SEX DIFFERENCES IN STRUCTURE OF THE PELVIC URETER

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A dissertation in partial fulfillment for the intercalated degree of Bachelor of Science in Anatomy of the University of Nairobi
Declaration

This dissertation is my original work and has not been presented for a degree in any other university.

Sign _______________________ Date 5th/10/2010

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Dedication

For Ma, Sharon and Oge, the three pillars around my heart.
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Summary

Background: Structure of the pelvic ureter plays a key role in the mechanism for prevention of vesico-ureteric reflux. Alterations of morphometric and histomorphological features such as length, obliquity and orientation of muscle fibers have been implicated in etiology of reflux. The oblique course and length of the intra-vesical ureter make up the passive mechanism while contraction of the distal longitudinal muscle layer contributes to the active component. A length: luminal diameter ratio of 5:1 is considered adequate to prevent reflux. Vesico-ureteric reflux is common in females than in males. The basis for this difference has not been elucidated. Anatomical sex differences in the pelvic ureter may partially explain the female preponderance to vesico-ureteric reflux. There is, however, scarcity of data on these differences in the organization of the pelvic ureter.

Objective: To determine sex differences in the structure of the pelvic ureter

Study design: A descriptive cross-sectional study.

Materials and Methods: A total of 100 ureters from adults (54 male and 46 female) at the Department of Human Anatomy University of Nairobi were studied. Eighty eight (48 male and 40 female) of these were cadaveric. These were used for morphometry. Twelve (6 male and 6 female), harvested during autopsy were used for histomorphology. Harvesting was performed through a midline anterior abdominal wall incision and cranial reflection of abdominal viscera. Length and angle at which intra-vesical ureter lies to the bladder was measured in millimeters and degrees respectively. Five millimeter sections were taken from juxta-vesical, mid and distal intra-vesical segments. These were fixed in formal saline solution and processed routinely for paraffin embedding. Seven micrometer sections were stained with Masson’s Trichrome to demonstrate orientation of smooth muscle cells and collagen fibers.
Morphometric data were analyzed using SPSS (Version 16.0, Chicago Illinois) for means and standard deviations. Sex differences in morphometry were determined using the Student’s t test. A value of $p \leq 0.05$ was considered statistically significant. Pearson’s correlation test was used to test for correlation between length and angle. A Two-tailed test was used to test for significance of the correlation co-efficient. A $p$-value < 0.01 was considered significant.

**Results:** The mean intra-vesical length of pelvic ureter in males was 18.69 mm compared to 14.81 mm in females ($p$-value of <0.000). The angle at which ureters lay to the bladder was 26.75° in males and in females 29.10° ($p$-value of 0.018). In all sections, the ureter displayed a mucosa, muscularis and adventitia. In males, the muscularis of juxta-vesical ureter displayed three layers namely an inner longitudinal, intermediate circular and an outer longitudinal. In females on the other hand, two layers namely an inner longitudinal and outer circular were observed. In the mid intra-vesical segment, the muscularis in males displayed two layers: an inner longitudinal and outer circular while in females only the longitudinal layer was present.

**Conclusion:** The pelvic ureter displays sex differences in structure. Its intra-vesical segment is longer and enters the bladder at a more acute angle in males than in females. In the males, the muscularis in the juxta-vesical and mid intra-vesical possess an additional longitudinal and circular layer respectively. These structural differences probably underlie the higher female: male predisposition to vesico-ureteric reflux.
Introduction

The pelvic ureter is that part which extends from the pelvic brim to the urinary bladder. It consists of a juxta and intra-vesical segments (Williams et. al, 2004). Intra-vesical segment has an oblique course through the bladder wall and measures 10-19 mm long in adults (Hutch, 1962). Ureteric wall consists of a mucosa, muscularis and the adventitia. The mucosal layer comprises a transitional epithelium and connective tissue lamina propria whose fibers are orientated parallel to the long axis of ureter (Williams et. al, 2004). The muscularis consists of an inner longitudinal layer, intermediate circular layer and outer longitudinal smooth muscle layers (Skandalakis’ et. al, 2004). Distally, the two outer smooth muscle layers and the adventitia anchor the ureter to bladder wall, forming the so called Waldeyer’s sheath (Cendron, 2009). The inner layer is continuous with the bladder wall (Thomson et. al, 1994) to anchor ureter at the trigone forming the superficial muscle (Roshani et. al, 1996). The adventitia, a fibrous coat composed of areolar and fibro elastic connective tissue, is continuous distally with fibrous structure of the bladder (William, et. al, 2004).

