THE HUMAN RECTUS SHEATH: FORMATION AND MICROSCOPIC ORGANISATION

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

By

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DECLARATION

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DEDICATION

To my father, the late Mr. Nesiphory Mwachaka,

for the pains he underwent in ensuring that I never missed school.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

AWRS----------------------Anterior wall of Rectus Sheath

AL --------------------------Arcuate line of Douglas

EO--------------------------External Oblique abdominis muscle

IO---------------------------Internal Oblique abdominis muscle

KNH -----------------------Kenyatta National Hospital

PWRS----------------------Posterior wall of Rectus Sheath

RA--------------------------Rectus Abdominis muscle

SPSS ----------------------Statistical Package for Social Sciences

TA --------------------------Transversus Abdominis muscle

SUMMARY

Background: The pattern of formation of the rectus sheath from the aponeuroses of external oblique, internal oblique and transversus abdominis muscles shows regional variations. The prevalence and location of the arcuate line which marks the inferior extent of the posterior wall of this sheath exhibits inter-population differences. Data on the Kenyan population however remains scarce and yet the location of this line may be important when harvesting rectus abdominis muscle for musculocutaneous flaps. Further, it is not known whether the variations on how the rectus sheath is formed and in the position of the arcuate line influence the histomorphology of this sheath. This knowledge may help explain the contribution of the rectus sheath in the biomechanics of the ventral abdominal wall.

Objective: To describe the pattern of formation and microscopic organization of the rectus sheath as well as location of the arcuate line.

Study design: A descriptive cross-sectional study.

Materials: Specimens were collected from eighty subjects of both gender aged 18 to 70 years. Of these, 31(16 male, 15 female) were collected from Chiromo and Nairobi City Mortuaries during autopsies. The remaining 49 (21 male, 18 female) were acquired from cadavers used for routine dissection by first year medical students at the Department of Human Anatomy, University of Nairobi.

Methods: The rectus sheath was exposed through a midline incision followed by dissection and clearance of the superficial fascia covering the anterior wall of this sheath. The pattern of formation of the sheath and position of the arcuate line were studied and documented. In the autopsy materials, five millimeter thick sections were

harvested and processed for light microscopy by paraffin embedding. From these, seven micrometer thick sections were cut then stained with Masson's Trichrome and Weigert's Resorcin Fuchsin to demonstrate collagen and elastic fibres respectively. Photographs were taken to illustrate the pattern of formation and the microscopic organisation of the rectus sheath.

Data analysis: Analogue photomicrographs taken were scanned using Hewlett Parkard® Scanjet scanner then entered into the Scion Image Beta 4.0.3.2 software (Scion Corporation, Fredrick, Maryland) for measurement of the thickness of the anterior and posterior walls of the rectus sheath. Data collected were coded then entered into SPSS computer software (version 15.0 Chicago, Illinois) for statistical analysis. The student t test was used in determining gender variations. A p-value ≤ 0.05 was considered significant.

Results: The anterior wall of the rectus sheath was aponeurotic in all cases while the posterior wall of the rectus sheath was aponeurotic in 71 (88.5%) cases, the rest were musculoaponeurotic. The internal oblique abdominis aponeurosis above the arcuate line split in all cases to enclose the rectus abdominis muscle. The arcuate line was present in 64 (80.4%) cases bilaterally as a single structure. This line was located closer to the umbilicus than the pubic symphysis, consistently at the last intersection of rectus abdominis muscle.

Microscopically, both walls of the rectus sheath were made of three distinct zones: superficial, intermediate and deep. The superficial and deep zones contained loosely arranged collagen and elastic fibres while the intermediate zones were made of compact bundles of collagen fibres. These bundles in the anterior wall of the rectus sheath were obliquely oriented above the arcuate line and transversely disposed below this line.

This corresponded with the alignment of the fascicles of internal oblique abdominis muscle. The intermediate zone in the posterior wall of the rectus sheath contained transversely oriented collagen fibre bundles in line with the fascicles of transversus abdominis muscle.

Conclusion: The microscopic organisation of the rectus sheath is determined by its pattern of formation and not by the position of the arcuate line. This sheath is mainly formed by the aponeuroses of internal oblique and transversus abdominis. Further, the arcuate line occurs more cranially and can accurately be located using the most distal intersection of rectus abdominis muscle. This information may be important to surgeons when harvesting rectus abdominis muscle for musculocutaneous flaps.

INTRODUCTION

The rectus sheath (RS) is a tendinous sheath formed by the aponeuroses of three anterolateral abdominal muscles: external oblique (EO), internal oblique (IO) and transversus abdominis (TA) (William et al., 1995). This sheath has two walls that enclose rectus abdominis (RA) muscle and its neurovascular bundle. The anterior wall of rectus sheath (AWRS) is firmly attached to RA while the posterior wall of rectus sheath (PWRS) is not (Sinnatamby, 2000). The functions of this sheath correspond to those of the muscles that form it which include generation and distribution of intra-abdominal forces (Hodges et al., 2000), passive respiration (Boriek et al., 2002), postural adjustments and truncal movements (Urquart et al., 2005).

The formation of the AWRS and PWRS usually varies according to the level of the anterior abdominal wall. Above the costal margin, only the AWRS made up of the aponeurosis of EO is present (William et al., 1995). Below the costal margin, IO splits into two laminae: a superficial lamina which blends with the aponeurosis of EO to form the AWRS and a deep lamina that fuses with the aponeurosis of TA to form the PWRS. This is the formation up to the arcuate line of Douglas which marks the inferior extent of the PWRS (Sinnatamby, 2000). Below the arcuate line, all the aponeuroses pass anterior to RA leaving the RA to lie directly on fascia transversalis (William et al., 1995).

Discrepancies in the pattern of formation of the rectus sheath below the costal margin have been observed. In a study by Monkhouse et al (1986), the aponeurosis of IO above the arcuate line of Douglas split into two laminae that enclosed the rectus abdominis (RA) muscle in up to 60% of the cases, while in 27.5% of the cases both laminae passed anterior to the RA. In the same study, TA split to enclose the RA in 12.5% of the cases. In addition, the rectus sheath was muscular in majority of the cases (57.5%) instead of being aponeurotic. Rizk (1991) reported that the fibres of the superficial lamina of IO decussated with those of EO aponeurosis in front of the midline of RA. Those of the deep lamina on the other hand decussated with TA aponeurosis lateral to the RA muscle.

The arcuate line of Douglas has been described as a sharp concave line that occurs bilaterally, midway between the umbilicus and the pubic symphysis (William et al., 1995). Some studies have however shown that the occurrence, number and location of the arcuate line exhibit inter-population differences (Monkhouse et al., 1986; Rizk, 1991; Rath et al., 1997; Cunningham et al., 2004). These workers reported a prevalence of this line in 15-100% of the cases. Double arcuate lines have also been seen in 15-47% of the cases (Rizk, 1991; Rath et al., 1997). The location of this line in relation to the umbilicus and pubic symphysis has also been documented as variable with the highest being at the level of the umbilicus and the lowest near the pubic crest (Monkhouse et al., 1986; Rizk, 1991; Cunningham et al., 2004).

The variations in the pattern of formation of RS and in the position of the arcuate line may influence the microscopic organisation of the connective tissue fibres therein. It is also possible that the fibre orientation and composition in the AWRS and PWRS differ as the former is firmly attached to the rectus abdominis muscle while the latter is not (William et al., 1995). This suggestion employs the fact that the pull by the anterolateral abdominal wall muscles is oblique while that of rectus abdominis is vertical (McArdle, 1997). Despite this, data on the microscopic organization of the human rectus sheath remains scarce. A study on the medial parts of this sheath reported presence of collagen fibres (Axer et al., 2001). These workers however did not pay attention to the lateral parts of the rectus sheath despite a report that medio-lateral variations in the formation of this sheath exist (Rizk, 1991). Although elastic fibres have been reported to coexist with collagen fibres in other structures in the anterior abdominal wall (Pulei, 2006), data concerning elastic fibres in the rectus sheath remains scanty.

The aim of this study was therefore to determine in the rectus sheath, its pattern of formation, location of the arcuate line and its microscopic organisation.

JUSTIFICATION

There are differing reports on how the rectus sheath is formed (Monkhouse et al., 1986; Williams et al., 1995) and where the arcuate line is located (Rizk 1991; Cunningham et al., 2004). It is not known whether the histomorphology of the rectus sheath is determined by the variations in its pattern of formation, location of the arcuate line or the association of AWRS and PWRS with the rectus abdominis muscle. The knowledge of the microscopic organization of this sheath may help explain the contribution of this sheath to the biomechanics of the anterior abdominal wall (Urquart et al., 2005; Hollinsky et al., 2006).

The prevalence and location of the arcuate line which marks the inferior extent of the posterior wall of this sheath exhibits inter-population differences (Monkhouse et al., 1986; Rizk, 1991; Rath et al., 1997; Cunningham et al., 2004). Data on the Kenyan population however remains scarce and yet the location of this line may be important when harvesting rectus abdominis muscle for musculocutaneous flaps (Cunningham et al., 2004).

HYPOTHESIS

Variations in the pattern of formation of the rectus sheath and the position of the arcuate line influence the histomorphology of the rectus sheath.

OBJECTIVES

BROAD

To describe the pattern of formation and microscopic organization of the rectus sheath as well as the location of the arcuate line.

SPECIFIC

In the rectus sheath of both male and females, to determine:

1. Its pattern of formation.
2. The prevalence and location the arcuate line.
3. Its microscopic organisation.

MATERIALS AND METHODS

Materials

The rectus sheaths were obtained from eighty adult subjects aged 18 to 70 years. Of these, 31 (16 male, 15 female) were collected from the Nairobi's City and Chiromo Mortuaries during autopsies which were conducted within 24 hours after death of the subjects. The remaining 49 (21 male, 18 female) were acquired from cadavers used for routine dissection by first year medical students at the Department of Human Anatomy, University of Nairobi. Five subjects were excluded from the study as they either had scars or wounds in the anterior abdominal wall.

