THE ROLE OF COLLAGEN COMPOSITION IN THE AETIO-PATHOGENESIS OF INGUINAL HERNIA

Dr. SIVA PRASANNA K

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Master of Surgery in General Surgery

Under the guidance of

Professor. Dr. VIMAL KUMAR GOVINDAN

Department of General Surgery

P.S.G Institute of Medical Sciences &Research, Coimbatore

Tamil Nadu Dr. M.G.R Medical University, Chennai

MAY 2018

ENDORSEMENT BY THE HOD, DEAN / HEAD OF THE INSTITUTION

This is to certify that this dissertation entitled **"THE ROLE OF COLLAGEN COMPOSITION IN THE AETIO-PATHOGENESIS OF INGUINAL HERNIA"** is a record of bonafide research work done by **Dr. Siva Prasanna K**, under the guidance of **Dr. Vimal Kumar Govindan,** in the Department of General Surgery, PSG Institute of Medical Sciences and Research, Coimbatore – 641004.

Dr. Premkumar .S
HOD & Professor of Surgery
PSG IMS&R,
Coimbatore – 641004.

Dr. S. Ramalingam
Dean,
PSG IMS&R,
Coimbatore – 641004.

CERTIFICATE

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Dr. Vimal Kumar Govindan
Guide
Professor of Surgery,
PSG IMS&R,
Coimbatore – 641004.

Dr. S. Shanthakumari,
Co guide
Professor of Pathology,
PSG IMS&R,
Coimbatore – 641004.

DECLARATION

I, **Dr. Siva Prasanna .K,** solemnly declare that this dissertation **"THE ROLE OF COLLAGEN COMPOSITION IN THE AETIO-PATHOGENESIS OF INGUINAL HERNIA"** is a bonafide record of work done by me in the Department of General Surgery, PSG Institute of Medical Sciences & Research, Coimbatore, under the guidance of Dr.Vimal Kumar Govindan, Professor of Surgery. This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai, in partial fulfillment of the University regulations for the award of MS Degree (General Surgery) Branch-I, Examination to be held in April 2018.

Dr. Siva Prasanna .K,

CERTIFICATE – II

This is to certify that this dissertation work titled **"THE ROLE OF COLLAGEN COMPOSITION IN THE AETIO-PATHOGENESIS OF INGUINAL HERNIA"** of the candidate **Dr. Siva Prasanna .K,** with Registration Number **221511502** for the award of Master of Surgery in the branch of General Surgery. I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **7%** of plagiarism in the dissertation.

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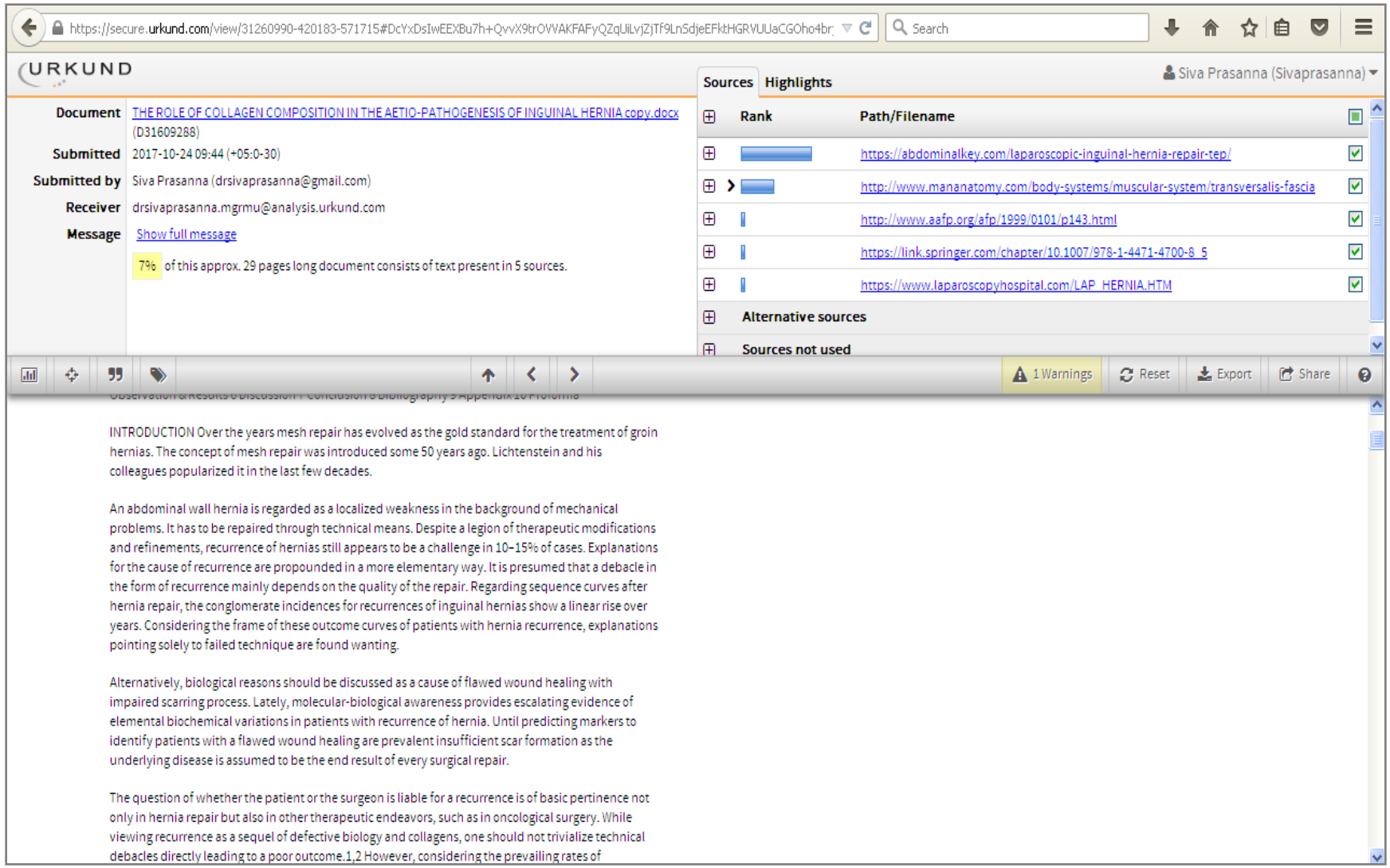