The intra-vesical part constitutes an important mechanism for prevention of vesico-ureteric reflux (VUR) (King et. al, 1974). This mechanism consists of passive and active components (Radmayr et. al, 2005). Oblique course of ureter and its length through the bladder constitutes the passive component (Thomson et. al, 1994), through valvular mechanism at the vesico-ureteric junction (VUJ) (Radmayr et. al, 2005). Studies have also reported that the sub mucosal length to luminal diameter ratio of 5:1 is adequate to prevent reflux during corrective surgery of refluxing ureters (Chiari et. al, 1997; Barrieras et. al, 1999). Refluxing ureters have therefore been shown to have a shorter intra-vesical length (Carr et. al, 1992), larger luminal diameters that are more laterally placed
(Cendron, 2009) and a less oblique intra-vesical course (Noble et. al, 1992). The Waldeyer’s sheath has also been implicated in prevention of reflux (Wood, 1953).

The active component on the other hand comprises the predominantly longitudinal muscle in intra-vesical part (Thomson et. al, 1994) which forms additional anti-reflux mechanism by acting as a valve at ostium of internal ureteric orifice (Roshani et. al, 1996; Shafik, 1997). In addition to the longitudinal muscle layer, a pre-vesical circular sphincter formed from the most distal circular ureteric muscle fibers has been demonstrated in an isolated study (Gil-Vernet, 1973). Thus refluxing ureters show a reduction (Douglas, 1964) with derangement in musculature and deprivation of innervation to the intra-mural segment of the pelvic ureter (Radmayer et. al, 2010).

Vesico-ureteric reflux, a condition five times more common in females than in males (El – Khatib et. al, 1990, Buckley et. al, 2008) has a high incidence of urinary tract infections and other sequelae including arterial hypertension and renal calculi (Kohler et. al, 1997). Although morphometric and histomorphological features of the pelvic ureter constitute important factors in the prevention of this condition, (Thomson et. al, 1994), sex differences in these have not been described. A histological study of the pelvic ureter would therefore provide a basis for comprehension of vesico-ureteric anti-reflux mechanisms. Further, comparison in structure of the pelvic ureter may explain the sex difference in incidence of reflux.
Broad Objective

To describe sex differences in the structure of the pelvic ureter

Specific Objectives

To determine in the pelvic ureter of males and females:

1. Length of the intra-vesical segment
2. Angle at which the intra-vesical ureter lies to the bladder
3. Regional differences in the histomorphology
Subjects and Methods

This study was carried out at the Department of Human Anatomy, University of Nairobi. Data collection was done between the months of March to May 2010. This study was done on 100 pelvic ureters (58 males and 46 females). These were obtained from black adult Kenyans aged 30 – 50 years. Subjects with any visible anomalies of the ureter including trauma, females with gravid uterus, previous urinary or ureteric surgery and autopsy carried out more than 48 hours post mortem were excluded from the study. Approval to carry out the study was granted by the Kenyatta National Hospital and University of Nairobi Ethics and Review Committee (KNH – UoN ERC) with a written consent for use of autopsy specimen being obtained from relatives of the deceased. For histomorphology, the pelvic ureter was obtained from 12 subjects (6 males, 6 females). These were collected from Chiromo Funeral Parlor during autopsies.

Eighty eight (48 males and 40 females) cadaveric hemi pelvis prosections were used to obtain the pelvic ureter for measurements of length and angle. Prosections with any form of urinary bladder abnormalities such as bladder wall trabeculation and nodulation or ureteric pathology were excluded from the study.
Methods

Exposure of urinary bladder

A midline skin incision from the xiphi-sternum to the pubis symphysis through the skin and the linea alba. The skin and anterior abdominal wall muscles were then retracted laterally to expose abdominal organs. The viscera were mobilized and reflected cranially to expose the peritoneum covering the posterior abdominal wall. The peritoneum was dissected away to expose the ureter in its entire length within the pelvic cavity.

The ureter was tied using a string 2 cm superior to the pelvic brim, cut above the tie and dissected out distally to its entry into the bladder. The ureters and the bladder were harvested en masse.

Tissue sampling

A horizontal incision was made on the anterior surface of the urinary bladder to expose the trigone.