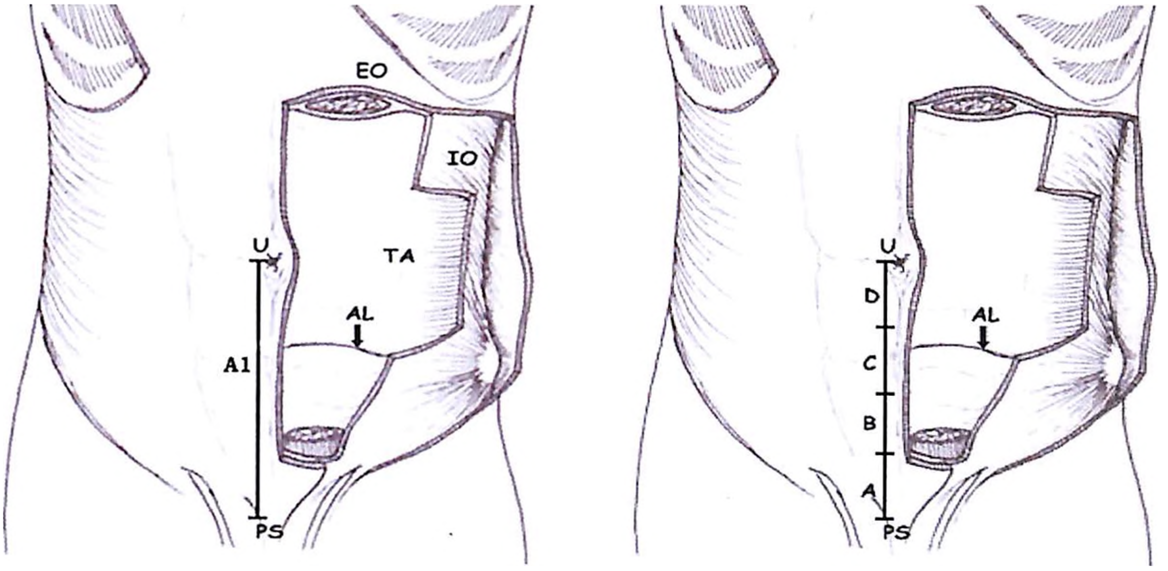
Ethical considerations

Approval to carry out the study was granted by the KNH-Ethics and Review Committee. A written consent for the use of postmortem material was given by the relatives of the deceased.

Methods

Gross dissection

The rectus sheath was exposed through a midline skin incision followed by dissection and clearance of the superficial fascia covering the AWRS. A transverse incision was made through the rectus sheath midway between the umbilicus and the costal margin. The rectus sheath was reflected laterally so as to view the PWRS which was then inspected for the presence of the arcuate line of Douglas. When the arcuate line was present, measurements were taken from the pubic symphysis to the umbilicus using a ruler (Figure 1A). This distance was then divided into four equal segments which were named A, B, C and D from the pubic symphysis upwards (Figure 1B). The position of the arcuate line was documented depending on the segment it was located. This sheath was then dissected laterally with the help of a dissecting lens so as to visualize its pattern of formation.



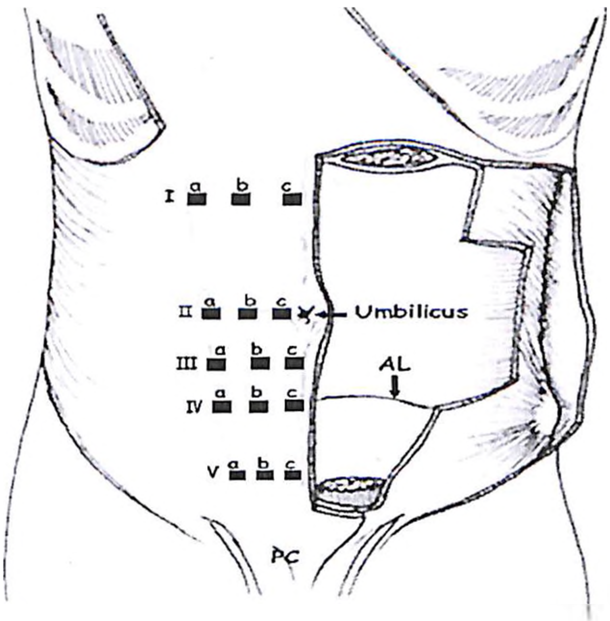
A B

Figure 1A & B: Anterior view of the anterior abdominal wall showing the location of arcuate line (AL).

EO, external oblique; IO, internal oblique; TA, transversus abdominis; PS- pubic symphysis, U- umbilicus; A1, distance from the umbilicus to the pubic symphysis.

Tissue sampling

Transverse segments of the rectus sheath, 5 mm thick, were taken from the autopsy material for microscopic examination. These segments were harvested from five levels (described in Figure 2). For each level, the segments were taken on the lateral (a), middle (b) and medial (c) parts of the rectus sheath. After tissue harvesting, the anterior abdominal wall was apposed in one layer.



KEY

- I – midway between umbilicus and costal margin
- II – at the umbilical region
- III - Midway between the umbilicus and arcuate line (or PC in cases when arcuate line was absent)
- IV – at the arcuate line
- V - Midway between arcuate line and pubic crest

Figure 2. Anterior view of the anterior abdominal wall showing areas where tissue sampling was done. AL, Arcuate line; PC, pubic crest.

Light microscopy

The segments harvested were fixed in 10% formaldehyde by immersion followed by dehydration through increasing concentrations of alcohol (starting from 70% to 100%). Clearing and infiltration using paraffin wax was followed by embedding. Seven micrometer thick serial sections were cut using a Lezlar® microtome (SM2400, Germany). These sections were floated, picked on slides and left for drying in an oven overnight after which they were stained using Weigert's Elastic stain with van Gieson counterstaining to elaborate the elastic fibers. Other sections were stained using Masson's Trichrome to demonstrate the collagen fibers (Drury et al, 1967). For the microscopic observations, a Leica® light microscope (BME model, Germany) was used.

Photography

Photographs illustrating the fiber orientation and composition of the rectus sheath were taken using a Fuji® digital camera (Finepix A900, 9 megapixels) and a Sony® analogue camera.

Data analysis

For histomorphometric analysis, the photomicrographs of the middle sections (Figure 2) taken at all levels were used. Analogue photomicrographs taken were scanned using Hewlett Parkard® Scanjet scanner (2200c model). The Scion Image Beta 4.0.3.2 software (Scion Corporation, Fredrick, Maryland) was used to measure the thickness of AWRS and PWRS. These measurements were randomly taken across the walls of the rectus sheath. Their r averages were documented. The data collected were coded then entered into SPSS computer software (version 15.0, Chicago, Illinois) for statistical analysis. The student *t* test was used in analyzing gender variations. A *p*-value of less than or equal to 0.05 was considered significant.

RESULTS

The rectus sheath was formed by the aponeuroses of external oblique, internal oblique and transversus abdominis muscles. This sheath enclosed the rectus abdominis muscle together with its neurovascular bundle (Figure 3). The anterior wall of the rectus sheath extended from the rib cage to the pubic crest. The posterior wall of the rectus sheath on the other hand spanned from the costal margin to the arcuate line or to the pubic crest when the arcuate line was absent. The pattern of formation of the rectus sheath as well as the position of the arcuate line exhibited variations.

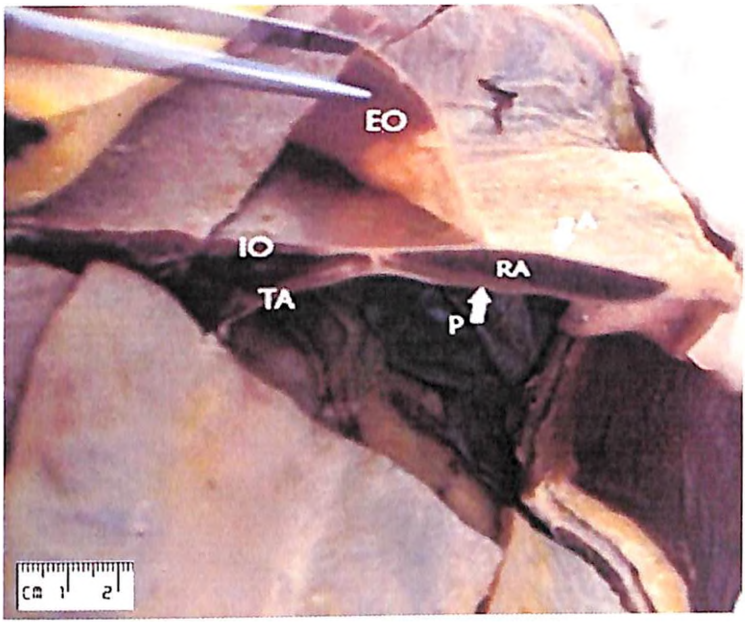


Figure 3. A section through the rectus sheath showing the formation of AWRS (A) and PWRS (P). EO, External oblique; IO, Internal oblique; RA, Rectus abdominis; TA, Transversus abdominis.

1. PATTERN OF FORMATION

a) Anterior wall of the rectus sheath

In all the cases, the anterior wall of the rectus sheath was aponeurotic and firmly attached to the rectus abdominis muscle (Figure 4A). These attachments were however strongest at the tendinous intersections of RA. Above the costal margin, the anterior wall of the rectus sheath was solely formed by the aponeurosis of external oblique abdominis muscle. Below the costal margin, the internal oblique abdominis muscle in all the cases split into two laminae: a superficial and a deep one (Figure 4A). The superficial lamina fused with the aponeurosis of the external oblique abdominis to form the anterior wall of the rectus sheath. When the arcuate line was present, all the anterolateral abdominal wall muscles passed anterior to rectus abdominis muscle to form the AWRS (Figure 4B). Three patterns of fusion of the superficial lamina of IO and EO aponeurosis were observed: at the lateral border of rectus abdominis, at the middle of the RA and diagonally from the pubic symphysis to the lateral border of RA towards the costal margin. Most of the cases had the diagonal fusion of EO with the superficial lamina of IO (Figure 5, 6).

b) Posterior wall of the rectus sheath

The posterior wall of the rectus sheath was made up of the aponeuroses of TA and the deep lamina of IO. Fusion of the TA with the deep lamina of IO was at the lateral border of RA muscle (Figure 4A). This wall was aponeurotic in 71(88.5%) cases, the rest were musculoaponeurotic. The musculoaponeurotic posterior walls of the rectus sheath were found in males only. In these cases, the muscle fibres were derived from transversus abdominis muscle and their content decreased caudally such that below the umbilicus the posterior wall of the rectus sheath was purely aponeurotic (Figure 4C&D).

