The current findings therefore provide evidence for the presence of cardiac muscle both in the TA and MA in males, and indistinct myofibres in the MA in females. None of these earlier studies grouped their specimens into sex, and hence it is difficult to ascertain whether there was any pattern in terms of sex variability. Comparative animal studies have also demonstrated presence of myocardium in other valvular apparatus. De Biasi (1984) reported presence of myocardium within proximal portion of AV valve cusps in mammals. Further, Gatonga et al (2009) demonstrated myocardium in the mitral valve cusps but only in males. Clearly, the annulus and other valvular apparatus previously reported as being merely fibrous structures lined by endothelium, contain distinct cardiac muscle fibres. What then 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 during cardiac cycle, and this may influence the timing and effectiveness of AV valve closure (Yacoub and Cohn, 2004). Myocardium within the fibrous structure of AV annuli may therefore serve to regulate annular contraction and relaxation during various phases of cardiac cycle. The myocardium may also play a 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).

Further significance of myocardium in the annulus was suggested by Racker (1991) who on his electrophysiological study, proposed that presence of myofibres in the annulus may be the anatomic substrate for circus movements in the genesis of atrial flutters. Therefore, the muscle fibres in TA and MA in males may be a possible origin of dysrrthymias. Comparatively in females, this possibility has no basis in the present study, as myofibres were indistinct in this sex. Thus comparing myocardial composition in male and female annuli, one may therefore argue that the composition in males may serve to support and strengthen their annuli, making them less predisposed to annular insufficiency and valvular failures. Further, extrapolating this argument to compare myocardial content in TA and MA, one may also argue that the distinct bundles present in TA may make it more pliable and structurally adapted to withstand hemodynamic forces compared to the strips in MA.

The interruption and density of elastic fibres in females observed in the present study may be functionally significant. Elastic fibres are found in areas subjected to stretch, and have unique feature of being extensible (Dobrin, 1978). These facts when put together may suggest that interruption of these fibres in female annuli may make them less adapted to withstand stretch and extensibility. Hence, when subjected to varying biomechanical forces as in ventricular systole, their annuli may have a higher chance of insufficiency and incompetence. Comparatively in males, the dense and regular arrangement of elastic fibres may serve to increase the structural adaptability of their annuli to withstand stretch and extensibility across several planes. However, this cannot be concluded without comparing the other zones of the annulus, as all zones play an integrated role.

Central Zone (Annular core)

The collagenous nature of this zone in both sexes in the present study, is in tandem with previous descriptions of this zone (Dudziak, 2009). There are so far no reports indicating variability in fibre organisation and composition in this part of the annulus. The unique finding in the present study is the existence of sex differences in myocardial fibre composition, and the organisation and density of CT fibres.

The cellular profile characteristically observed in tricuspid annulus in males, could be fibroblastic in nature, due to their elongated nuclei (Dudziak, 2009). This author showed that fibroblasts are widely distributed in human annular and valvular tissue, and studies which have been done on these cells in culture, have indicated 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. If the same argument is extrapolated to compare cellularity in TA and MA, one may also argue that TA may be better adapted hemodynamically.

Further, the myocardium contained in this zone in males, was interposed between two layers of elastic fibres on its inner and outer aspects, in a manner to suggest functional interaction. Similar organisation between muscle and elastic fibres has been described in lamellar units of elastic arteries (Clark and Glagov, 1985). These are functional units which confer elastic properties to these vessels, and are important in their functional integrity. On this basis therefore, this feature may be important in conferring mechanical strength to male annuli. Comparatively in females, there was no muscle in this zone and elastic fibres were scarce and discontinuous, a feature which may make female annuli less flexible and less adapted to withstand stretch and extensibility. However, this cannot be fully concluded without considering the other zone of the annulus where valves derive their main support, the annulo- valvular junction.

Annulo- valvular zone (AVZ)

The predominant collagenous nature of this zone in the present study has been previously reported (Puff, 1960). The current study further adds information on presence of elongated cells in male annuli in this zone hitherto unreported, raising questions whether this may make annulo- valvular junction in this sex more pliable to hemodynamic changes during cardiac cycle.