A circular incision was made on the posterior wall of the urinary bladder to section out the ureters at the trigone. The course of the intra-vesical ureter was then dissected out from the bladder wall and three 5 mm sections of the ureter were taken at the juxta-vesical, mid and distal intra-vesical portions. These sections were then processed for light microscopy.

Processing for Light Microscopy

The segments harvested were fixed in 10% formal saline solution by immersion followed by dehydration through increasing concentrations of alcohol (starting from 70% to 100%) for a period of one hour each. Subsequent clearing was done in 50: 50 absolute alcohol: toluene solution for one hour followed by immersion in two washing of toluene for fifteen minutes each. Infiltration was done using paraffin wax (Paraplast®, McCormick Scientific LLC, USA) followed by embedding.
Seven micrometer thick serial sections were cut using a Lezlar® microtome (SM2400), Germany). These sections were floated in a water bath at 55°c, picked on slides smeared with egg albumin and left for drying in an oven overnight at a temperature of 38°c after which they were stained using Masson’s Trichrome to demonstrate the collagen fibers and orientation of muscle fibers (Drury et al. 1967). Slides were examined using a Leica® light microscope (BME model, Germany).

Photography

Micrographs of representative slides showing the organization of smooth muscle cells and collagen fibers in the pelvic ureter were taken using a Fuji® digital Camera (Finepix A900, 9 megapixels). These were uploaded into a personal computer and edited using Macromedia® Fireworks MX®. Descriptions and comparisons of micrographs were done for sex and regions of the pelvic ureter.

Determination of intra-vesical ureter length and angle Q

Measurements of intra-vesical length and angle Q were taken by two observers and averages obtained for the two sets of values. For intra-vesical length, a probe was inserted through the internal ureteric orifice and length of the intra-vesical ureter was marked and measured using a rule to the nearest 0.5 mm. The angle at which the ureter lies to the bladder (Q) was measured using a protractor as an angle subtended between a horizontal plane through the inter – ureteric ridge and a diagonal axis passing through the intra – vesical ureter (Figure 1).
Figure 1: Schematic diagram showing a hemi-section of the posterior bladder wall at the trigone. The angle Q was measured in between the horizontal (←→) and diagonal (↕) axes subtended for intra-vesical ureter (IV)
Data Analysis and Presentation

Observations and measurements were coded, tabulated and analyzed using SPSS version 16.0. for means ± standard deviations. Student’s T test, at 95% confidence interval was used to test for significant differences in the intra-vesical ureter length and the angle at which the intra-vesical ureter lies to the bladder with regards to sex. A p – value of < 0.05 was considered significant. Pearson’s correlation test was used to test for association between mean length and mean angle. A Two-tailed test was used to test for significance of the correlation co-efficient. A p – value < 0.01 was considered significant.

Tables and boxplots were used to present these measurements.
Results

One hundred and twenty two ureters from sixty one individuals were available for this study at the Department of Human Anatomy. Twenty two were excluded from the study due to difficulty in identification of internal ureteric orifice (4) and obvious pathologies of the ureters and bladder (8). One hundred were therefore studied. The pelvic ureters, in all cases, were bilateral muscular tubes extending from the pelvic brim to the urinary bladder wall. The ureters coursed within the pelvic cavity to pierce the posterior wall of the urinary bladder and traverse the bladder wall to terminate at the internal ureteric orifice. The ureters varied in morphometry and morphology depending on region and sex.

Sex differences in length of intra-vesical ureter

The mean length of the intra-vesical ureter was 16.99 mm (standard deviation 3.459, range 9 mm – 25 mm.) It was longer in males with a mean value of 18.81 mm (standard deviation of 3.266, range 9 mm – 25 mm.) compared to 14.80 mm (standard deviation of 2.198 and range 10 mm – 19 mm) in females (Fig. 2). This difference in length between the sexes was statistically significant (p-value < 0.001) (Table 1).