LEGENDS

Figure 4A: A transverse section of the rectus sheath and its contents below the costal margin. Note that internal oblique (IO) split to enclose the rectus abdominis muscle (RA). Its superficial lamina fuses with external oblique (EO) near the middle of the RA while the deep lamina fuses with transverses abdominis (TA) at the lateral border (LB) of RA.

Figure 4B: A transverse section through the rectus sheath below the arcuate line. Note that the PWRS is deficient at this level. The arrow points at the anterior wall of the rectus sheath. RA, rectus abdominis; PS, pubic symphysis.

Figure 4C: A transverse section through the rectus sheath showing a musculoaponeurotic PWRS. Note the medial part is aponeurotic (A) while the lateral part is muscular (M).

Figure 4D: Diagram showing a musculoaponeurotic type of PWRS (arrow). Rectus abdominis (RA) muscle has been reflected laterally. M, muscular part; P, aponeurotic part; GB, gall bladder.

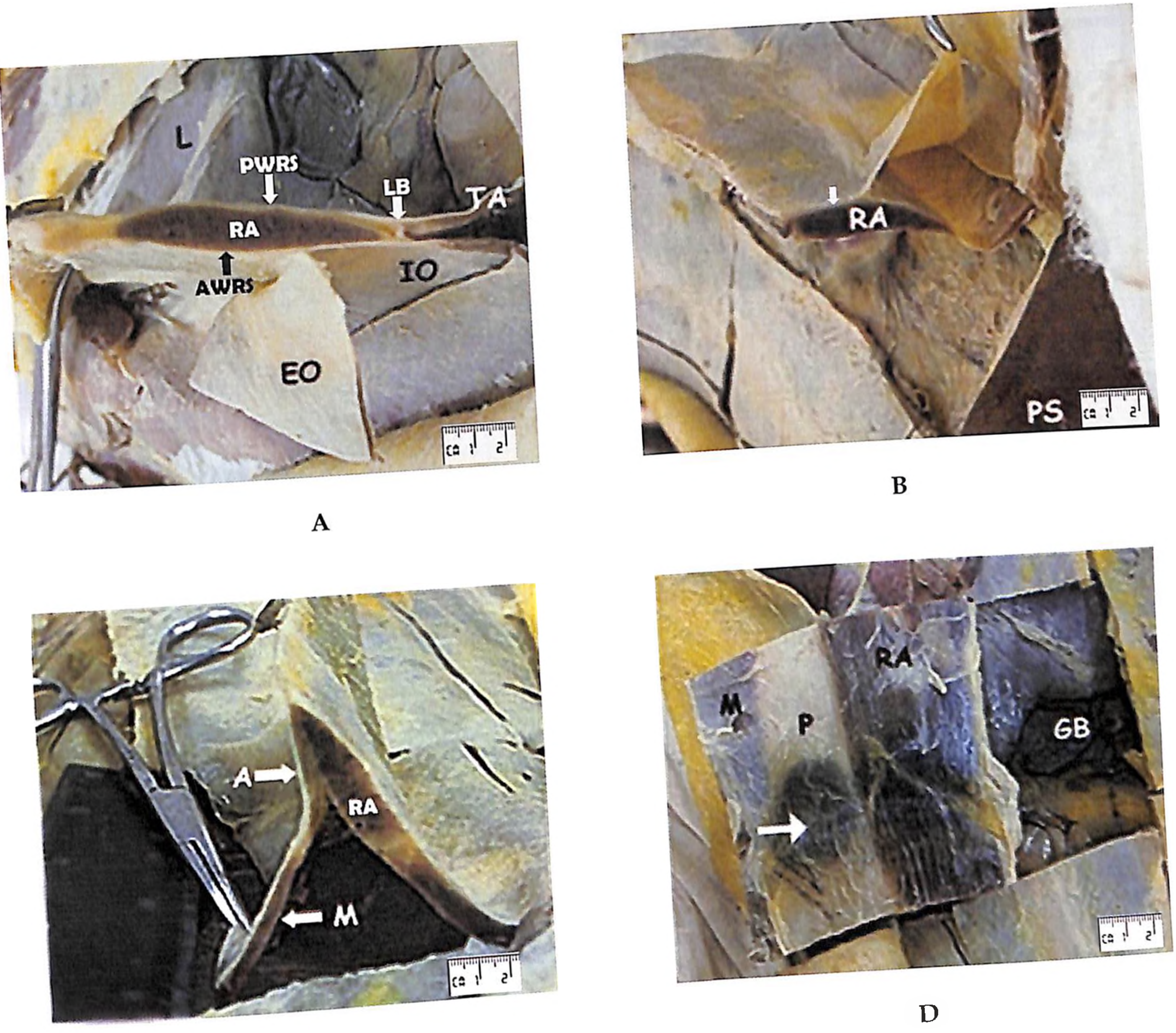


Figure 4A-D: Pattern of formation of the rectus sheath

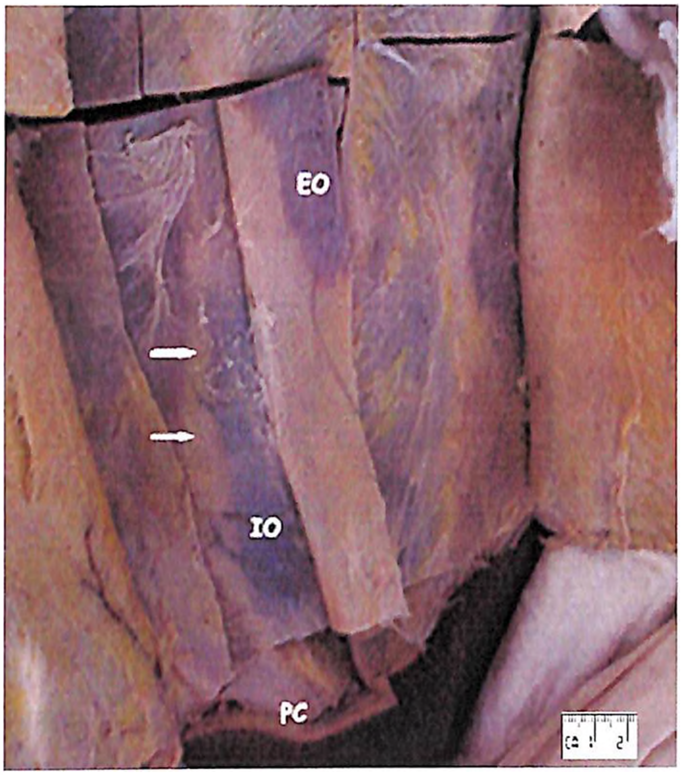


Figure 5: Anterior view of the rectus sheath showing the diagonal fusion of external oblique (EO) with the internal oblique (IO). EO has been reflected medially. The arrows point at the linea semilunaris that marks the lateral border of rectus abdominis muscle. PC, pubic crest.

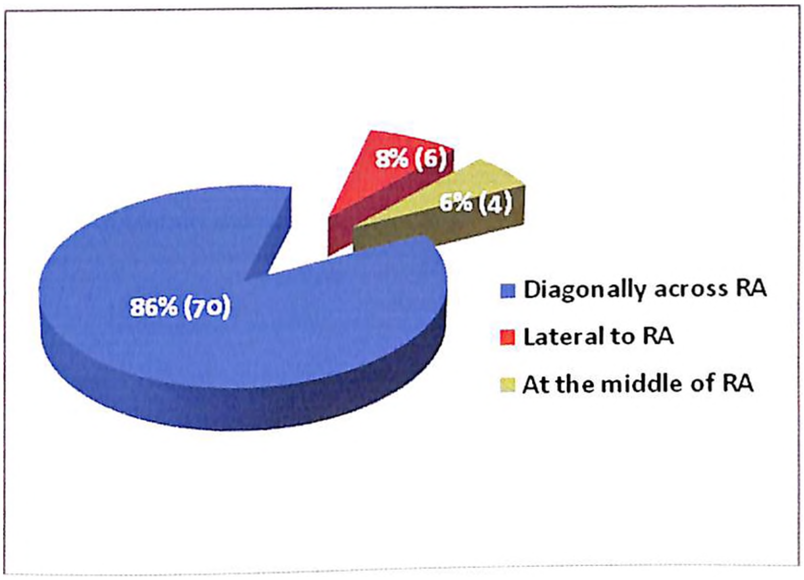


Figure 6: Pattern of fusion of external oblique with superficial lamina of internal oblique. RA, rectus abdominis muscle.

2. LOCATION OF THE ARCUATE LINE

The arcuate line was present in 64 (80.4%) cases (Figure 7). In these cases, the line occurred bilaterally as a single structure and was consistently found at the most distal intersection of the rectus abdominis muscle. In the majority of the cases (52), the arcuate line occurred in segment C and D (Figure 8A&B). Although none of the females had their arcuate lines in segment A, this was not statistically significant (p=0.308).

