

Sex Variation in Occurrence of Myocardium in Human Mitral Valve Cusps

Variación del Sexo en la Aparición del Miocardio en Cúspides de la Válvula Mitral Humana

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SUMMARY: Mitral valve cusps consist mainly of connective tissue and myocardium. Connective tissue fibres within the cusps have been demonstrated to exhibit sex variations in organisation. Mitral prolapse, a disease attributed to defects within the cusps occurs more commonly in females. Sex variations in valvular myocardium however remain to be studied. Possible variations in its organisation may enhance understanding of sex differences in prevalence of mitral prolapse. The aim of this study was to determine anatomical variations of mitral valvular myocardium by sex; by mean a comparative cross-sectional study. A total of 48 (27 male and 21 female) adult heart specimens obtained during autopsy at Nairobi City and Chiromo mortuaries after ethical approval were used. Valvular cusps were then harvested. Four – millimetre sections were made at the area of maximum width for both anterior and posterior cusps. These were processed for paraffin-embedding and sectioning and stained with Masson's trichrome and Weigert's resorcin fuchsin with Van Gieson counterstain to demonstrate cardiac muscle, collagen and elastic fibres. Both anterior and posterior cusps demonstrated three histological laminae, that is, atrialis, fibrosa and ventricularis. Only lamina atrialis contained cardiac muscle. This lamina in males was characterised by a transversely oriented subendothelial myocardial strip while that in females contained compact longitudinal elastic fibres but no muscle. The presence of cardiac muscle in the lamina atrialis may be relatively protective against mitral valve prolapse in males compared to females.

KEY WORDS: Mitral valve; Cusp; Structure; Variation; Sex.

INTRODUCTION

Human mitral valve cusps have a base, a middle zone, an edge and they mainly consist of connective tissue and myocardial fibres (Walmsley, 1978). Investigations on connective tissue components have demonstrated sex variation in human heart valves (Keller *et al.*, 2006). Further, mitral prolapse, a disorder with a prevalence of up to 6 – 7% in the general population (Arcari, 2004) occurs up to twelve times more commonly in females compared to males. This sex predisposition may be attributed to differences in the organisation of valvular myocardium.

There are no previous studies on possible gender variation in organisation of valvular myocardium. This study therefore aimed at investigating possible anatomical variations based on gender in the microscopic organisation of mitral valvular myocardium.

MATERIAL AND METHOD

Forty-eight specimens (27 male and 21 female, age range 25 – 45 years) were used for histology. Hearts from male and female subjects were examined separately. From each specimen, the anterior and posterior mitral valve cusps were excised en bloc from the mitral annulus with the myocardium at the base of the cusps still attached. This was in order to facilitate examination of the transition between the atrioventricular wall of the heart and base of the cusps. Four millimetre sections centred on the area of maximum width on each cusp were obtained (Fig. 1).

Valve cusp tissue sections were fixed for a period of 72 hours by immersion in 10% formal saline. Dehydration was done in ethyl alcohol of increasing concentrations, starting with 70% alcohol to absolute alcohol, each change lasting one hour.

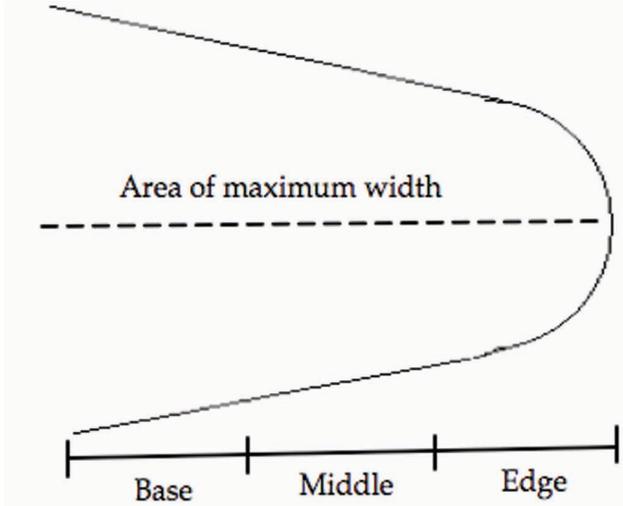


Fig. 1. Showing area of maximum cusp width, base, middle and edge zones of a mitral valve cusp.