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Range</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>length of intra-vesical ureter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>18.81</td>
<td>3.266</td>
<td>9 – 25 mm</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Female</td>
<td>40</td>
<td>14.80</td>
<td>2.198</td>
<td>10 – 19 mm</td>
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Figure 2: Box plot of sex dimorphism in length of intra-vesical ureter

Angle of the ureter to the bladder (Q)

The ureter entered the bladder at a mean angle of 27.59° (standard deviation of 4.770°, range 20° - 40°). In males, this angle was narrower with a mean of 26.50° (standard deviation of 5.251°, range 20° - 40°) in comparison to females mean 28.90° (standard deviation of 3.781°, range 20° - 39°) (Fig. 3). The difference in angle Q between sexes was statistically significant (p-value 0.018). (Table 2)
Table 2 Angle at which the ureter entered the bladder (Q)

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
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<td>40</td>
<td>28.90°</td>
<td>3.781</td>
<td>20 – 39°</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3: Box plot of sex dimorphism in angle at which the ureter lies to the bladder
Pearson's correlation test was applied to test for correlation between mean length and angle and a value of -0.333. The strength of this correlation was considered significant (p-value 0.002). (Fig 2)

**Figure 4: Scatter gram of length of intra-vesical ureter against the angle of ureter to bladder**
Histomorphology

The wall of pelvic ureter comprised three layers namely mucosa, muscularis and adventitia (Fig. 5a). Its mucosa was made of transitional epithelium with predominantly cuboidal cells and an underlying lamina propria. The lamina propria consisted of dense irregular connective tissue fibers with cell aggregates (Fig. 5b). The smooth muscle cells in the muscularis layer on the other hand displayed an inner longitudinal and outer circular smooth muscle (Fig. 5c). Individual muscle layers were set in bundles by connective tissue septae extending from the lamina propria. These layers were continuous circumferentially around the lumen of the ureter. Within the muscularis, blood vessels with their associated nerve fiber bundles were observed in the connective tissue (Fig. 5d). The adventitial layer consisted of loose connective tissue with adipocytes. Blood vessels were interspersed in this layer with surrounding venules and nerve fiber bundles (Fig. 5e).
Legend

Figure 5A - E: Micrographs of transverse sections through different segments of the ureter:

A: Juxta-vesical segment of the pelvic ureter in a female showing general organization with its layers: Mucosa (Mu), muscularis (Msc) (Masson’s Trichrome stain; magnification x40)

B: Juxta-vesical segment of the pelvic ureter in a female showing mucosal organization: transitional epithelium (Ep) and an underlying lamina propria (Lp) with cellular aggregates (Masson’s Trichrome stain; magnification x400)

C: Mid intra-vesical segment of the pelvic ureter in a male showing orientation of muscular bundles by connective tissue septae from lamina propria: an inner longitudinal (Lm) and circular (Cm) muscle layers are demonstrated. Lu shows the side of the lumen (Masson’s Trichrome stain; magnification x40)

D: Juxta-vesical segment of the pelvic ureter in a male showing a blood vessel (Bv) in association with nerve fibers (arrowhead) in muscularis (Msc) (Masson’s Trichrome stain; magnification x40)

E: Juxta-vesical segment of the pelvic ureter in a male showing organization of the adventitia of a blood vessel (Bv), and associated nerve fibers (Nf) (Masson’s Trichrome stain; magnification x40)
Figure 5

(A) Mu
Lumen
Msc

(B) Ep
Lp
Ca

(C) Cm
Lm
Lu

(D) Msc

(E) Nf
Bv
Bv
Regional Differences

Regional differences observed within the juxta-vesical and intra-vesical portions of the pelvic ureter were predominantly in the organization of layers within the muscularis.

In the juxta-vesical segment, the muscular coat appeared to be set into three interconnecting layers: an inner longitudinal, intermediate circular and outer longitudinal with some fibers obliquely oriented. The muscle fibers were more compact towards the lumen (Fig. 6a).

A distinct connective tissue layer separates the ureteric muscular coat from the Waldeyer’s sheath. This layer has blood vessels and associated nerve fiber bundles (Fig. 6d).

An additional muscle layer, forming the Waldeyer’s sheath, is separated into bundles by connective tissue septae. The muscle bundles are predominantly longitudinal. Other muscle bundles have an oblique and circular orientation. In some areas, circumferential orientation of muscle fibers was interrupted by connective tissue as shown in the (Fig. 6d). Nerve fiber bundles were interspersed within this sheath. This sheath was also observed in the mid and distal intra-vesical segment, however the muscular bundles were predominantly longitudinal and less compact as compared to the juxta-vesical portion (Fig. 6e – f)

The mid intra-vesical ureter demonstrated connective tissue septae extending from the lamina propria to the muscular. This muscular coat appeared, however, to be set into two interconnecting layers: an inner longitudinal and outer circular (Fig. 6b). Circular oriented muscle fibers however did not completely surround the ureteric lumen.