INTRODUCTION Over the years mesh repair has evolved as the gold standard for the treatment of groin hernias. The concept of mesh repair was introduced some 50 years ago. Lichtenstein and his colleagues popularized it in the last few decades.

An abdominal wall hernia is regarded as a localized weakness in the background of mechanical problems. It has to be repaired through technical means. Despite a legion of therapeutic modifications and refinements, recurrence of hernias still appears to be a challenge in 10–15% of cases. Explanations for the cause of recurrence are propounded in a more elementary way. It is presumed that a debacle in the form of recurrence mainly depends on the quality of the repair. Regarding sequence curves after hernia repair, the conglomerate incidences for recurrences of inguinal hernias show a linear rise over years. Considering the frame of these outcome curves of patients with hernia recurrence, explanations pointing solely to failed technique are found wanting.

Alternatively, biological reasons should be discussed as a cause of flawed wound healing with impaired scarring process. Lately, molecular-biological awareness provides escalating evidence of elemental biochemical variations in patients with recurrence of hernia. Until predicting markers to identify patients with a flawed wound healing are prevalent insufficient scar formation as the underlying disease is assumed to be the end result of every surgical repair.

The question of whether the patient or the surgeon is liable for a recurrence is of basic pertinence not only in hernia repair but also in other therapeutic endeavors, such as in oncological surgery. While viewing recurrence as a sequel of defective biology and collagens, one should not trivialize technical debacles directly leading to a poor outcome.1,2 However, considering the prevailing rates of

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INTRODUCTION

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AIM OF THE STUDY

To compare the composition of collagen in the tissues – Skin and Transversalis fascia from the abdominal wall of patients with inguinal hernia and age matched controls with no hernia.

To compare the Collagen I/Collagen III ratio in transversalis fascia and skin in both cases and controls.

REVIEW OF LITERATURE

HISTORY OF INGUINAL HERNIA SURGERY

Hernia (from Greek hernios – meaning to bud or offshoot) is a well known and a widely dealt with surgical disease throughout history.[7]It is one of the diseases that haunted humanity from the very beginning. Ancient surgeons treated hernias based on their size and the threat they posed to the patient. Although the natural course of the disease is insidious it eventually reaches the size that severely impairs the patient ability to perform daily activities (Fig1). This is why surgeons and physicians alike were trying to find the solution for this highly disturbing and if left untreated deadly condition.

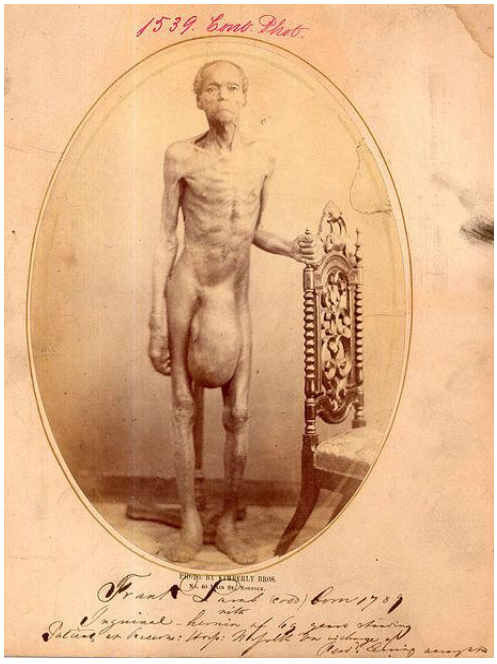


Fig 1: Frank Lamb, a slave in North Carolina with a 69 year history of groin swelling. Nevertheless, he was made to toil in the plantations of the southern states of America