Clearing was done with toluene (S.G. 0.866) for two hours followed by wax impregnation for 12 hours at 58°C. Embedding was done in fresh molten paraffin wax (Paraplast®, McCormick Scientific LLC, USA) using metal moulds. These were then fixed on wooden blocks to facilitate cutting on a sledge microtome. A Leitz Wezlar® microtome was used for this purpose to produce 7 – micron

thick sections. These sections were floated in warm water at 45°C to enhance spreading upon which they were fixed on slides. For 12 hours, the slides were left to dry in an oven at 38 °C.

Dewaxing was done using xylene, and the segments rehydrated using xylol, descending concentrations of alcohol, from absolute up to 70%, and then running water. Masson's trichrome stain was used to study the myocardial organisation of the mitral cusp and Weigert's resorcin fuchsin with van Gieson counterstaining used to demonstrate elastic fibres (Drury *et al.*, 1967). Observation was done using a bright-field light microscope (Leica® model BME, Germany) at magnifications x40, x100 and x400. Transverse sections were also done by adjusting orientation of tissue blocks was adjusted accordingly, and processing for light microscopy repeated.

RESULTS

The mitral valve cusps in both gender comprised a connective tissue core covered by endothelial linings. The core consisted of three laminae namely atrialis, fibrosa and ventricularis (Fig. 2).

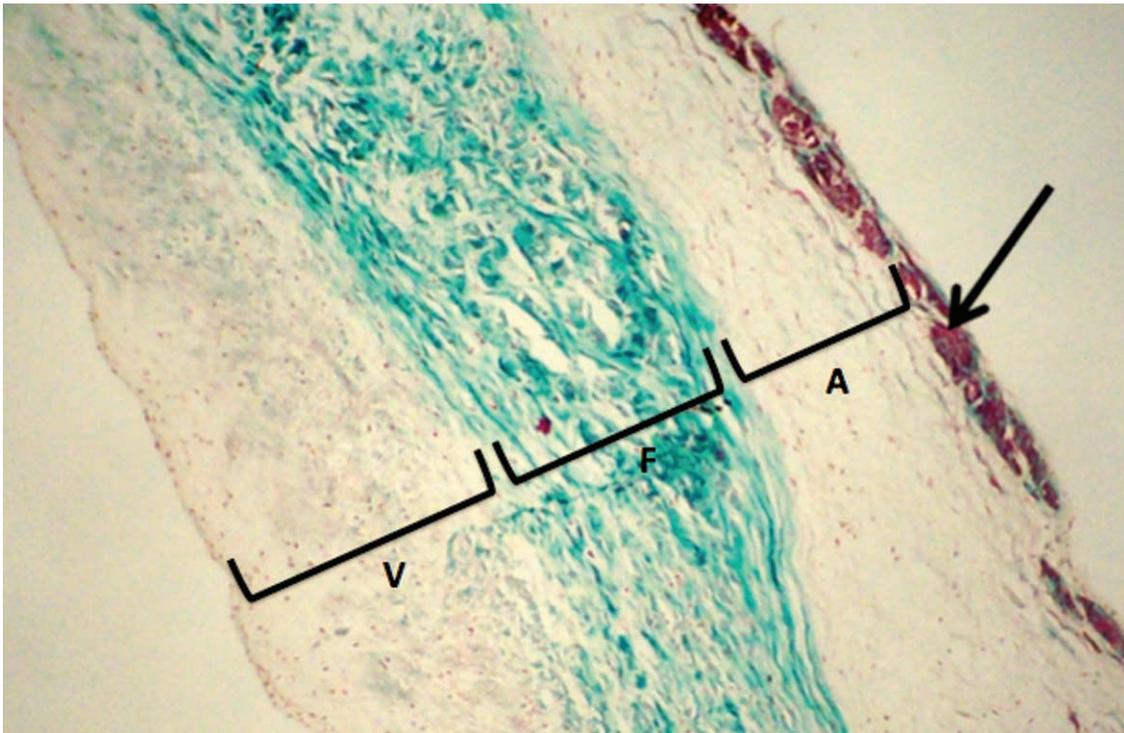


Fig. 2. Longitudinal section of a cusp from a male subject showing the general layout of laminae ventricularis (V), fibrosa (F) and atrialis (A) using Mason Trichrome stain (X100). Note the arrow pointing on a strip of myocardium.