The muscularis in the distal intra-vesical was organized in bundles with connective tissue septae extending from lamina propria (Fig. 6c). These bundles had a longitudinal orientation
Figure 6A – F: Micrographs of transverse sections through different segments of pelvic ureter.

A: Juxta-vesical segment of the pelvic ureter in a male showing organization of the muscularis into three layers. From the lumen (L), inner longitudinal (Lm), intermediate circular (Cm) and outer longitudinal (Lm) (Masson’s Trichrome stain; magnification x10).

B: Mid intra-vesical segment of the pelvic ureter in a male showing orientation of muscles into layers: an inner longitudinal (Lm) and outer circular (Cm). L shows the side of the lumen (Av) the side of the adventitial layer (Masson’s Trichrome stain; magnification x40).

C: Distal intra-vesical segment of the pelvic ureter in a male showing longitudinal muscle fibers adjacent to the lumen (Lu). (Av) shows the side of the adventitial layer (Masson’s Trichrome stain; magnification x40).

D: Juxta-vesical segment of the pelvic ureter in a male showing connective tissue with blood vessels (Bv) and nerve bundle (arrowhead) separating the ureteric muscularis from the Waldeyer’s sheath (Ws) with muscle fibers oriented longitudinally (Lm) and obliquely (Om). Connective tissue separating the muscularis from the sheath (star) interrupts continuity of muscle fiber (Masson’s Trichrome stain; magnification x40).

E: Mid intra-vesical segment of the pelvic ureter in a male showing connective tissue separating the ureteric muscularis from the Waldeyer’s sheath (Ws) with muscle fibers oriented longitudinally. (Masson’s Trichrome stain; magnification x40).

F: Distal intra-vesical segment of the pelvic ureter in a male showing connective tissue separating the ureteric muscularis from the Waldeyer’s sheath (Ws) with muscle fibers oriented longitudinally. (Masson’s Trichrome stain; magnification x40).
Sex Differences

The juxta-vesical ureter in the female displayed predominantly a single longitudinal muscle layer with a connective tissue septae separating it from the Waldeyer’s sheath. The circular muscle layer separating the inner from the outer longitudinal muscle layer seen in males (Fig. 7a) was not distinct in females (Fig. 7b).

In mid intra-vesical section, the female ureter displayed predominantly single longitudinal muscle layer that was arranged in bundles by connective tissue septae from the lamina propria (Fig. 7d). In the male ureter, an additional circular muscle layer appeared external to this longitudinal muscle layer forming an incomplete ring around the ureter (Fig. 7c).

The muscularis of distal intra-vesical section in females consisted of a single layer of longitudinally arranged muscle layer organized in bundles (Fig. 7f). In contrast, the male ureter showed an additional circular muscle layer beyond the longitudinal layer (Fig. 7e).
Figures 7A – F: Micrographs of transverse sections through the pelvic ureters of males and females

A: Juxta-vesical segment of the pelvic ureter in a male showing organization of the muscles into three layers. From the lumen (L), inner longitudinal (Lm), intermediate circular (Cm) and outer longitudinal (Lm) (Masson’s Trichrome stain; magnification x100)

B: Mid intra-vesical segment of the pelvic ureter in a male showing orientation of muscles into layers: an inner longitudinal (Lm) and outer circular (Cm). L shows the side of the lumen (L) while (Av) the side of the adventitial layer (Masson’s Trichrome stain; magnification x100)

C: Distal intra-vesical segment of the pelvic ureter in a male showing orientation of muscles into a single longitudinal (Lm) layer. L shows the side of the lumen while (Av) the side of the adventitial layer (Masson’s Trichrome stain; magnification x40)

D: Juxta-vesical segment of the pelvic ureter in a female showing organization of the muscularis into a single longitudinal layer (Lm), from the lumen (L) with adventitia (Av) as the outer layer (Masson’s Trichrome stain; magnification x100)

E: Mid intra-vesical segment of the pelvic ureter in a female showing orientation of muscles into a single longitudinal (Lm) layer. L shows the side of the lumen while (Av) the side of the adventitial layer (Masson’s Trichrome stain; magnification x100)

F: Distal intra-vesical segment of the pelvic ureter in a female showing orientation of muscles into a single longitudinal (Lm) layer. L shows the side of the lumen while (Av) the side of the adventitial layer (Masson’s Trichrome stain; magnification x40)
Discussion

Morphometry and histomorphology of the pelvic ureter have been implicated in etiology of vesico-ureteric reflux (Carr et al., 1992). This condition has demonstrated disparity based on sex (El-Katib et al., 1990). Accordingly, this study proceeded to describe differences in the pelvic ureter structure that probably underlie this disparity. Indeed observations in the current study have revealed subtle sex differences in morphometry and histomorphology of the pelvic ureter between males and females.