Age related changes in histomorphology

The age- related decrease in annular cellularity and elasticity observed in the present study, has been previously reported (Pomerance, 1967). The increased collagen fibre density with increasing age, may be caused by a decrease in the rate of fibre turnover with subsequent accumulation and cross- linking (Drury, 1967). This may be associated with fibrosis, a common cause of recurrent valve failures (Dalane, 1990). Further, increased collagen fibre density may also exert forces on elastic fibres hence distorting them (Fukuda, 1989), as shown in the present study. Elastic fibres have also been shown to undergo age- related degenerative changes such as loss of fibrillar structure, and increase in foreign deposits and inclusions (De Cavarlho, 1996), which may also alter their overall functional capacity. The present study also depicted a reduction in myocardial content of the annulus with increasing age. This is not entirely surprising considering a general decline in heart function in the old (Dalane, 1990). This may therefore imply reduced annular flexibility and contractility with increasing age.

Another important addition in the present study is the age- related difference in collagen fibre discontinuity in TA and MA. Collagen is the main load- bearing component of annular and valvular tissue, and is responsible for biomechanical strength, especially tensile strength (Balguid, 2007). Collagen fibre discontinuity may therefore be associated with less resistance to tensional forces and compromised structural integrity. This arrangement, as observed in MA, may therefore predispose it to structural failures more than tricuspid.

CONCLUSION

Gross morphometry of AV annuli show no significant sex variation, although there is trend towards larger annuli in males, attributable to larger heart sizes. Nonetheless, the age specific annular measurements provide baseline values which may be useful for annular reconstruction and local prosthesis design. Microscopic organisation, notably presence of myocardium, cellular profiles and elastic fibre density, show remarkable sex variation. Hence a greater predisposition to AV valve failures in females may be attributed to these differences. The age- related decrease in cellularity, elasticity and myocardial composition, as well as increased collagen fibre density and irregularity, may provide anatomical basis for the higher incidence of valvular failures with increasing age. The differences in myocardial content, cellular density, and collagen fibre discontinuity, may explain why valve failures are more common in mitral than tricuspid.

SUGGESTIONS FOR FURTHER STUDIES

- Characterization of cellular components in the annulus by histochemical and electron microscopic techniques in order to ascertain their function
- Studies on structure-function relationships in order to ascertain the roles played by different components of annular tissue in the overall functional integrity of entire fibrous skeleton of the heart.
- Characterisation of presence/absence of nerve fibres in order to determine the interplay between muscle and nervous tissue in the annulus

REFERENCES

Angelini A, Ho SY, Anderson RH. A histological study of the atrioventricular junction in hearts with normal and prolapsed leaflets of the mitral valve. Br Heart J 1988; 59: 712–716.

Balguid A, Rubbens MP, Mol A, Bank RA, Bogers JJ, Van Kats JP, De Mol AJ, Baaijens FP, Bouten CV. The role of collagen cross-links in biomechanical behavior of human aortic heart valve leaflets – relevance for tissue engineering. Tissue Eng 2007; 13: 1501 – 1511.

Carpenter A.J, Margarita Camacho. Valvular heart disease in women: The surgical perspective. J Thorac Cardiovasc Surg 2004; 127 :4-6

Clark JM, Glagov S. Transmural organization of the arterial media. The lamellar unit revisited. Arterioscl Thromb Vasc Biol 1985; 5: 19 – 34.

Dalane W. Kitzman and William D. Edwards. Age-Related Changes in the Anatomy of the Normal Human Heart. J of Gerontol 1990; 2: 33-39

De Biasi S, Vitellaro – Zuccarello L, Blum I. Histochemical and ultrastructural study on the innervation of human and porcine atrio-ventricular valves. Anat Embryol 1984; 169: 159 – 165

De Cavalho Filho ET, de Cavalho CA, de Souza RR. Age- related changes in elastic fibres of human heart. Gerontol 1996; 42: 211-217

Dobrin PB. Mechanical properties of arteries. Physiol reviews 1978; 58: 397 – 460.

Drury RAB, Wallington EA, Cameron R. Connective tissue fibers. In: Carleton's Histological Techniques, 4th ed. New York: Oxford University Press, 1967; 166 – 181.

Dudziak M, Skwarek M, Hreczecha J, Jerzemowski J, Grybiak M. Microscopic study of right fibrous annulus. Folia Morphol 2009; 1: 32-35.

Farry JP, Simon AL, Ross AM, Cohen LS, Wolfson S. Quantitative angiographic assessment of the mitral annulus in the prolapsing leaflet syndrome. Circul 1975; 52: 11-12,

Filip DA, Radu A, Simionescu M. Interstitial cells of the heart valves possess characteristics similar to smooth muscle cells. Circul Res 1986; 59: 310 – 320.