There are five eras described for the evolution of Hernia surgery.[7]

The first era dates back to Ancient Egypt where the first documented description of a hernia was discovered in the Papyrus of Ebers. A cough impulse was first described in this manuscript. Galen of Greece also contributed a lot to understanding hernia after this period. Hippocratic corpus states that hernia was the result of drinking water from large rivers.

The second era began during the renaissance in Europe. Anatomy as a science flourished during this era and surgeons had a deeper insight into the subject of hernia. The mainstay of treatment remained the use of various types of inguinal belts that were supposedly designed to maintain the hernia sac inside the body cavity. To successfully apply the herniary belt the hernia was first manually reduced and then, the custom-made herniary belt was applied. The use of hernia belts was widespread and even today it can be found in some regions of the world. The wide popularity of the belts was maintained because the surgical option for the cure of inguinal hernia was extremely dangerous and unfortunately not very convincing.

The third era is termed ‘Era of Hernia repair under tension’. One of the first attempts to solve inguinal hernia by the means of surgical knife came from the famous 14th century Italian anatomist, Gabriele Fallopio. Fallopio advanced the

idea of wide excision of the sac with the ensheathed skin and all its contents, securing the neck with a significant suture (so-called golden stitch).This technique did not enjoy widespread acceptance, especially among patients because it resulted in castration and sometimes in the permanent stoma from the cut intestinal loop. The risk of death from bleeding and peritonitis was also an important limiting factor of this primitive technique. This is why many 'barber-surgeons' of that time suggested that the Fallopio operation should be considered only for marked hernias, which could not be held even with the strongest and sturdiest bands at their right place.[8,9]Another attempt worth mentioning was by the London surgeon Claudius Amyand who in 1735 operated on an 11-year-old boy. The patient suffered from a right inguinal hernia complicated by a fecal fistula. The operation performed by Amyand is important for two reasons. First, it is the earliest description of a hernia containing a vermiform appendix (known even today as Amyand's hernia). And second, it is the earliest documented appendicectomy in the history of surgery. The child died due to sepsis a few days later.

The age of asepsis brought about by Joseph Lister marked the beginning of this era. The three cardinal features of hernia surgery performed during this period are

1. Strict adherence to aseptic techniques.
2. High ligation of the hernial sac.

3. Narrowing the internal inguinal ring.

The results of surgeries performed during this era were disappointing. There was a high recurrence rate and significant mortality. The introduction of antisepsis, asepsis, anesthesia and anatomical clarity allowed for safer procedures. Among techniques that received some fame among renaissance surgeons, we should mention the techniques of William Wood, Vinzenz Czerny and James Heaton. Heaton was performing injection of the mixture of white oak and morphine into the hernia sac to obtain its fibrosis.[10]Czerny was performing the high ligation of the hernia sac and complete closure of the internal inguinal ring with sutures.[11]In the Wood's method, the surgeon was supposed to double ligate hernial sac to perform a natural 'plug' and use it to close internal inguinal ring. Although these techniques looked appealing at first, virtually all patients experienced hernia recurrence. All these procedures had an unacceptable mortality rate of over 7%.[11]

The fourth era began with Eduardo Bassini introducing the technique of strengthening the posterior wall of the inguinal canal. This became the fourth and a vital component of hernia surgeries performed thereafter. Bassini suffered a bayonet wound to his right groin that resulted in a caecal fistula. He remained a patient at Pavia for more than 6 months. His Caecostomy healed but during this time he studied meticulously the anatomy of the inguinal region. He realized that

the problem was due to the distorted anatomy and physiology rather than the technique.

Bassini had presented his first results in Padua, Italy during the surgical congress in Genoa and after a few years in Germany to gain a wider audience[12].The significance of Bassini repair lies in the fact that most of the procedures that were conceived later were modifications of this hallmark procedure.

The Halsted's modification left the spermatic cord in the subcutaneous plane so that the surgeon can perform repair of the posterior wall using deep transfixation sutures. This resulted in troublesome urinary fistulae.

The Polish technique propounded by Slawinski of Warsaw consisted of dissecting, ligating and cutting only the neck of the sac. The divided sac is left insitu. This procedure was carried out on a cardinal who would later become Pope Pio IX.