Length of intra-vesical ureter

Length of the intra-vesical ureter constitutes part of the passive anti reflux mechanism (Roshani et al., 1996). Observations from the current study revealed a mean length of 16.90 mm. This is within the range reported by Hutch (1962). It is however lower than 23 ± 0.6 mm reported by Roshani et al. (1999). Similarity with Hutch’s findings can probably be attributed to similar methodology. On the other hand, the wide difference between findings of the current study and those of Roshani et al. (1999) may be explained by differences in nature of the specimens: fresh autopsy ureters bathed in physiological saline as opposed to formalin fixed samples used in the current study and the methodology used that is Endoluminal ultrasonography (ELUS). In males, this length averaged 18.96 mm compared to 14.81 mm in females. There are studies that have demonstrated relatively shorter intra-vesical lengths in refluxing ureters (Carr et al., 1992; Murawski et al., 2007). This could imply that a shorter intra-vesical ureter in females compared to their male counterparts probably accounts for their predisposition to VUR.
Angle at which intra-vesical ureter lies to the bladder

The mean angle at which the intra-vesical ureter lies to the bladder in current study was 27.84°. This was much higher than 11 ± 0.5° reported by Roshani et al. (1999) who calculated this angle from bladder wall thickness and intra-vesical ureteric length. A notable observation of the current study was that females had a wider angle implying that the course of the intra-vesical ureter was less angulated as compared to that of males. The obliquity of the ureter forms part of the passive anti-reflux mechanism (Roshani et al., 1996) achieved through neutralization of a tendency of the ureteral roof to separate from floor. This tendency is due to the increased surface of the wall in a bladder filled with urine (Peremans, 1966). Consequently, the wider angle is less efficient at preventing VUR (Noble et al., 1992).

A negative correlation was found between length and angle Q of the intra-vesical ureter. Therefore an increase in length would result in a decrease in angle Q. A literature report has correlated the propensity to reflux in the Pax2 1Neu+/- mouse with a shortened intra-vesical ureter that has lost its angulated entry into the bladder wall (Murawski et al., 2007). In combination with a shorter intra-vesical length, a wider angle Q can explain in part predisposition of females to VUR.
Histomorphology

Observations of the current study, similar to literature reports (Skandalakis' *et al.*, 1994; Williams *et al.*, 2004), revealed three layers in the pelvic ureter: mucosa, muscularis and adventitia. The mucosa consisted of transitional epithelium and an underlying lamina propria with dense irregular connective tissue. This organization of the lamina propria is at variance with the literature report that the fibers are regularly arranged parallel to the long axis of the ureter (Williams *et al.*, 2004). The orientation of connective tissue fibers is greatly influenced by mechanical activities around them (Viidik, 1978). Therefore irregular dense connective tissue in the lamina propria suggests that there are multidirectional forces acting on the lamina propria. Contraction of the muscularis with consequent peristaltic propulsion of urine probably contributes to multidirectional nature of these forces.

Ureteric muscle has been reported to run spirally with the longitudinal muscle having a smaller pitch than the circular muscle (Burkitt *et al.*, 1993). The muscular layer in the current study was organized in bundles of circular and longitudinal oriented smooth muscle cells along its course. Peristaltic contractions of the longitudinal muscle layer cause a passive distension and shortening of the ureter upstream and downstream of the urine bolus respectively (Osman *et al.*, 2009). This, followed by circular muscle layer contraction serve to propel and inject urine through the ureter into the bladder (Osman, *et al.*, 2009).