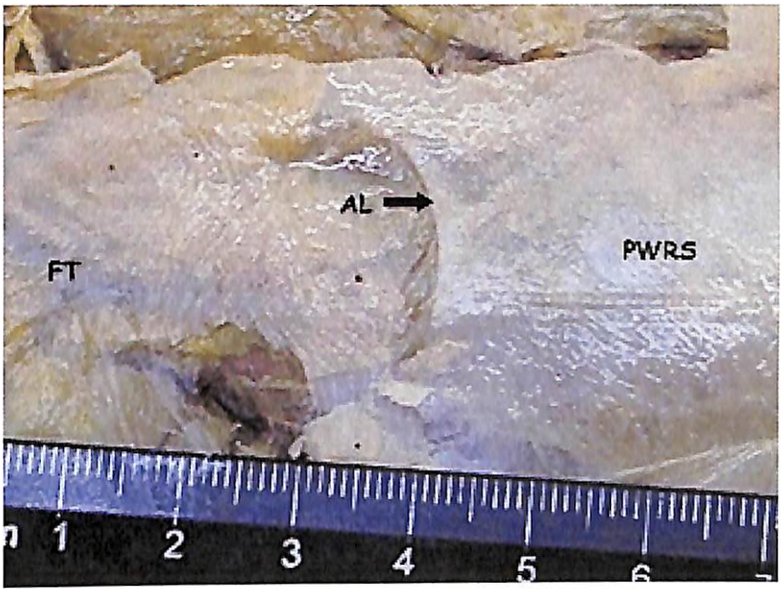
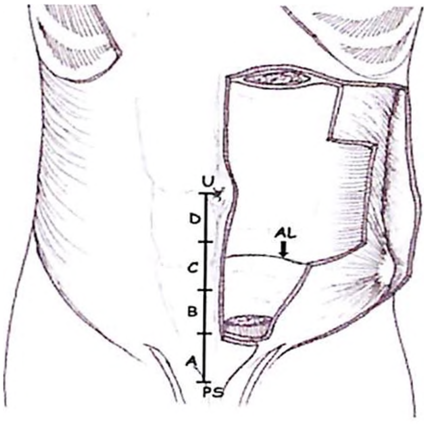
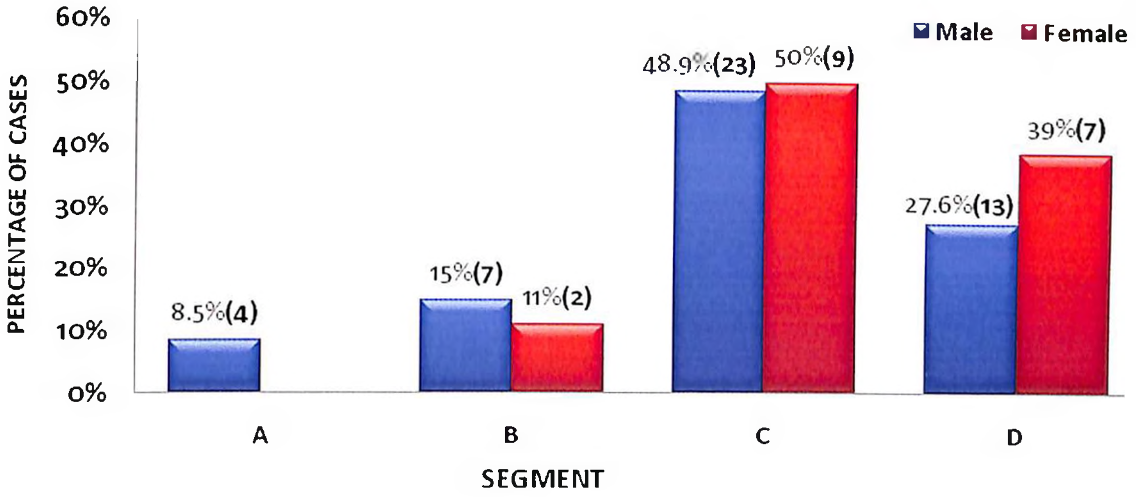


Figure 7: Photograph demonstrating the position of arcuate line (AL). Note that below the arcuate line, rectus abdominis lies on fascia transversalis (FT). PWRS, posterior wall of rectus sheath.



8A



8B

Figure 8A: Diagram showing how the line between pubic symphysis (PS) and umbilicus (U) was divided into four equal segments: A, B, C and D. AL, arcuate line.

Figure 8B: Bar chart showing the location of the arcuate line in the different segments among males and females.

LEGENDS

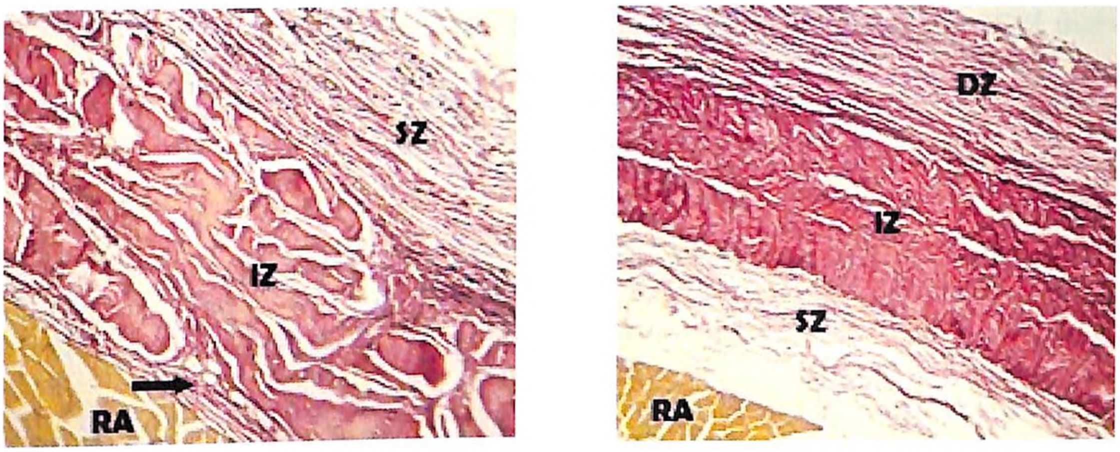
Figure 9A: Photomicrograph of a transverse section of the AWRS above the arcuate line in a 30 yr old male (Weigert elastic stain with Van Gieson counterstain, mag. X35). The arrow points at the deep zone. SZ, superficial zone; IZ, intermediate zone; RA, rectus abdominis.

Figure 9B: Photomicrograph of a transverse section of the PWRS above the arcuate line in a 30 yr old male (Weigert elastic stain with Van Gieson counterstain, mag. X35). SZ, superficial zone; IZ, intermediate zone; DZ, deep zone; RA, rectus abdominis.

3. MICROSCOPIC ORGANISATION

GENERAL ORGANIZATION

Both the AWRS and PWRS contained collagen and elastic fibres which were organized into three distinct zones: superficial, intermediate and deep, in relation to skin (Figure 9A&B). The superficial and deep zones in both the AWRS and PWRS contained loosely arranged collagen and elastic fibres. The intermediate zones on the other hand were made of compact bundles of collagen fibres (Figure 9A&B). The make up of the walls of the rectus sheath showed mediolateral (from linea semilunaris towards linea alba) and craniocaudal (from the costal margin towards the pubic crest) differences. Gender differences in the fibre composition and orientation were absent.



A B

Figure 9A&B: General microscopic organization of the rectus sheath.

MEDIOLATERAL VARIATIONS

a) Anterior wall of the rectus sheath

Above the arcuate line and at the lateral border of rectus abdominis muscle, there was a clear cut separation between the aponeuroses of EO and the superficial lamina of IO (Figure 10A). These aponeuroses contained collagen and elastic fibres. Most of the collagen fibres were organized into compact bundles of variable sizes with an oblique orientation. Surrounding the collagenous bundles were collagen and elastic fibres (Figure 10B). There were more elastic fibres in the aponeurosis of EO than IO. The elastic fibres were mainly located superficial to the collagen fibre bundles of EO and together with some loosely arranged collagen fibres, formed the superficial zone of the AWRS (Figure 10C). These elastic fibres decreased towards the linea alba (Figure 10D&E). At the point of fusion of the aponeurosis of EO and IO, collagen fibres at the interface of these aponeuroses intermingled leaving no plane of separation (Figure 10C). The thickness of collagen fibre bundles of EO decreased distal to the fusion leaving the superficial lamina of IO to form the intermediate zone of the AWRS (Figure 10F). Other levels of the rectus sheath studied displayed similar mediolateral differences.

b) Posterior wall of the rectus sheath

The aponeuroses of TA and deep lamina of IO were made of collagen and elastic fibres. Like in the AWRS, most of the collagen fibres were organized into bundles. At the lateral border of RA, the bundles of the deep lamina of IO intermingled with those of TA (Figure 11A). Distal to the fusion of these aponeuroses, the collagen fibre bundles of both IO and TA were transversely aligned (Figure 11B). The elastic fibres were concentrated deep to the collagen fibre bundles of transversus abdominis. Together with some loosely arranged collagen fibres they formed the deep zone of the PWRS. These fibres progressively decreased towards the linea alba (Figure 11C&D).

LEGENDS

Figure 10A: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male at the linea semilunaris (Masson's Trichrome stain, mag. X100). IO, superficial lamina of internal oblique; EO, external oblique aponeurosis; RA, rectus abdominis muscle.

Figure 10B: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male (Weigert Elastic stain with Van Gieson counterstaining, mag. X100). Note that collagen (C) fibre bundles are surrounded by elastic fibres (arrow).

Figure 10C: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male at the lateral border of rectus abdominis muscle (Weigert Elastic stain with Van Gieson counterstaining, mag. X100). Note the differences in the orientation of collagen fibre bundles of external oblique (EO) and internal oblique (IO). The arrow points at the band of elastic fibres forming the superficial zone. C, collagen fibres at the interface of EO and IO.

Figure 10D: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male at the lateral border of RA (Weigert Elastic stain with Van Gieson counterstaining, mag. X400). Note the abundance of elastic fibres in the superficial zone (arrow).

Figure 10E: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male at the middle of the rectus abdominis muscle (Weigert Elastic stain with Van Gieson counterstaining, mag. X400). Note the decrease in the elastic fibres (arrow).

Figure 10F: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 19 year old male at the point of fusion of EO with IO (Weigert Elastic stain with Van Gieson counterstaining, mag. X100). Note that distal to the fusion of EO with IO, collagenous bundles of IO diminish. Elastic fibres in the superficial zone are present but also decrease.