Canadian surgeon E.Shouldice advocated strengthening the posterior wall using four layers of fasciae and aponeurosis. This was considered the 'last big step' in a barrage of Bassini repair modifications. In his world-renowned clinic, he performed thousands of hernia repairs. He insisted that anyone keen on performing the surgery should visit his clinic and witness the 'Canadian' repair. His patients

were ambulant in 3 days and recurrence rate 3% as against patients in other centers who were advised strict bed rest for 3 weeks and had a dismal recurrence rate of 20%. His patients include several prominent monarchs and presidents.

Next began the era of tensionless hernia surgery. The tension in the suture line was reduced initially by incisions over the rectus sheath. Norman Tanner brought about this method as a tension reducing approach to conventional surgeries performed then. His 'slide operation' was meant for both inguinal and femoral hernias[8]. The Bassini operation was later denounced in many centers and remained only in academic interest.

There were more than 70 modifications for the Bassini repair. This very plethora of modifications is by itself a hostile piece of evidence against the Bassini repair. In anatomy, the function of muscles is motion, locomotion and stabilization and not a buffer for a weakened posterior wall.

Reasons for the failure of Bassini repair are many. Uniting muscle with fascia is against the rules of anatomy. This can take place only when the areolar tissue in the region has been meticulously removed and tight tying of sutures thus effecting a fibrin reaction and connective tissue formation. The surface area of contact between muscle and fascia also needs to be greater.

The Bassini operation fails[13]if applied to cases not demanding a reinforcing procedure like cases of indirect hernia where the muscles are undamaged by distension and the rings sound. In the direct hernias where it is required, the conjoined tendon may be weak or have anomalous insertions into the rectus sheath. This makes repair in the medial portion (the area most prone to recurrence) demanding. Even when used efficiently the muscle does not function properly as it is pulled away from the normal line of direction of its fibers. Slack sutures are ineffective and tight sutures cause pressure necrosis of the vital lower part of the conjoined tendon.

The era of plastics was ushered into herniology and foreign materials were introduced as means of reducing tension between tissues.

The terms ‘onlay’ and ‘sublay’ were in reference to the position of mesh in relation to the posterior wall ofthe inguinal canal. The ‘onlay’ technique consisted of placing the mesh superficial to the posterior wall and ’sublay’ position requires formation of space for mesh in the preperitoneal space.

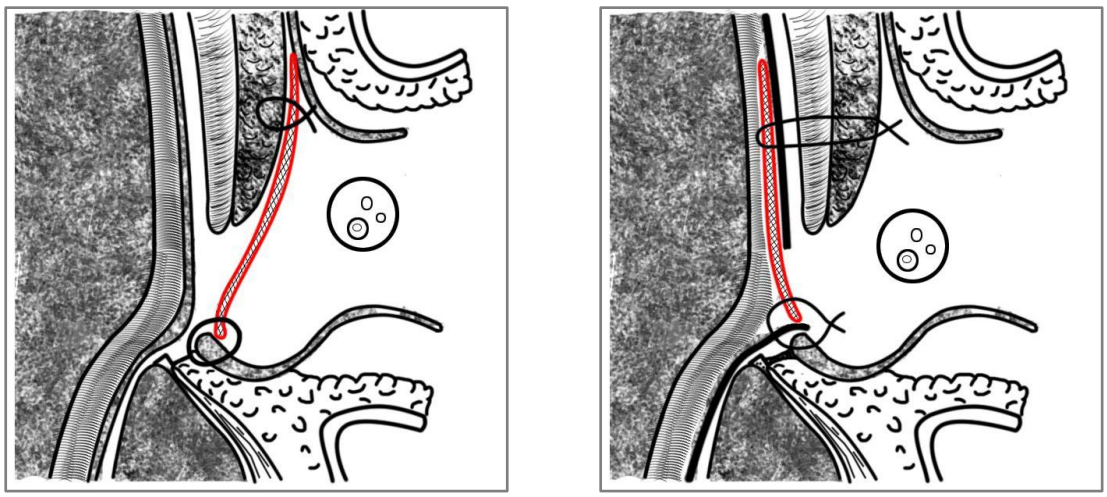


Fig 2: Onlay [A] and Sublay [B] positions of mesh

Irving Lichtenstein published his paper on 1000 surgeries performed using[14] Marlex mesh. Marlex is a high-density polyethylene which is loosely woven to form a mesh which is quite pliable. It is well tolerated by the tissues even in the presence of infection.

The Marlex mesh currently available is made of knitted polypropylene fiber, which is more pliable and has a higher melting point [335 F]. This permits sterilization by autoclaving. The original Marlex mesh had to be boiled in water, cold-sterilized, or gas sterilized for reuse [melting point 270F]. It stimulates fibroblastic proliferation.