Fukuda Y, Masuda Y, Ishizaki M. Morphogenesis of abnormal elastic fibres in lungs of patients with panacinar and centriacinar emphysema. Hum Pathol 1989; 20: 652- 659

Gatonga, P, Odula, P. O, Saidi, H, Mandela, P. Sex Variation in Occurrence of Myocardium in Human Mitral Valve Cusps. Int. J. Morphol 2009; 4: 1217-1222,

Glower DD, Bashore TM, Harrison JK, Wang A, Gehrig T. Pure annular dilatation as a cause of mitral regurgitation: a clinically distinct entity of female heart disease. J Heart Valv Dis 2009 3: 284-288

Guyton AC, Hall JE. Textbook of medical physiology, 10th ed. Philadelphia: Saunders; 2000.

Istvan Szentkiralyi, Arpad Peterffy, Zoltan Galadja. Importance of stabilization of mitral annulus in mitral valve repair. J. Thoracic cardiov. Surg 2008; 4: 1102-1103

Jar S. Mitral regurgitation; emedicine, June 2009

Martin Misfield and Hans- Hinrich Sievers. Heart valve macro and microstructure. Phil Trans. R. Soc 2007; 362: 1421-36

Maurice Enriquez, Cary W Akins, Alec Vahanian. Mitral regurgitation. Lancet 2009; 373: 1382–94

Moore KL, Dalley AF. Clinically Oriented Anatomy. 4th ed. Philadelphia: Lippincott williams and Wilkins, 2006; 151-155.

Mutlak Diab , **Jonathan Lessick, Shimon Reisner, Doron Aronson, Salim Dabbah, Yoram Agmon**. Echocardiography-based Spectrum of Severe tricuspid Regurgitation. 2007; 4: 405-408

Ormniston JA, Shah PM, Tei C, Wong M. Size and motion of mitral valve annulus in man. A two dimensional echocardiographic method and findings in normal subjects. Circul 1981; 64: 113-120

Peter S. Rahko. Prevalence of Regurgitant Murmurs in Patients with Valvular Regurgitation Detected by Doppler Echocardiography. Ann Intern Med 1989; 111: 466-472

Pomerance A. Ageing changes in human heart valves. Br Heart J 1967; 29: 222-231

Puff A. Functionelle Anatomie des Herzens. In: Borst HG, Klinner W, Senning A eds. Herz und herznahe Gefasse, Springer Verlag, Berlin Heidelberg, New York 1978 pp. 35–38.

Puff A. Uber das functionelle Verhalten des Annulus Fibrosus bei der Volumenandergung des Herz hohlen und die Konsequenzen fur einen Klappeneresantz.Thoraxchirurgie 1972; 20: 185–198.

Racker DK, Ursell PC, Hoffman BF Anatomy of the tricuspid annulus. Circumferential myofibers, the structural basis for atrial flutter in a canine model. Circul 1991; 84: 841–851.

Roberts W.C. Morphologic features of the normal and abnormal mitral valve. Am J Cardiol 1983; 51:1005.

Sairanen H, Louhimo I. Dimensions of the heart and great vessels in normal children. A post-mortem study of cardiac ventricles, valves and great vessels. Scand J Thorac Cardiovasc Surg 1992; 26: 83–92.

Silver MD, Lam JHC, Ranganathan N, Wigle ED. Morphology of the human tricuspid valve. Circul 1971; 43: 333–348.

Singh B, Mohan JC. Atrioventricular valve orifice areas in normal subjects: determination by cross- sectional and Doppler echocardiography. Intl J Cardiol 1994 44: 85-91

Skwarek, J. Hreczecha, M. Dudziak, J. Jerzemowski, M. Szpinda, M. Grzybiak. Morphometric features of the right atrioventricular orifice in adult human hearts. Folia Morphol 2008; 1: 53–57

Williams PL, Banister LH, Berry MM, Collins P, Dyson M, Dussek JE, Fergusson MWJ. Gray's Anatomy. London. Churchill Livingstone. 38th edition 1995

Yacoub, M. H, Cohn, L. H. Novel approaches to cardiac valve repair. From structure to function. Circul 2004; 109: 942–950.