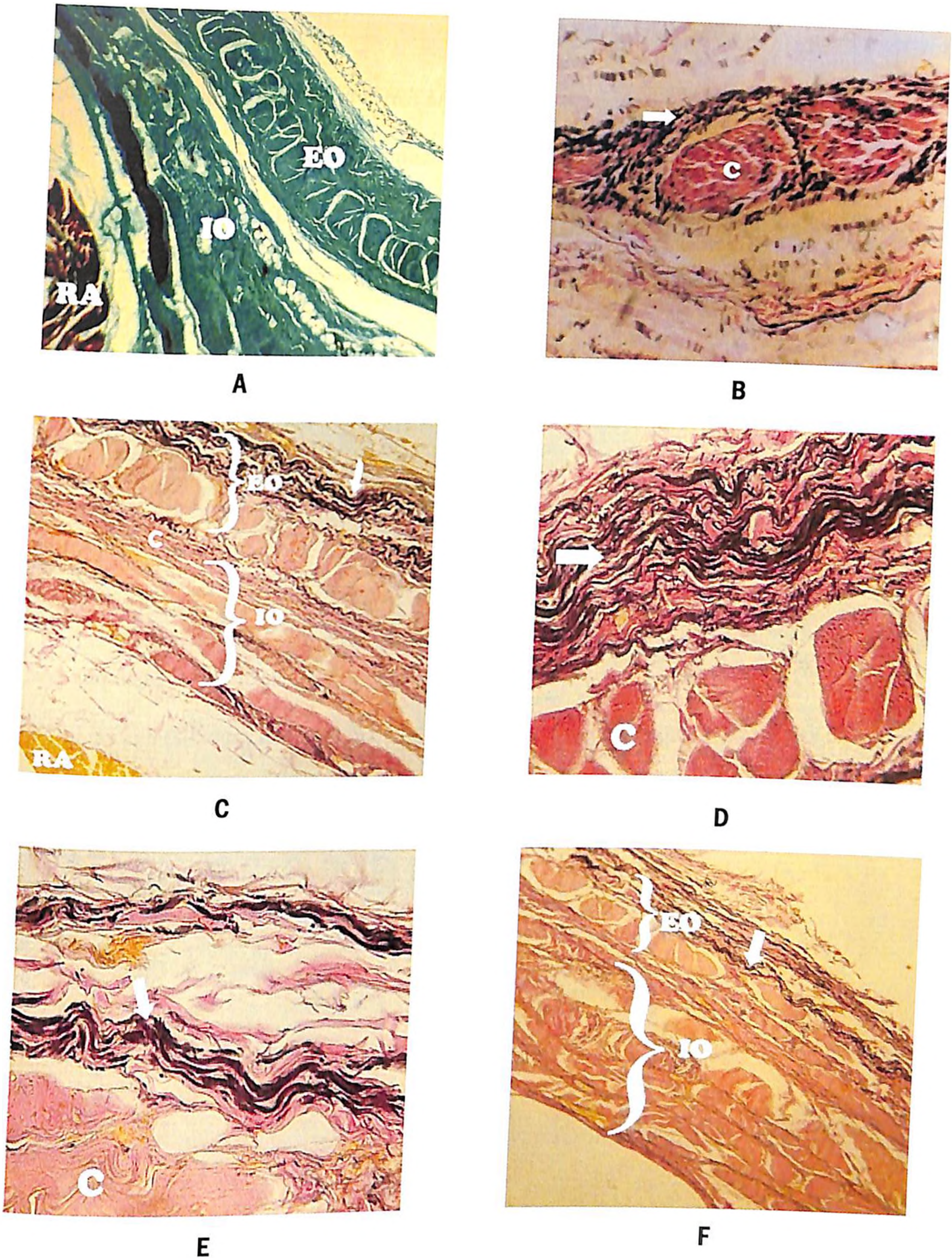


Figure 10A-F: Mediolateral differences in the histomorphology of the AWRS

LEGENDS

Figure 11A: Photomicrograph of a transverse section of the PWRS midway between the umbilicus and the arcuate line in a 19 year old male at the lateral border of rectus abdominis muscle (Masson's Trichrome stain, mag. X100). Bundles of the deep lamina of internal oblique and transversus abdominis which form the intermediate zone (IZ) intermingle. RA, rectus abdominis; DZ, deep zone; SZ, superficial zone.

Figure 11B: Photomicrograph of a transverse section of the PWRS midway between the umbilicus and the arcuate line in a 19 year old male at the middle of rectus abdominis muscle (Masson's Trichrome stain, mag. X100). Note that the collagen fibre bundles are transversely oriented.

Figure 11C: Photomicrograph of a transverse section of the PWRS midway between the umbilicus and the arcuate line in a 19 year old male at the lateral border of rectus abdominis muscle (Weigert Elastic stain with Van Gieson counterstaining, mag. X400). Note that the deep zone (DZ) has a preponderance of elastic fibres.

Figure 11D: Photomicrograph of a transverse section of the PWRS midway between the umbilicus and the arcuate line in a 19 year old male at the middle rectus abdominis muscle (Weigert Elastic stain with Van Gieson counterstaining, mag. X400). Note that the deep zone (DZ) has less elastic fibres compared to that in figure 11C.

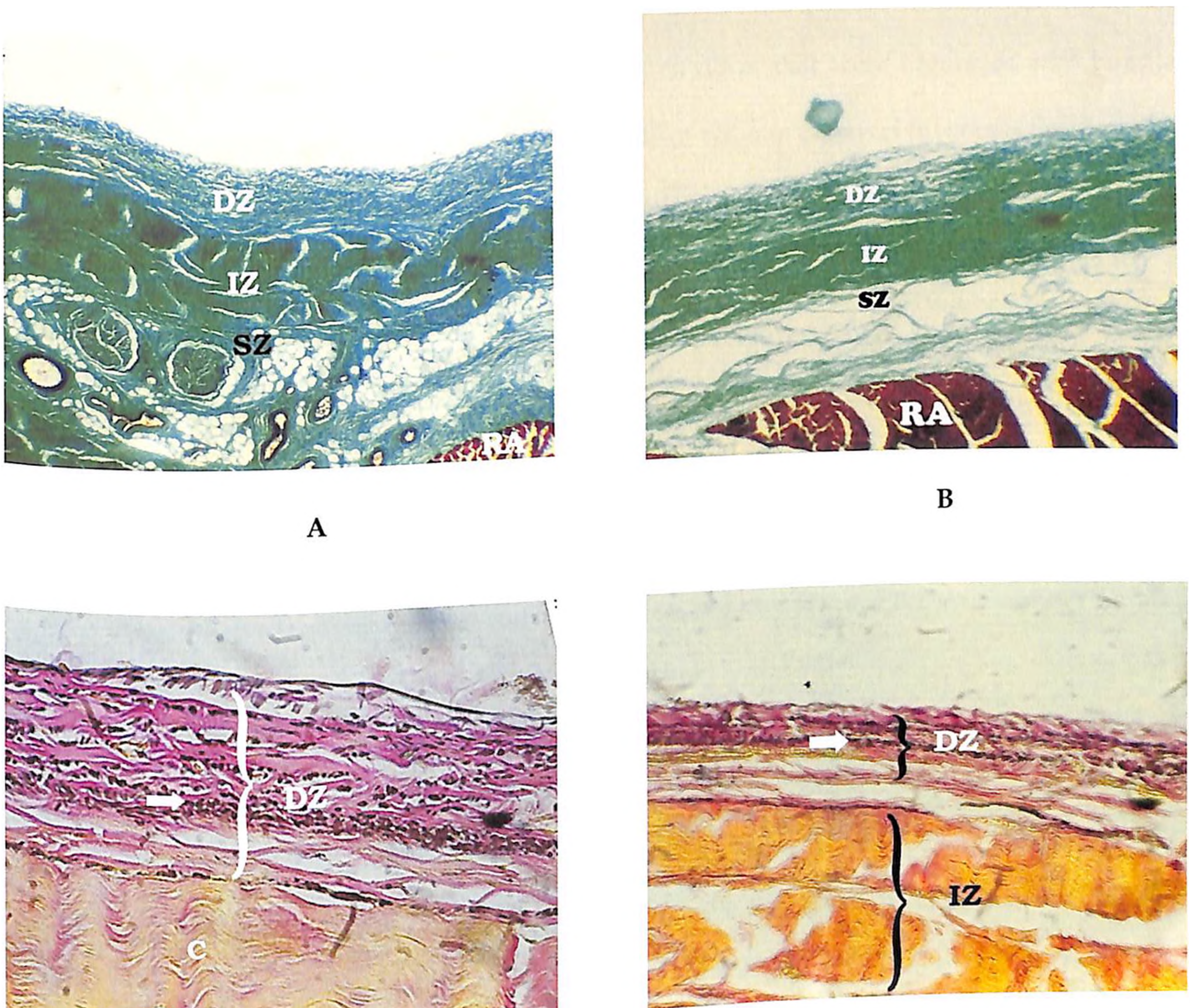


Figure 11A-D: Mediolateral differences in the histomorphology of the PWRS

CRANIOCAUDAL VARIATIONS

a) Anterior wall of the rectus sheath

The superficial zone comprised elastic and collagen fibres that were irregularly spaced (Figure 9A). In the supra-umbilical regions, these elastic fibres were obliquely disposed while those of the infra-umbilical regions were transversely oriented (Figure 12A&B). The intermediate zone was made of collagen fibres that were organized into bundles. Above the arcuate line, this zone was made of oblique collagen fibre bundles that were arranged in rows (Figure 12C&D). The angulation of these bundles increased toward the costal margin such that some of these bundles appeared longitudinal (Figure 12C). At the arcuate line, these bundles ran transversely (Figure 12E). In addition, these bundles exhibited branching and also contained collagen fibres that were wavy (Figure 12F). This organization was seen in the regions below the arcuate line but the only difference was that the collagen fibre bundles in this zone were thicker. The deep zone in all regions contained irregularly spaced collagen and elastic fibres (Figure 12E).

b) Posterior wall of the rectus sheath

The superficial and deep zones were made of irregularly arranged collagen and elastic fibres (Figure 13A). The deep zone was more extensive than the superficial zone and contained more elastic fibres (Figure 13A&B).The intermediate zone contained collagen fibres that were organized into bundles that were transversely oriented (Figure 13C). The thickness of this zone decreased towards the arcuate line (Figure 13D&E). Throughout the PWRS, the collagen bundles were transversely arranged irrespective of the position of the arcuate line. In addition, these bundles displayed branching and contained collagen fibres that were wavy (Figure 13C).