Lichtenstein himself said to incise a strong posterior layer and, then, to reconstruct it as in the Bassini, Shouldice or McVay repair is inappropriate, disruptive and even meddlesome. The application of a wide sheet of harmless

prosthetic mesh, one which serves only to strengthen such a floor, is harmless and should reduce the incidence of recurrences.[15]

More recently in 1999, Arthur Gilbert described a technique that allows placing a mesh both in 'onlay' and 'sublay' position. His Prolene Hernia System consisted on introducing a complex mesh build from two meshes of distinctive shapes connected with a small tube. This allowed reinforcing the posterior wall of the inguinal canal both from pre-peritoneal site and from the 'onlay' position.[16,17]

PREPERITONEAL REPAIRS:

Rene Stoppa in1969 developed a technique of Giant prosthetic reinforcement of the visceral sac. This technique was supposed to be applied to large, complicated and bilateral inguinal hernias and consisted on implanting a large polyester mesh in pre-peritoneal connective tissue between the peritoneum and fascia transversalis. The incision of choice for preperitoneal access was a low midline incision and the mesh need not be fixed with sutures due to its size and intraabdominal pressure maintaining it in situ.[18]Another preperitoneal hernia repair was described in 1976 by Lloyd Nyhus from Chicago. Unlike in the Stoppa method, the incision was made above the inguinal ligament. A similar incision was used also for a pre-peritoneal placement of a sutureless mesh by Robert Kugel from Olympia in his technique described in 1999 and coined Kugel Hernia Patch.[19]

LAPAROSCOPIC REPAIR:

With the advent of computer chip technology, laparoscopic picturing and treatment of inguinal hernia got introduced in the surgical field.Ralph Ger was the first in 1982 to report a transabdominal closure of an inguinal hernia defect during a laparoscopy for other reasons. Some years later, in 1989, the gynecologist S.Bogojavalensky showed a video demonstrating the laparoscopic intraabdominal incision of the peritoneal hernia sac, subsequently closing the visible muscular defect with a rolled-up piece of polypropylene mesh.

The early 1990's saw a rapid rise in the number of publications, confirming the feasibility of laparoscopic hernia repair. Although the first interventions were limited to a plug and patch repair, later transabdominal approaches opted for the fixation of a large preperitoneal mesh, either sutured or stapled to the posterior muscular wall. A first attempt was made by applying a synthetic mesh to the peritoneal wall and got the name IPOM (IntraPeritoneal Onlay Mesh).

Another approach involved making an intraperitoneal U-type incision in the peritoneal wall and introducing the mesh in a preperitoneal position. It became known asthe TAPP technique (TransAbdominal PrePeritoneal approach).Soon other surgeons proposed a complete extraperitoneal insertion of the preperitoneal mesh, namely Dulucq in 1992, Ferzli et al. in 1992, Himpens in 1992, and Barry

Mac Kernan and Laws in 1993.It became known as the TEP technique (Total ExtraPeritoneal approach). Even a special balloon dissector was introduced to facilitate this extraperitoneal approach.

ANATOMY OF THE ABDOMINAL WALL

The abdominal wall is made up of muscles and fascia which cover the anterolateral area between the xiphoid process and a line that goes through the iliac crests, femoral arches, and pubis.