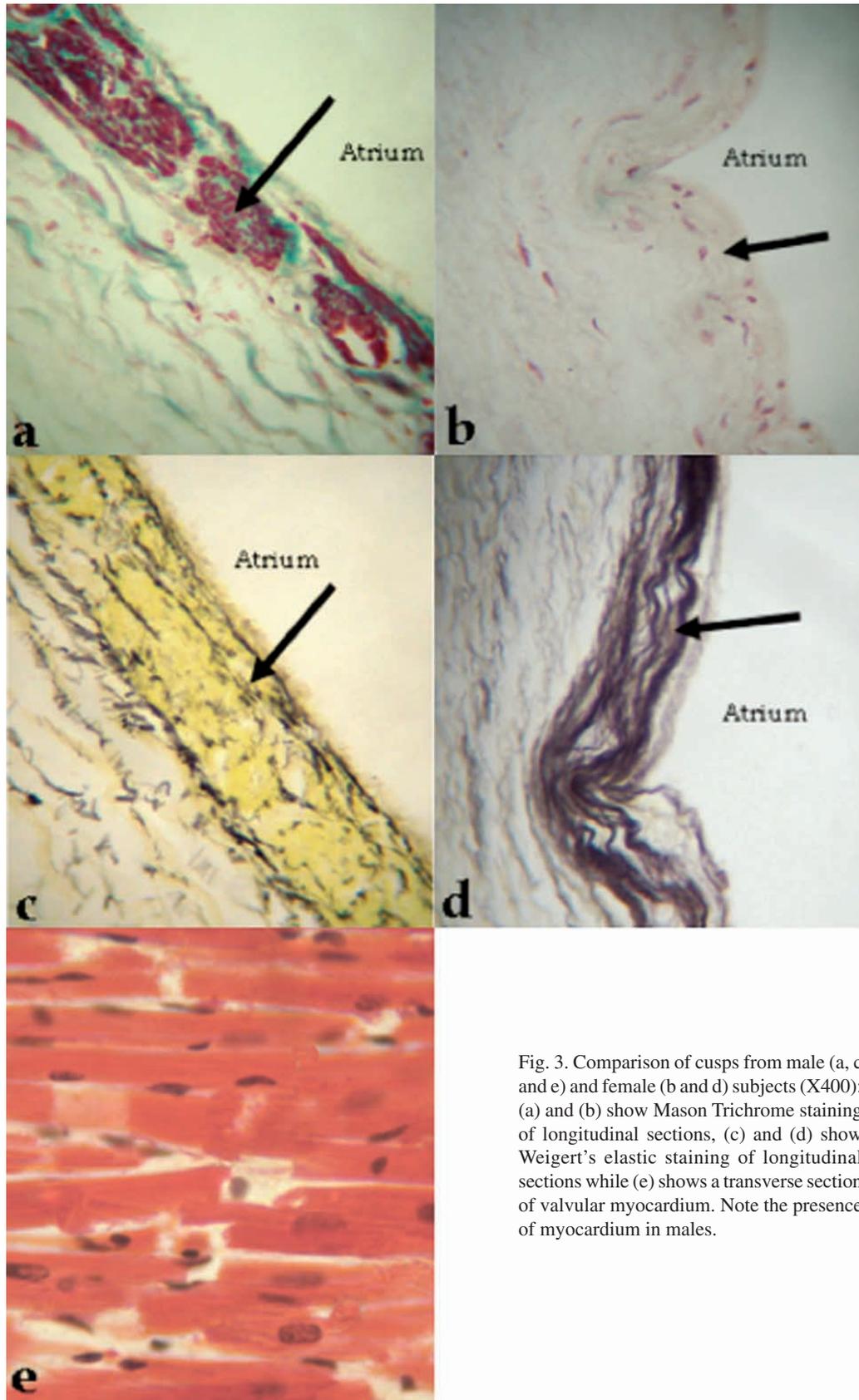


Fig. 3. Comparison of cusps from male (a, c and e) and female (b and d) subjects (X400): (a) and (b) show Mason Trichrome staining of longitudinal sections, (c) and (d) show Weigert's elastic staining of longitudinal sections while (e) shows a transverse section of valvular myocardium. Note the presence of myocardium in males.