LEGENDS

Figure 12A: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the costal margin in a 40 year old male (Weigert elastic stain with Van Gieson counterstain, mag. X250). Note that the elastic fibres are predominantly oblique (arrow).

Figure 12B: Photomicrograph of a transverse section of the AWRS midway between the umbilicus and the arcuate line in a 40 year old male (Weigert elastic stain with Van Gieson counterstain, mag. X250). Note that some elastic fibres are transversely disposed (arrow).

Figure 12C: Photomicrograph of a transverse section of the AWRS midway between the costal margin and the umbilicus in a 30 year old female (Masson's Trichrome stain, mag. X100). Note that the collagen bundles in the intermediate zone are longitudinally disposed and are arranged in rows. SZ, superficial zone; IZ, intermediate zone; DZ, deep zone.

Figure 12D: Photomicrograph of a transverse section of the AWRS at the umbilicus in a 30 year old female (Masson's Trichrome stain, mag. X100). Note that the collagen bundles are all oblique.

Figure 12E: Photomicrograph of a transverse section of the AWRS at the arcuate line in a 30 year old female (Weigert Elastic stain with Van Gieson counterstaining, mag. X100). Note that the collagen bundles are transverse.

Figure 12F: Photomicrograph of a transverse section of the AWRS at the arcuate line in a 30 year old female (Masson's Trichrome stain, mag. X250). Note that the collagen bundles exhibit branching (arrow) and contain collagen fibres that are wavy (star).

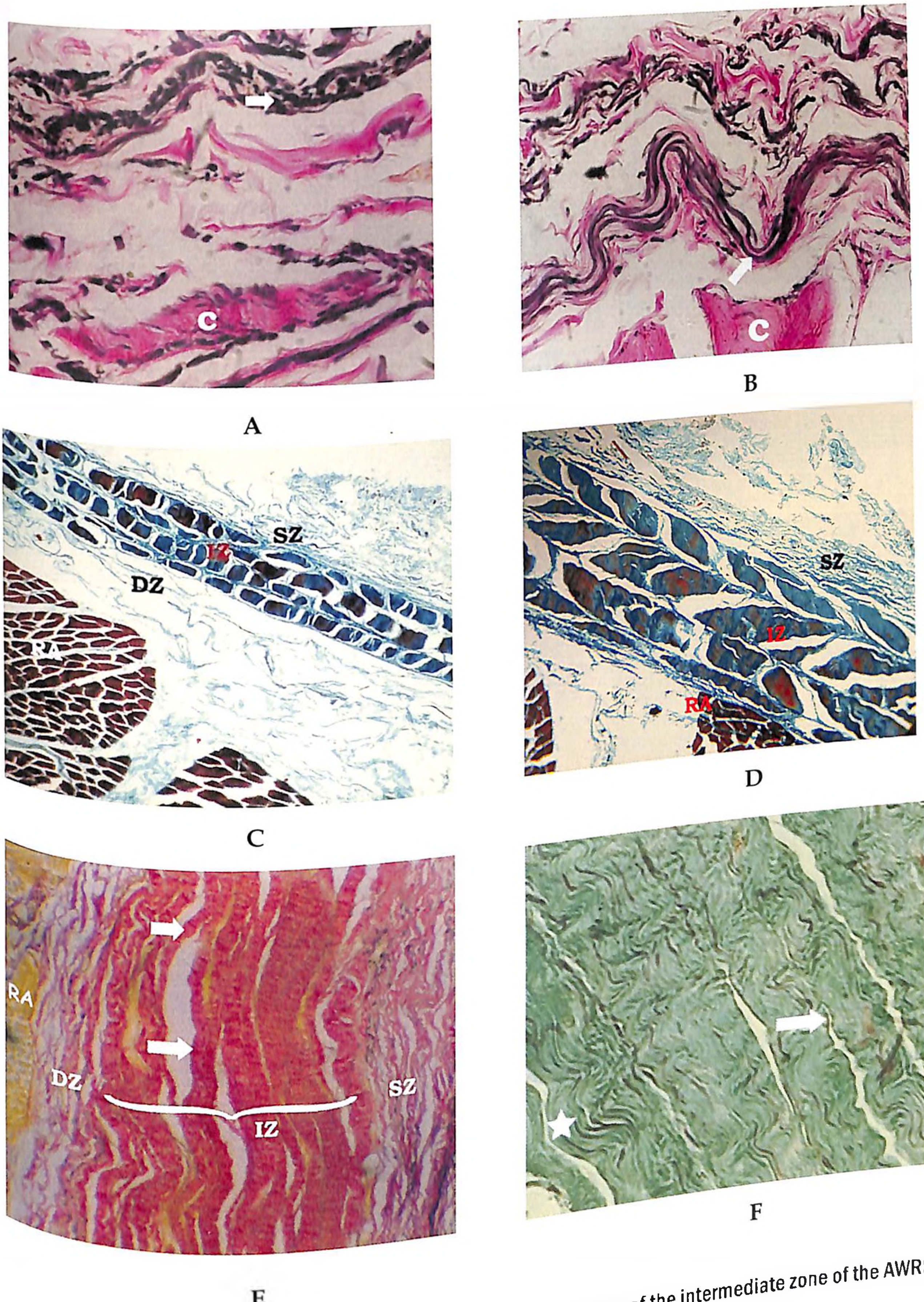


Figure 12A-F: Craniocaudal differences in the histomorphology of the intermediate zone of the AWRS

HISTOMORPHOMETRY

Measurements of the middle segments of the walls of the rectus sheath revealed that the thickness of the AWRS steadily increased towards the pubic crest while that of the PWRS was fairly constant up to the umbilicus. Below this point, the PWRS gradually decreased in thickness. Summation of the thickness of AWRS and PWRS revealed that the rectus sheath below the arcuate line was thicker than that in the above this line (Figure 14).

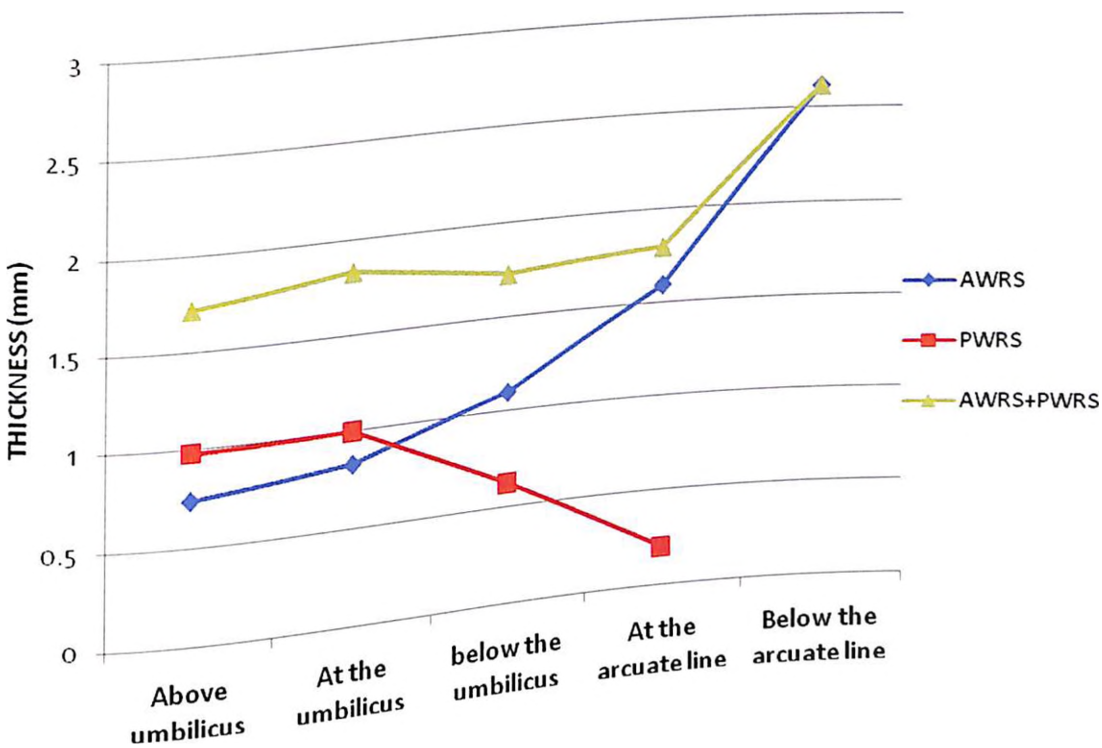


Figure 14: A line graph showing the craniocaudal changes in the thickness of the anterior wall of the rectus sheath (AWRS) and the posterior wall of the rectus sheath (PWRS).

DISCUSSION

PATTERN OF FORMATION

Observations of the present study have revealed that the pattern of formation of the rectus sheath is different in three regions namely; above the costal margin, between the costal margin and the arcuate line and below the arcuate line. In this study, the aponeurosis of internal oblique abdominis muscle between the costal margin and the arcuate line split to enclose the rectus abdominis muscle in all cases. This is consistent with the findings by Rizk (1980) but differ with those by Monkhouse et al (1986) who observed three different patterns of formation of this sheath. In the study by the latter workers, only 60% of the cases conformed to the pattern in the present study. In 27.5% of the cases, the internal oblique abdominis did not split but passed anterior to the rectus abdominis leaving the transversus abdominis to form the posterior wall of the rectus sheath. Still in the same study, transversus abdominis split to enclose the rectus abdominis in 12.5% of the cases.

The pattern of formation of the rectus sheath may be explained by its embryogenesis (Walmsley, 1937; Skandalakis et al., 1997). The anterior abdominal wall is first formed by ectoderm and parietal mesoderm which form somatopleura (Skandalakis et al., 1997). According to these workers, the ventral abdominal wall muscles are derived from the lower thoracic myotomes that migrate ventrally invading the somatopleura during the sixth week of intra-uterine life. Rectus abdominis muscle develops first from the tips of these myotomes then the rest of the mesodermal sheet divides into two strata: internal oblique stratum and external oblique stratum (Walmsley, 1937). The internal oblique stratum gives rise to transversus abdominis and internal oblique abdominis muscles while the external oblique stratum develops into external oblique abdominis muscle (Walmsley, 1937). Later in development, the distal parts of the muscles become aponeurotic (Rizk et al., 1982). The aponeurosis of the internal oblique abdominis muscle attaches to the primordial rectus sheath and grows on both anterior and posterior surfaces towards the linea alba forming its superficial and deep lamina (Walmsley, 1937). External oblique becomes placed anterior to the superficial lamina of internal oblique because it is derived from the external oblique stratum that lies ventral to the internal oblique stratum (Rizk et al., 1982). Transversus abdominis muscle on the other hand assumes a position posterior to the deep lamina of internal oblique as it is derived from the internal oblique stratum (Skandalakis et al., 1997).