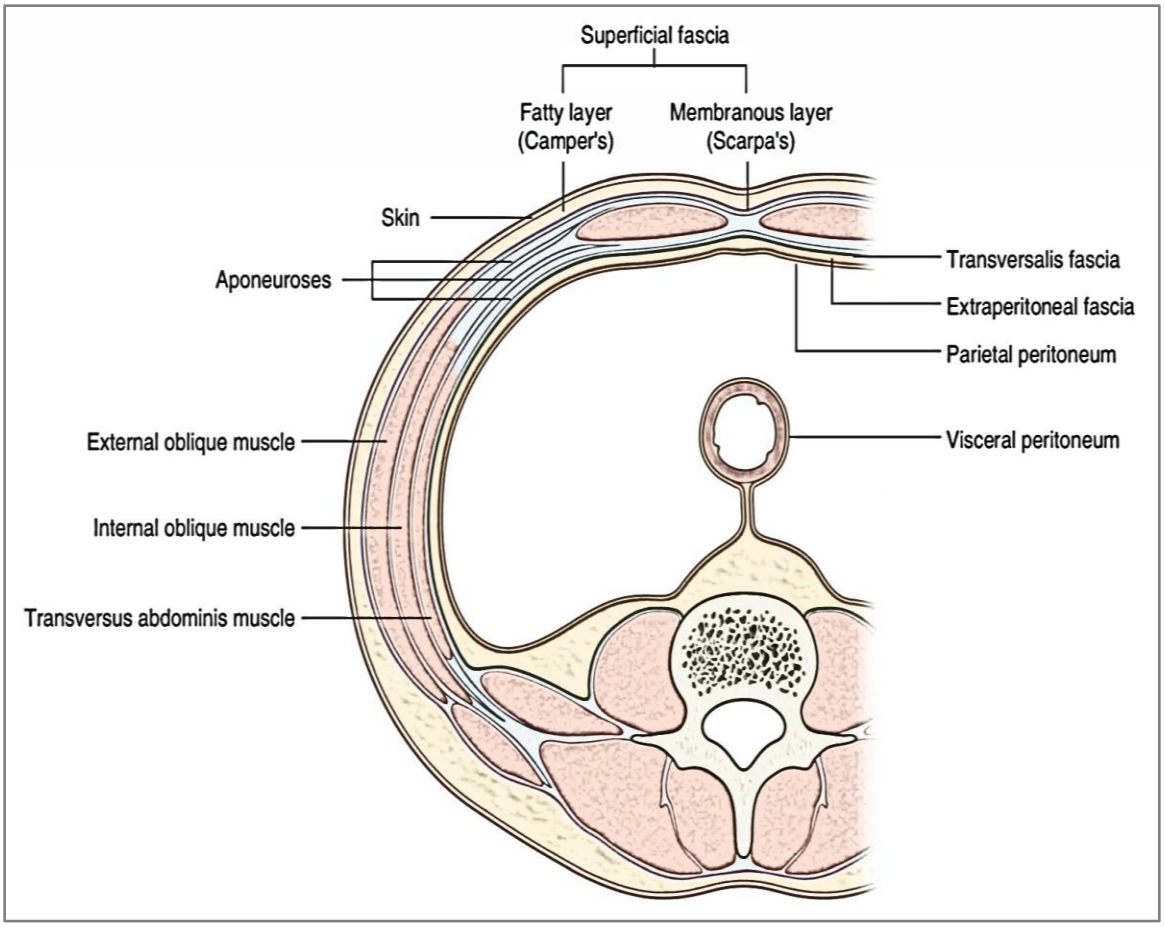


Fig 3: Layers of the abdominal wall

Skin (Integument)

The epidermis has great ability to regenerate because its nutrition comes via diffusion from the underlying vascular planes. Arterial vessels form a subdermal plexus from which some branches leave to enter the subcutaneous tissue. In the subdermal plexus, arteriovenous anastomoses exist; some of these are glomus under the control of the autonomous nervous system. The area least irrigated is the midline of the abdomen as the plexus comes from the back to the front area. The subdermal lymphatics are anastomosed at all levels, so a free exchange is produced between regions.

Subcutaneous Tissue (Adipose Tissue or Hypodermis)

This consists of areolar tissue and/or unilocular white adipose tissue according to the constitution of the individual and the subject's nutritional status and according to factors of hereditary.

The mobilization and deposition of lipids are influenced by nerve factors (nor adrenaline, which activates the lipase) and hormonal factors (insulin, thyroid hormones, glucocorticoids, and pituitary hormones).

In the subcutaneous tissue, the blood vessels are the vessels from perforating cutaneous branches of the direct and subdermal plexus. The nerves are perforating branches coming from the intercostal and first lumbar nerves.

Musculoaponeurotic Plane

This is composed of the Transversus abdominis, Internal oblique muscle, External oblique muscle and Rectus abdominis. Transverse and oblique muscles go forward internally and externally forming the rectus sheath and the white line. Blood supply of these muscles depends on the epigastric vessels, which come up from the external iliac vessels to get to the internal mammary artery and vein, in the thorax. Epigastric vessels are included in the rectus muscle inside the sheath.[20-25]

Extraperitoneal or Subperitoneal Space

This is placed between the inner surface of the abdominal wall, roofed by its fascia, and the parietal peritoneum. It encloses vessels, nerves, organs, and extra-peritoneal adipose tissue, in a variable arrangement according to regions and subjects.

We can identify the following regions or extra-peritoneal spaces[26-31]:

- Lateroperitoneal spaces: at the level of the iliac fossa internal to the external iliac vessels, gonadal, and nerve genitocrural.
- Preperitoneal spaces: at the round ligament and lower and include prevesical space (Retzius) and paravesical space (Bogros).

- Pelvic subperitoneal space: comprised of a visceral mediastinum, laterovisceral spaces.
- Retroperitoneal spaces

Transversalis fascia is a thin layer of fascia lining the transversus abdominis muscle. It lies between the transversus abdominis and the extraperitoneal fascia.It is imperative to understand that the transversalis fascia, the diaphragmatic fascia, the iliacus fascia, and the pelvic fascia form one continuous lining to the abdominal and pelvic cavities. They are named according to the structures they are associated with. The femoral sheath in the lower limbs is formed from this fascia, in addition to the iliacus fascia

Borders and attachments of transversalis fascia:

- **Posterior aspect:** In the posterior aspect, the transversalis fascia is lost in the fat covering the posterior surface of the kidneys.
- **Inferior aspect:** Inferiorly, the transversalis fascia is attached to the iliac crest in its entire length and posterior margin of the inguinal ligament.
- **Medial to femoral vessels:** In this region, the fascia is thin and attached to the pubis and pectineal line. It descends in front of the femoral vessels to form the anterior wall of the femoral sheath.