Adventitia was made up of loose fatty areolar tissue with blood vessels and nerve bundles being interspersed in this layer. A fibro muscular layer was observed to extend the course of the pelvic ureter between the muscularis and adventitia similar to literature reports (Peremans, 1966; Roshani *et al.*, 1996). This layer is a specialized fibrous tissue with muscle from the middle layer of bladder wall (Peremans, 1966) and extends 3 to 4cm into the juxta-vesical ureter (Osman *et al.*, 2009). The connective tissue layer between the sheath and ureteric muscular layer acts as a lubricant that
facilitates gliding of the ureter into the bladder for urine bolus expulsion playing a role in reflux prevention (Wood, 1953). This organization of connective and contractile tissue elements has been proposed to influence behavior of physiologic processes (Roshani et al., 1996) in the ureter such as prevention of reflux.

Regional Differences in Pelvic Ureter

The pelvic ureter displays gradual changes in histomorphology along its course. Juxta-vesical portion possesses an inner longitudinal, intermediate circular and outer longitudinal muscle bundles arranged circumferentially around the ureter. This three layer muscle arrangement is similar to that reported by Skandalakis et al., (1994) for the proximal ureter. Contractions of the longitudinal followed by circular muscle layer propel the bolus of urine through the ureter with subsequent injection into the bladder (Osman, et al., 2009). The intra-vesical segment on the other hand, reveals longitudinally and sparse circularly arranged muscle fibers. This is similar to studies reporting a reduction in proportion of the outer two ureteric muscle layers with continued progression towards the external ureteral hiatus in the bladder (Roshani et al., 1999; Osman, et al., 2009). These two layers and periureteral adventitia merge with the bladder wall in the upper part of the ureteral hiatus to anchor the juxta-vesical portion of the ureter to the bladder (Osman, et al., 2009).

Muscle fibers of Waldeyer's sheath in juxta-vesical ureter were arranged circumferentially around the ureter contrary to report stating that this layer only extends to the ventro-medial portion of the ureter (Roshani et al., 1996; Osman, et al., 2009). Other anatomical studies on the pelvic ureter have, however, shown complete circumferential organization of this sheath (Tanagho et al., 1963). This concurs with observations in the current study. In addition to the inner muscularis, the Waldeyer's sheath was also observed to lie adjacent to the ureter within the intra-vesical segment. Macroscopic
dissection by Tanagho et al. (1963) demonstrated this sheath as extending into the intra-vesical segment up to the middle layer of detrusor muscle referred to as the deep trigone. Osman, et. al, (2009) however described this sheath as terminating external to the bladder as an anchorage of the juxta-vesical ureter to the bladder wall. Waldeyer's sheath supports the ureter and reinforces ureteric contraction (Tanagho et al, 1968). Concentric arrangement of smooth muscle cells in this layer probably augments this function.

Sex Differences in Pelvic ureter

In the male juxta-vesical segment, an additional outer longitudinal and a prominent intermediate circular muscle layers were observed. This was in contrast to an inner longitudinal and an indistinct outer circular layer in females. The arrangement of muscularis in males is similar to that demonstrated for the proximal ureter (Skandalakis et. al, 1994). The female juxta-vesical ureter however displayed the gradual reduction in the outer two layers of muscle with observations revealing predominantly longitudinal muscle fibers. Contraction of longitudinal muscle layers will cause a consequent reduction in length of the ureter (Roshani et. al, 1996) downstream of urine bolus. This coupled with contraction of circularly oriented smooth muscle cells will propel and inject urine through the ureter (Osman et. al, 2009). It is therefore probable that the additional longitudinal muscle layer augments this function improving delivery of bolus of urine to the next segment of the ureter.

The mid intra-vesical segment in males displayed an additional circular oriented muscle layer in comparison to the sole longitudinal layer in females. This difference is largely unreported in literature. The circular oriented muscle bundles observed in this current study within the male intra-vesical ureter are likely to be a VUR preventing factor through peristaltic motion (Tsuchida and
Kimura, 1964, 1967) which combined with the longitudinal muscle fibers are likely to be additional features in prevention of VUR in this group. Circular oriented muscle fiber bundles at this section of the pelvic ureter have not been reported in literature. Studies cite that only the longitudinal muscle layer is present in the muscularis of the intra-vesical ureter (Douglas, 1964; Roshani et al. 1996). Circular muscle layers have been observed in high pressure areas such as the rectosigmoid junction which exhibit presence of a physiologic sphincter (Shafik, 1996). The bladder is one such high pressure system during micturition and presence of circular oriented muscles within the intra-vesical segment would suggest presence of a muscular sphincter.