A layer of transversely oriented cardiac muscle fibres forming a subendothelial zone observed in males (Fig. 3a) was interposed between longitudinal elastic fibre layers both on its outer and inner aspects (Fig. 3c). Towards the edge of the valve, myocardial fibres decreased as elastic fibres increased (Fig. 3a). These elastic fibres at first assumed a

haphazard orientation but further along the length of the cusp, they were organised into distinct lamellae (Fig. 4b).

Comparatively, in females, the subendothelium contained longitudinally oriented elastic fibres (Figs. 3a and 3b). There was no muscle present in this layer.

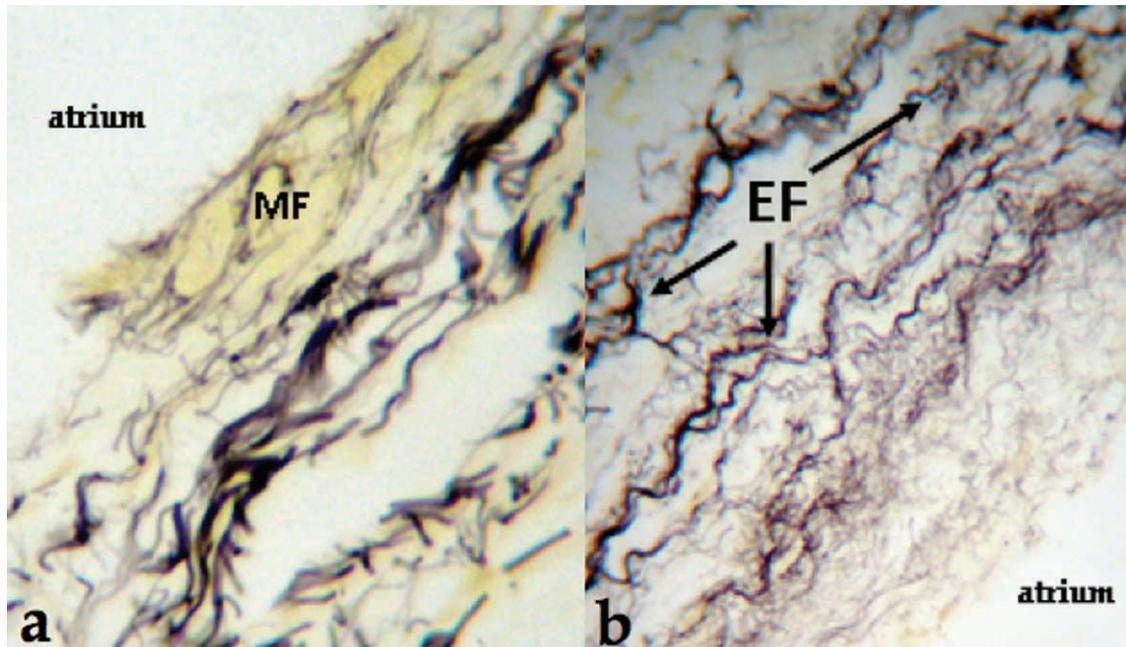


Fig. 4. Longitudinal sections of cusps from males with (a) showing decreased myocardial fibres in the middle zone (Weigert's elastic stain, X400) and (b) showing elastic fibre lamellae and absence of muscle at the edge (Weigert's elastic stain, X400).

DISCUSSION

The results of this study have elaborated gender variation in valvular myocardium of males and females in a sample of Kenyans. These results have provided, probably for the first time, data on the adaptive capabilities of the valves to hemodynamic stress in the different genders, and a possible morphological basis for the patterns of pathology affecting them. Previous comparative studies on these valves have only described interspecies variations with little mention of gender considerations (Walmsley; Ozbag *et al.*, 2005; Degandt *et al.*, 2007).

In the current study, presence of myocardium in the atrial subendothelium of both anterior and posterior cusps, extending from the base to the middle zone was observed in males. The subendothelium in females was devoid of muscle. Instead, it was composed of characteristically dense

longitudinal elastic fibres. This finding is unique when compared to the findings of other workers. Montiel (1970) reported presence of cardiac muscle in the anterior cusp. Muresian *et al.* (2006) described myocardium in the mitral valve as being found only in the posterior cusp. The current findings therefore provide evidence of presence of cardiac muscle in both anterior and posterior cusps of males. None of these earlier studies grouped their specimens into gender, and therefore it is difficult to ascertain whether there was any pattern in terms of gender variability. Comparative animal studies demonstrate the presence of myocardium within the proximal portion of cusps in atrioventricular valves of mammals (De Biasi, 1984). Clearly, a structure previously reported as being merely an endothelial-lined fibrous structure (Harisios & Wooley, 2000) is widely endowed with muscle. What then is the function of the valvular myocardium?