The morphology of the adult rectus sheath can not be fully explained by its embryology. As observed in the present study and by other workers, AWRS is firmly attached to RA while the PWRS is not (William et al., 1998; Sinnatamby, 2000). The maturation of the rectus sheath could be influenced by the mechanical activities of the muscles of the anterior abdominal wall. The upper fascicles of transversus abdominis act as a circular compressor of both thoracic and abdominal cavities (Hodges et al., 2000). Their location deep to the rectus abdominis coupled by the fact that the PWRS is not firmly attached to the RA may be a biomechanical adaptation. As evidenced by physiological studies, these fascicles of TA are recruited before other muscles of anterior abdominal wall during truncal flexion and respiration (Hodges et al., 1997; Urquart et al., 2005). Contraction of TA therefore increases the separation between the PWRS and the RA. This may facilitate smooth gliding of the rectus abdominis muscle.

As seen in the present study and in other studies (Rizk, 1980; Monkhouse et al., 1986), the aponeuroses of transversus abdominis and internal oblique abdominis below the arcuate line blended with that of external oblique abdominis muscle to form the anterior wall of the rectus sheath. The lower fascicles of TA and IO support abdominal viscera and generate forces that compress the sacro-iliac joints for postural stability (Richardson et al., 2002). Electromyographic (EMG) studies on the TA have shown greater tonic activity in the lower fibres during upper limb movements (Hodges et al., 1999). Rapid flexion of the upper limb produces a brief challenge to postural stability of the trunk with most of the effects being felt at the sacroiliac joints (Bouisset et al., 1987). Contraction of lower fibres of TA and IO flexes the spine leading to stabilization of the sacroiliac joints (Richardson et al., 2002). The rectus abdominis is also recruited during these maneuvers (Hodges et al., 1997). The anterior position of these aponeuroses is thus the most favorable as the fibres act on the most curved surface with the contracted RA acting as a rigid support.

In the current study, three patterns of fusion of EO with superficial lamina of IO were observed: diagonally across RA, at the lateral border of RA and at the middle of RA. TA on the other hand fused with the deep lamina of IO at the lateral border RA. These patterns of fusion are hitherto unreported. It is probable that these observations have an embryologic basis (Walmsley, 1937). According to this author, the TA becomes tightly adherent to the deep lamina of IO all the way form the lateral border of rectus abdominis muscle because they have a common origin, the internal oblique stratum. The three patterns of fusion of the aponeuroses of external oblique and superficial lamina of internal oblique muscle could be a result of the different embryonic origin of external oblique.

The diagonal fusion of external oblique and the superficial lamina of internal oblique which was seen in most of the cases in the present study may have been as a result of the flexion lines caused by movements of the trunk and those of lower limbs. According to Walmsley (1937), the hypogastric part of external oblique lies between the iliac bones hence moves with the pelvis but is not influenced by the movements of the lower limbs. Consequently all the flexion lines of the trunk lie above it while those of the lower limbs are below it. The aponeurosis of external oblique therefore has no major function in the hypogastric region (Walmsley, 1937). Consequently, it may not need to fuse with the superficial lamina of internal oblique all the way from the lateral border of rectus abdominis.

The posterior wall of the rectus sheath in the current study was aponeurotic in 88.5% of the cases, the rest were musculoaponeurotic. This is at variance with the findings by Monkhouse et al (1986) who found aponeurotic sheaths in 42.5% of the cases, the rest were muscular. The dissimilarity in the findings could be as a result of the methods used. Monkhouse et al (1986) were not clear on their definition of muscular sheaths. In the present study however, the posterior wall of the rectus sheath was not entirely muscular but musculoaponeurotic with the muscular content decreasing caudally towards the arcuate line. This observation may be an adaptation of the transversus abdominis muscle in generating intra-abdominal pressure. Pertinent to this is the report by Muramatsu et al (2001) that skeletal muscles contain contractile elements (muscle fibres) and elastic components (aponeuroses or tendons). The elastic component interacts with the contractile element (Kubo et al., 2000) and functions as a store of elastic energy when the muscle contracts (Alexander, 1984). It is plausible therefore that the aponeurotic part of transversus abdominis stores energy when this muscle contracts and releases the energy when the muscle relaxes. This leads to build up of pressure in the lower abdomen.

The musculoaponeurotic PWRS were found only in male individuals. This could be as a result of hormonal influence. In general, males contain more muscular tissue than females (Komi et al., 1978, Griggs et al., 1989). These workers attributed the increase in muscle to the more pronounced contractile elements and metabolic enzymes in males as a result of testosterone.

LOCATION OF THE ARCUATE LINE

The arcuate line of Douglas in the present study was seen in most of the cases. These findings are in tandem with the observations in other studies (Table 1). A study in an Iraqi population reported a low prevalence of the arcuate line (Rizk, 1991). This worker observed that this line in some cases was replaced by what he called a 'thick aponeurotic bundle'. These aponeurotic bundles were however not seen in the current study.

Table 1: Prevalence of the arcuate line among different populations

Author	Study population	Sample size	Arcuate line	
			Present (%)	Absent (%)
Monkhouse et al (1986)	British	56	100	0
Rizk (1991)	Iraqi	40	15	85
Rath et al (1997)	French	30	100	0
Cunningham et al (2005)	American	18	94	6
Present study	Kenyan	80	80.4	19.6

The occurrence of the arcuate line has been associated with the shifting of fibres from PWRS to AWRS below the umbilicus (Rizk, 1991). In support of this suggestion, the histomorphometric observations in the current study revealed a gradual decrease in the thickness of PWRS below the umbilicus. Usually the shifting ends abruptly, forming a sharp crescenteric line (Rizk, 1991). In some cases however, the shifting of these fibres may be incomplete such that the PWRS extends all the way to the pubic crest.

Although the arcuate line in the current study occurred as a single structure in all cases, some studies have reported double arcades (Rizk, 1991; Rath et al., 1997). These workers attributed the presence of double arcuate lines to the fact that the deep lamina of internal oblique abdominis crosses to the anterior wall of the rectus sheath at a higher point than the transversus abdominis. The upper arcade would then be formed by the aponeurosis of internal oblique abdominis muscle while the lower one would be formed by transversus abdominis muscle (Rath et al., 1997).

It was noted that the arcuate line was closer to the umbilicus than the pubic crest. These findings are in accord with those of Cunningham et al (2004) but are at variance with the previous descriptions that the arcuate line consistently occurs midway between the umbilicus and pubic symphysis (Williams et al., 1998; Sinnatamby et al., 2000). The variability in the occurrence of the arcuate line has been associated with shifting of fibres from the posterior wall of the rectus sheath to the anterior wall (Rizk, 1991). The observation that the arcuate line may occur more cranial suggests that the shifting of these fibres may be influenced by form of rectus abdominis muscle (Walmsely, 1937). Pertinent to this, in the present study, a more consistent landmark for this line was the most distal intersection of rectus abdominis. In this regard the last tendinous intersection of rectus abdominis muscle may be used as a guide for the arcuate line during rectus abdominis musculocutaneous harvest (Cunningham et al, 2004).

MICROSCOPIC ORGANISATION

Observations of the current study have revealed that the human rectus sheath contains both collagen and elastic fibres. This is in agreement with previous workers (Axer et al., 2001; Szczesny et al., 2006). The view generally held is that collagen fibres provide tensile strength (Diamant et al., 1972; Parry et al., 1978) while elastic fibres allow stretch and recoil of tissues to their original shapes and sizes (Kielty et al., 2002). Accordingly, as suggested by McArdle (1997), the fibroelastic composition of the rectus sheath may be a design to enable it to withstand high levels of biomechanical stresses emanating from the pull of the muscles and also to tolerate as sudden changes in the intra-abdominal pressure in a manner similar to other structures in the anterior abdominal wall such as the linea alba (Pulei, 2006).

Further, the rectus sheath is organized into three distinct zones. Although this is hitherto unreported, other structures in the anterior abdominal wall such as the linea alba exhibit zonation (Axer et al., 2001; Pulei, 2006). The basis for the zonation largely lies on the pattern of formation of the rectus sheath as was observed in the present study. Disparities in fibre composition and orientation in the various zones however suggests that these zones are modified for specific functions. This is pertinent in that the superficial and deep zones of AWRS contain loosely arranged collagen and elastic fibres. Loosely arranged connective tissue fibres function to anchor tissues to one another (Purslow et al., 1998). It is therefore possible that these superficial and deep zones function in anchoring the rectus sheath to the superficial fascia of the anterior abdominal wall and to the rectus abdominis muscle respectively.

The random arrangement of collagen and elastic fibres in the deep zone of the AWRS in the present study suggests a functional adaptation. In support of this is the report by Viidik (1978) that the orientation of connective tissues fibres is greatly influenced by the mechanical activities of tissues around. By virtue of its position, this zone is subjected to multidirectional forces emanating from the pull of rectus abdominis muscle as well as that from the three anterolateral muscles (McArdle, 1997). Accordingly, the multidirectional orientation of collagen and elastic fibres may be designed to accommodate these forces.

Elastic fibres were concentrated in the superficial zone of AWRS and the deep zone of PWRS. It is possible that these are the zones that are subjected to more stretching forces. This proposition derives from the fact that elastic fibres permit long range deformability and passive recoil of tissues (Kielty et al., 2002). It is probable that superficial zone of the AWRS, being the most superficial zone of the rectus sheath, is stretched more when the rectus abdominis contracts during respiration (Walmsely, 1937) and in postural adjustments (Hodges et al., 1997). Presence of elastic fibres in this zone may be designed to allow for passive recoil of the rectus sheath after contraction of rectus abdominis. The deep zone of the PWRS, being the deepest zone, is also likely to be stretched before other zones when intra-abdominal pressure increases. Presence of elastic fibres in this zone may allow gradual transmission of stresses generated by increase in intra-abdominal pressures to the collagenous components of the rectus sheath.