In both males and females, the distal intra-vesical ureter consisted of a longitudinal muscle layer. This organization of the muscular layer is in tandem with most literature reports (Peremans, 1966; Tanagho et al. 1968; Roshani et al. 1996). Longitudinal muscle bundles in the intra-vesical ureter decreases length by progressive telescopic mechanism with consequent obstruction of ureteric lumen upstream of urine bolus (Roshani et al. 1996). This prevents retrograde flow of urine from the bladder back into the ureter.
Conclusion

The pelvic ureter displays sex differences in structure. Its intra-vesical segment is longer and enters the bladder at a more acute angle in males than in females. In the males, the muscularis in the juxta-vesical and mid intra-vesical possess an additional longitudinal and circular layer respectively. These histomorphologic and morphometric differences probably constitute part of underlying factors that predispose females to vesico-ureteric reflux.
Study Limitations and Suggestions for further studies

Autopsy specimens are not ideal for histological analysis as tissues may undergo autolysis leading to poor quality sections for staining. To delimit this, harvesting of tissues was done within forty eight hours after death of subjects. Tissue shrinkage that occurs in cadaveric specimen affected measurements taken. However, the shrinkage factor was the same for all specimens included as only cadaveric specimen were used in morphometry. Further, the angle at which the intra-vesical ureter lies to the bladder could be affected by the capacity of urine in the urinary bladder. A study should therefore be designed to measure these lengths and angles by imaging techniques in order to obtain more accurate results that could be applied in vivo.
References


Viidik A. On the correlation between structure and mechanical function of soft connective tissue. *Verhandlugen Anatomischen Gesellschaft*, 1978; 72: 75 - 76


Appendix 1 – Data Collection Sheet – Morphometry

Study number:

Sex: M _____ F ______

Side: R _____ L ______

Intra-vesical ureteric length (mm): ______

Angle at which ureter lies to bladder (°): ______
Appendix 2 – Data Collection Sheet – Histomorphology

Case number: _________

Sex: M _____ F ______

Age group: 18-38 _____; 39-59 _____; >59____

Side: R _____ L _____

<table>
<thead>
<tr>
<th>Extra-vesical portion:</th>
<th>No. of muscle layers and orientation</th>
<th>Collagen fiber orientation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transverse section</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix 3 — Consent Form

Study Number ____________

Aim of the Study

The study will examine the structure of the pelvic ureter

Benefits

This study will be useful in understanding the basis of why females are affected more by vesico-ureteric reflux

Confidentiality and Ethics

The incision made to access the specimens will be precise and stitched appropriately. Only the segments necessary for the study will be harvested and 5 mm sections will be used. Once the segments have been utilized they shall be buried at Lang’ata Cemetery. The deceased’s identity will be confidential and no information concerning him/her will be published.

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

Signature/ Thumbprint: _____________

(Relative to the deceased)

I, the investigator, having explained in detail the purpose of the study; hereby submit that privacy of the data recorded shall be maintained and no details will be revealed, apart from those related to the study.

Signature: ______________

Date: ______________

In case of any questions contact the researcher through: 0724694369
Appendix 4: Kibali cha Ruhusa

Nambari ya uchunguzi: ____________

Lengo la Uchunguzi

Lengo la uchunguzi huu ni kufafanua maumbile ya mrija unaoelekeza mkojo kutoka kwende figo hadi kibofuni.

Manufaa

Uchunguzi huu utachangia pakubwa katika kuelewa kiini cha maradhi ya mrija huu kati ya wanawake.

Maadili na Usiri


Nadhibitisha kwamba nimeyafahamu aliyonieleza mtafiti na nimekubali kwa hiari yangu mweneyewe marehemu asaidie katika uchunguzi huu.

Sahihi/ Kidole cha gumba:

(Jamaa ya marehemu)

Mimi mtafiti nimewaeleza jamaa ya marehemu kuhusu uchunguzi huu ipasavyo.

Sahihi ya mtafiti: ____________

Tarehe: ____________

Ikiwa kuna maswali, wasiliana na mtafiti kupitia: 0724694369
Research proposal: Clearance “Gender Differences in structure of Pelvic Ureter” (UP51/02/2010)

Your above revised proposal refers.

This is to inform you that permission has been granted by the KNH/UON-Ethics & Research Committee to carry out research on “Gender differences in structure of Pelvic Ureter”.

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

DR. L. W. MUCHIRI
AG. SECRETARY, KNH/UON-ERC

Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
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