APPENDIX 1: DATA SHEET

GROSS MORPHOMETRY

Sex: M: ☐ F: ☐

Age group: ≤21 ☐ 22- 45 ☐ 46- 70 ☐

Heart weight: --------- g External length: --------- cm

- Tricuspid Annulus circumference and area: -------------cm; ....cm^2
- Mitral Annulus circumference and area: ------------- cm; ....cm^2

HISTOMORPHOLOGY

Sex: M: ☐ F: ☐

Age group: ≤21: ☐ 22- 45: ☐ 46- 70: ☐

Reference annulus at microscopy	Collagen fibre org	Elastic fibre org	Myocardial org
Tricuspid/ Mitral			
1. Central zone 2. Annulo- myocardial zone 3. Annulo- valvular zone			

Appendix 2: Consent form

Aim of the study

The annulus is part of heart skeleton playing an important role in supporting heart valves. Natural and artificial valve failures have been attributed to internal problems in the annulus and females are more affected. Structure of the annulus in relation to these problems remains largely unexplored. This study attempts to describe possible risk factors in the annulus that may help understand the incidence of these complications and plan for them.

Benefits

This study may be useful in understanding the problem mechanism affecting normal and artificial heart valves leading to their failures and reoperations.

Humble request

In order to carry out this study, we will need specimens during autopsy. If you agree for your next of kin to participate, measurements will be taken with the organ intact and only small blocks will be extracted for histology. There will be no mutilation or whole organ extraction. The tissue blocks will then be buried at Lang'ata cemetery.

Confidentiality

The name of the deceased will remain confidential and no information regarding him/ her will appear on either the data sheets or the final thesis.

I the undersigned have been explained to and understood the above and willingly accept to let the deceased participate in the study.

Signature of the researcher .... Signature of guardian ....

Appendix 3: Cheti cha ruhusa

Lengo la uchunguzi

Kiungo kidogo cha moyo katika uchunguzi huu hupatikana ndani ya moyo. Umuhimu wa kiungo hiki ni katika kudhibiti kiungo kinacho elekeza damu moyoni. Athari ya udhibiti wa kiungo hiki husababisha maafa abayo ni vigumu kuyamiliki. Maumbile ya kiungo hiki hayajulikani kwa kina.

Manufaa

Ujuzi wa maumbile ya kiungo hiki utachangia pakubwa katika kueleweka kwa maafa ya moyo yanayokiathiri. Isitoshe, uchunguzi huu utasaidia kuongeza ujuzi wa matibabu.

Maombi

Hili ni ombi la ruhusa ya kutumia kiungo kidogo cha moyo katika mwili wa marehemu ili kufanya utafiti huu. Mtafiti mkuu atakupa maelezo zaidi kuhusu uchunguzi huu. Tafadhali fanya uamuzi kuhusu utumizi wa kiungo hiki katika mwili wa marehemu. Iwapo una swali uliza mtafiti.

Kumwachia marehemu kutumika katika uchunguzi huu si lazima na hauna gharama yoyote.

Iwapo utakubali kutumika kwa kiungo hiki katika mwili wa marehemu:

- Hakuna kiungo kizima kitakachotolewa
- Vipimo vya kiungo hiki vitafanywa na vipande vidogo kuchukuliwa kwa uchunguzi kutumia darubini.
- Vipande vikibaki vitazikwa katika makaburi ya lang'ata.

Sahihi ya Mtafiti .... Sahihi ya mwenye mwili............

Appendix 4: Ethical approval letter

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd.
P.O. Box 20723, Nairobi.
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi.
Email: KNHplan@Ken.Healthnet.org

Ref: KNH-ERC/ UA/173

8th April 2010

El-busaidy Hemed Mohamed
Third year Medical Student
Dept. of Human Anatomy
University of Nairobi

Dear Mohamed

Research proposal: Clearance "Age and Sex variations in the structure of Atrioventricular Annuli" (UP49/2/2010)

Your above proposal refers.

This is to inform you that permission has been granted by the KNH/UON-Ethics & Research Committee to carry out research on "Age and sex variations in the structure of Atrioventricular Annuli".

By a copy of this letter, I am requesting the relevant persons to accord you the professional support and other materials that may be useful to your research.

Yours faithfully,

PROF A N GUANTAI
SECRETARY. KNH/UON-ERC

c.c. Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The HOD, Records, KNH
Supervisors: Dr. Paul Odula, Dept. of Human Anatomy, UON
Dr. Hassan Said, Dept. of Human Anatomy, UON