In addition, the elastic fibres in the superficial zone of AWRS and deep zone of PWRS were more concentrated on the lateral parts of the rectus sheath. A similar medio-lateral variation has been reported in the linea alba (Pulei, 2006). This asymmetry in the concentration of elastic fibres suggests that the stretching forces acting on the anterior abdominal wall are not uniformly distributed. These forces appear to decrease towards the linea alba.

Apart from medio-lateral variations in the concentration of elastic fibres in the superficial zone of the AWRS, there was a cranio-caudal increase in these fibres. As elucidated by McArdle (1997), the assumption of upright nature in human beings lead to generation of more intra-abdominal pressure in the lower abdomen as a result of the weight of abdominal viscera. Consequently, presence of more elastic fibres in the lower parts of the rectus sheath could be an adaptation for conferring this region with stretchability to accommodate these forces.

The intermediate zones of the AWRS and PWRS were made of compact bundles of collagen fibres. This organization has also been reported by Axer et al (2001) and appears to be a feature of tissues subjected to biomechanical stresses such as tendons and ligaments (Parry et al., 1978; Birk et al., 1989). It is possible that the intermediate zones of AWRS and PWRS are designed to provide tensile strength to the rectus sheath. Pertinent to this is the report by Parry et al (1978) that the presence of collagen fibres in compact bundles increases the cross-linkages in these fibres leading to increase in tensile strength.

The collagen bundles in the intermediate zone of AWRS exhibited craniocaudal asymmetry. These bundles ran in the same direction as the fascicles of internal oblique muscle (Williams et al., 1995). The lower fascicles of this muscle are transversely disposed while the upper ones slant upwards and medially to be inserted in the costal margin. Regarding the posterior wall of the rectus sheath, the collagen fibre bundles were transversely aligned. This corresponds to the orientation of transversus abdominis muscle (William et al., 1995). As seen in the present study, the deep lamina of internal oblique joins transversus abdominis then they run together all the way to the linea alba. This observation may be a result of common embryologic origin of internal oblique and transversus abdominis muscles (Walmsley, 1937).

The collagen fibre bundles in the PWRS also ran in the same direction as the upper fascicles of transversus abdominis. Consequently, the muscle that exerts more effect in the posterior wall of the rectus sheath is transversus abdominis. It is probable that the entire rectus sheath above the arcuate line takes part in all movements of transversus abdominis such as in respiration (Hodges et al., 1997; Urquart et al., 2005). In this way, contraction of

TA pulls the deep lamina of IO because they are fused. This traction force then draws the superficial lamina of IO together with rectus abdominis muscle. This phenomenon is observed during normal respiration and supports the classification of the upper abdominal wall into a respiratory portion (Rath et al, 1997).

Moreover, the intermediate zone of AWRS from the arcuate line downwards was made of thick bundles of collagen fibre that are transversely oriented. The form of collagen bundle diameter distribution has been related to mechanical properties of tissues (Parry et al., 1978). According to these workers, tissues designed to have high tensile strength have thicker bundles of collagen fibres. Consequently, the modifications in the AWRS below the arcuate line may constitute an adaptation for resisting intra-abdominal pressure that is higher in this region (McArdle, 1997). Pertinent to this are the histomorphometric measurements carried out in the present study that showed a sudden increase in the thickness of the anterior wall of the rectus sheath below the arcuate line.

Collagen fibre bundles in the intermediate zone of PWRS and that of AWRS below the arcuate line exhibited branching. In addition, the collagen fibres in these bundles were wavy. The undulating nature of collagen fibres endow elasticity to tissues, preventing damage e.g. to a tendon during the initial rapid stage of muscular contraction (Diamant et al., 1972; Parry et al., 1978). The network organisation of the collagen fibres may be an adaptation for distributing forces arising from contraction of TA and EO.

CONCLUSION

The microscopic organisation of the rectus sheath is largely influenced by its pattern of formation. The chief muscles that form the rectus sheath are internal oblique and transversus abdominis. These muscles complement each other in forming the anterior and posterior walls of the rectus sheath. The fibre composition and orientation above the arcuate line are suited for generating intra-abdominal pressure while those below this line are for resisting the intra-abdominal pressure generated.

The arcuate line occurs more towards the umbilicus than the pubic crest. In addition, this line corresponds to the most distal intersection of the rectus abdominis muscle. In this regard, the last intersection of the rectus abdominis muscle may be used as a guide for arcuate line during rectus abdominis musculocutaneous flap harvest.

STUDY LIMITATIONS

Autopsy specimens are not ideal for histological analysis as the tissues may undergo autolysis leading to their shrinkage and change of normal parameters such as the thicknesses of the walls of the rectus sheath. This was however delimited by harvesting the tissues within 24 hours after death of the subjects. The anterior wall of the rectus sheath in most cases detached from the rectus abdominis muscle during sectioning of the tissue blocks. This may have influenced the orientation of connective tissue fibres in the deep zone of the anterior wall of the rectus sheath.

SUGGESTIONS FOR FURTHER STUDIES

This study did not focus on subjects below eighteen years. Age related quantitative and qualitative changes in connective tissue fibres have been reported. A study in this age group could provide a basis for comparison of age related changes in the human rectus sheath. In addition, the stains used in this study mainly depicted the general organisation of the connective tissue fibres. The architecture of these fibres could be demonstrated better using scanning electron microscopic techniques.

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APPENDIX I

DATA SHEET FOR GROSS

Case number__________ **Gender** *M/F* **Age** ______

1. **Arcuate line** ☐ *Present* ☐ *Absent*

   Other ______________________________

2. **Distance from the pubic symphysis to the umbilicus** _______**(mm)**

3. **Location of arcuate line (segment)**______

4. **Pattern of formation of RS**

   ☐ *IO splitting to enclose RA*

   ☐ *TA splitting to enclose RA*

   ☐ *Both lamina of TA posterior to RA*

5. **Formation of AWRS**

   ☐ *Aponeurotic*

   ☐ *Muscular*

6. **Formation of PWRS**

   ☐ *Aponeurotic*

   ☐ *Muscular*

Other comments:

APPENDIX II

DATA SHEET (HISTOLOGY)

Case number ____________________ Gender M/F Age ________

1) Above the Umbilicus

a) Medial part

____

____

____

b) Middle part

____

____

____

c) Lateral part

____

____

____

2) At the umbilicus

a) Medial part

____

____

____

b) Middle part

____

____

____

c) Lateral part

____

____

____

3) Below the umbilicus /At the arcuate line

a) Medial part

b) Middle part

c) Lateral part

4) Below the arcuate line

a) Medial part

b) Middle part

c) Lateral part

APPENDIX III

CONSENT FORM

Study No: ____________

Aim

To study the rectus sheath (in the anterior abdominal wall) in males and females.

Benefits

The findings in this study will contribute useful knowledge regarding surgery involving the anterior abdominal wall. You will not incur any costs or risks by allowing your next of kin to take part in this study. Denial of consent is duly respected.

Procedure for tissue harvesting

The procedure for tissue harvesting is like that during post mortem. The rectus sheath lies below the skin. So as not to disfigure the anterior abdominal wall the rectus sheath will be separated from the skin. Very small blocks ($0.25cm^2$) of tissue will then be harvested after which the anterior abdominal wall will be closed by stitching. No other organ will be harvested. Further, the used blocks of tissues will be buried in Lang'ata cementry.

Confidentiality

All information will be treated with utmost confidentiality. The name of the subject will not appear on the data sheets or in 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/ thumbprint: ____________ Date: ____________

I, the investigator having explained in detail the purpose for 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: ____________

APPENDIX IV

KIBALI

Nambari ya uchunguzi ________

Lengo

Lengo la uchunguzi huu ni kufafanua mabadiliko ya kimaumbile kulingana na jinsia katika ukuta wa misuli ulio mbele ya tumbo.

Manufaa

Matokeo ya uchunguzi huu yatachangia kwenye upasuaji ukuta huu wa misuli. Kumwachia marehemu atumike katika uchunguzi huu si lazima na hauna gharama yeyote.

Utaratibu

Utaratibu wa kukusanya vipande vitakavyotumiwa katika uchunguzi huu ni kama ule wa kuchunguza chanzo cha kifo cha marehemu (post mortem). Mwili wa marehemu hautaharibiwa kwani ukuta huu wa misuli uko chini ya ngozi, ambayo haitakatwa. Pia, ni vipande vidogo vitakavyochukuliwa kwa uchunguzi. Baada ya haya, ukuta huu utashonwa pamoja. Hakuna kiungo kingine kitachukuliwa. Vipande vitakavyobaki vitazikwa katika makaburi ya Lang'ata.

Nathibitisha nimeyafahamu aliyonieleza mtafiti na nimekubali kwa hiari yangu mwenyewe kwamba marehemu atumike kwenye uchunguzi huu.

Tarehe ________________

Sahihi ________________

Mimi mchunguzi nimeeleza kwa kina lengo la uchunguzi huu na naapa kutimiza usiri wa matokeo yote ya uchunguzi huu.

Tarehe ________________

Sahihi ________________

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31st May 2007

Philip Maseghe Mwachaka
Third year medical Student
Dept. of Human Anatomy
University of Nairobi

Dear Philip

RESEARCH PROPOSAL: "THE HUMAN RECTUS SHEATH: FORMATION AND CONNECTIVE TISSUE FIBRE COMPOSITION." (UP56/3/2007)

Your above revised research proposal refers.

This is to inform you that permission has been granted by the KNH-Ethics & Research Committee to carry out research on "The Human Rectus Sheath: Formation and Connective Tissue Fibre Composition".

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 sincerely

AN Guantai

PROF A N GUANTAI
SECRETARY. KNH-ERC

c.c. The Deputy Director CS, KNH
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