Valves of the heart were for a long time considered passive structures, moving only in response to hemodynamic forces generated by cardiac contractions (Guyton & Hall, 2000). Current thoughts indicate that the mitral valve is capable of independent contraction during stages of the cardiac cycle and may therefore be capable of compensatory adjustments that influence timing and effectiveness of valve closure (Williams & Jew, 2004). Myocardium within the proximal portion of valvular cusps has been functionally ascribed to control of cusp movement (De Biasi *et al.*). These myocytes may play a role in valvular function, either in supporting the cusp or being actively involved in contraction and relaxation of the valve during the cardiac cycle. Cardiac muscle in the atrial subendothelium may therefore play a supportive role in maintaining the integrity of the valve. Contraction of this muscle may aid in retraction and hence closure of the valve following ventricular systole. This cardiac muscle is anchored by elastic fibres on both the inner and outer aspects, in a manner to suggest functional interaction. Similar organisation between muscle and elastic fibres is seen in lamellar units of elastic arteries (Clark & Glagov, 1985). These are functional units conferring elastic properties to these vessels and important in functional integrity. On this basis therefore, this feature may be important in conferring mechanical strength to mitral valves.

Further significance of myocardium in mitral cusps

was raised by Wit *et al.* (1979). These workers showed that action potentials of normal human valve muscle also resemble atrioventricular nodal potentials. They postulated that the human mitral valve can then initiate both automatic and triggered impulses in the presence of catecholamines. This property may clinically cause dysrhythmias. The atrial subendothelium in males may therefore be a possible origin of dysrhythmias. Comparatively, in females, this possibility has no basis in the current study.

In conclusion, presence of valvular myocardium shows remarkable gender variation. Hence, disparity in mitral valvular pathology may partly be attributed to this variation. These findings may be further improved by characterizing cellular components by electron microscopic and histochemical techniques. Other studies on difference populations may elaborate any possible ethnic variations in the distribution on mitral valvular myocardium. To the same extent, comparative studies on the other heart valves may enhance understanding of this valvular component.

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RESUMEN: Las cúspides de la valva mitral están constituidas principalmente por tejido conectivo y miocardio. Se ha demostrado que las fibras del tejido conectivo de las cúspides presentan diferencias en la organización según sexo. El prolapso mitral, una enfermedad atribuida a defectos en las cúspides es más frecuente en las mujeres. Las variaciones de sexo en el miocardio valvular sin embargo aún no se han estudiado. Las posibles variaciones en su organización puede mejorar la comprensión de las diferencias de sexo en la prevalencia de prolapso mitral. El objetivo de este estudio fue determinar las variaciones anatómicas del miocardio valvar mitral por sexo, por medio un estudio comparativo de corte transversal. Un total de 48 los especímenes de corazón adulto (se utilizaron 27 hombres y 21 mujeres), obtenidos durante autopsias en la ciudad de Nairobi y desde el depósito de cadáveres Chiromo, después de la aprobación ética. Las cúspides valvares fueron extraídas. Fueron realizadas secciones de cuatro milímetros en el parte más ancha de las cúspides anteriores y posteriores. Estas fueron procesadas por inclusión en parafina, corte y tinción con tricómico de Masson y fucsina-resorcina de Weigert con contraste Van de Gieson, para demostrar la presencia de músculo cardíaco, fibras colágenas y elásticas. Tanto cúspides anteriores como posteriores demostraron tres láminas histológicas, es decir, atrial, fibrosa y ventricular. Sólo en la lámina atrial se observó músculo cardíaco. Esta lámina en los hombres se caracterizó por una banda de orientación transversa de miocardio subendotelial, mientras que en las mujeres se observaron fibras longitudinales elásticas compactas, pero no músculo. La presencia de músculo cardíaco en la lámina atrial puede servir relativamente como protección contra el prolapso de la válvula mitral en los varones en comparación con las mujeres.

PALABRAS CLAVE: Valva mitral; Cúspide; Estructura; Variación; Género.

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