- **Beneath the inguinal ligament:** In this region, it is strengthened by a band of fibrous tissue that loosely connects to the inguinal ligament and forms the iliopubic tract.

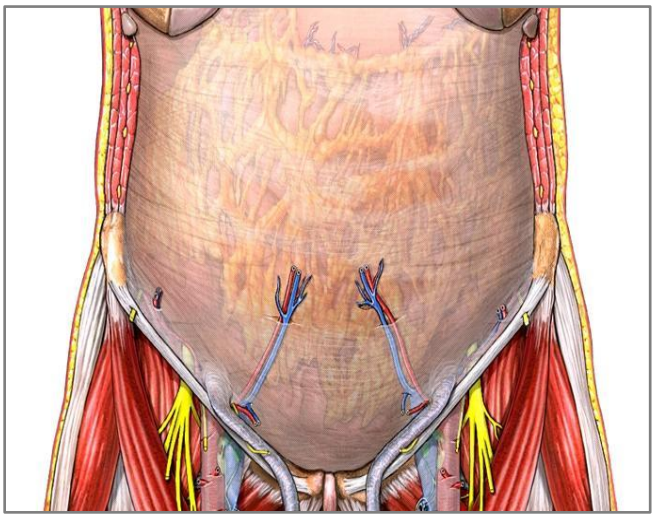


Fig 4a: Fascia transversalis

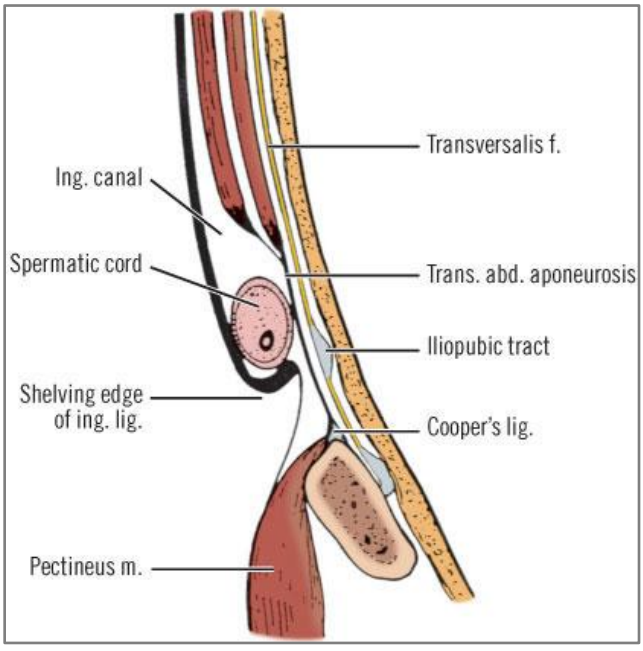


Fig 4b: Sagittal section – Inguinal canal

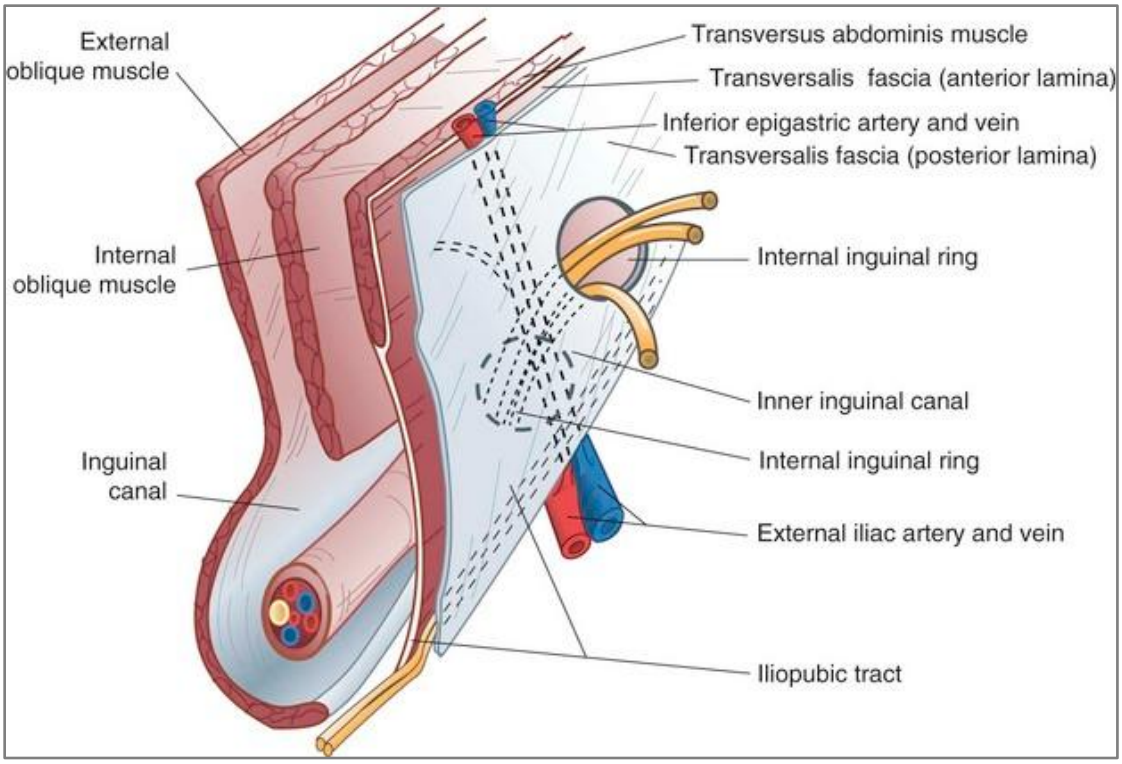


Fig 4c: Relation of the fascia to the structures in the Inguinal Canal

The iliopubic tract[32] (Thomson ligament) is a band in the posterior inguinal region distinct from the inguinal ligament and is sometimes known as the deep crural arch. The advent of laparoscopic approaches to herniorrhaphy has recently heightened awareness of the importance of the iliopubic tract. The histological composition of the iliopubic tract is strikingly different from the inguinal ligament, being high in elastin relative to collagen.

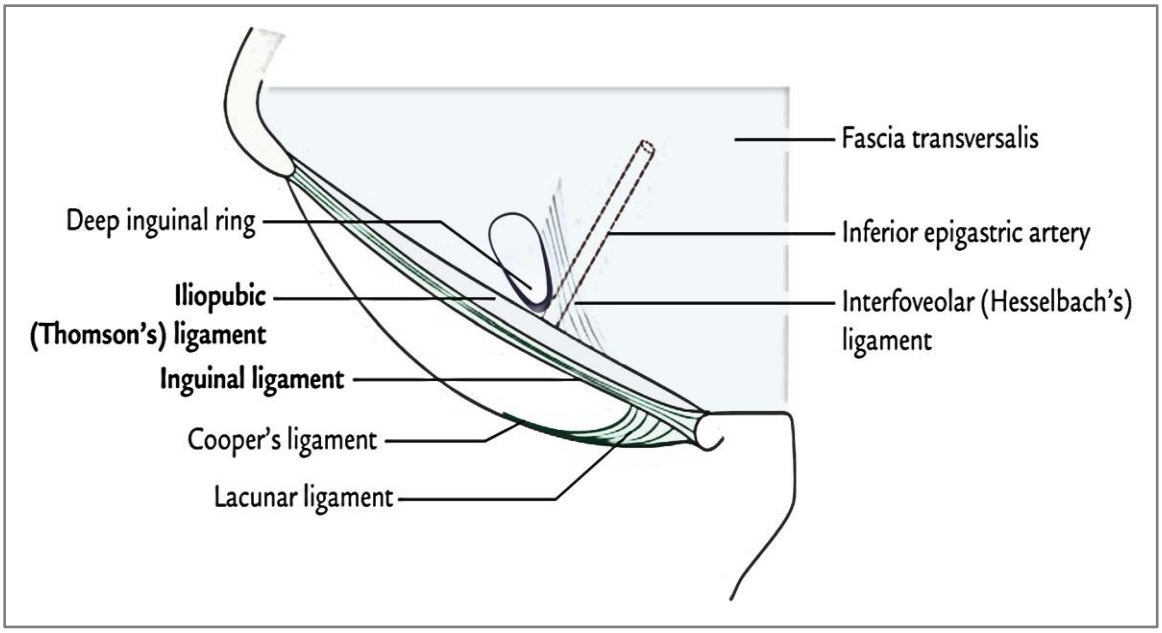


Fig 5: Iliopubic tract

Opening in the transversalis fascia (Deep inguinal ring):

The transversalis fascia is called the Achilles heel of the abdomen. It has an oval opening near the midpoint of the inguinal ligament Fig 4c. This opening is known as the deep inguinal ring. It transmits the spermatic cord (in males) or round ligament of the uterus (in females).

The opening in the fascia is not visible externally. It is very hard to locate during dissection of a cadaver. The reason is that transversalis fascia is prolonged on the structures passing through the opening as the internal spermatic fascia. Internally there is an established passage but externally one can see nothing.

Peritoneum–This holds the intraperitoneal organs and is divided into areas and regions that are useful in surgical exploration and intraperitoneal pathways and structures ideal for transperitoneal/ extraperitoneal approach.

Areas of Weakness the in the inguinal region:

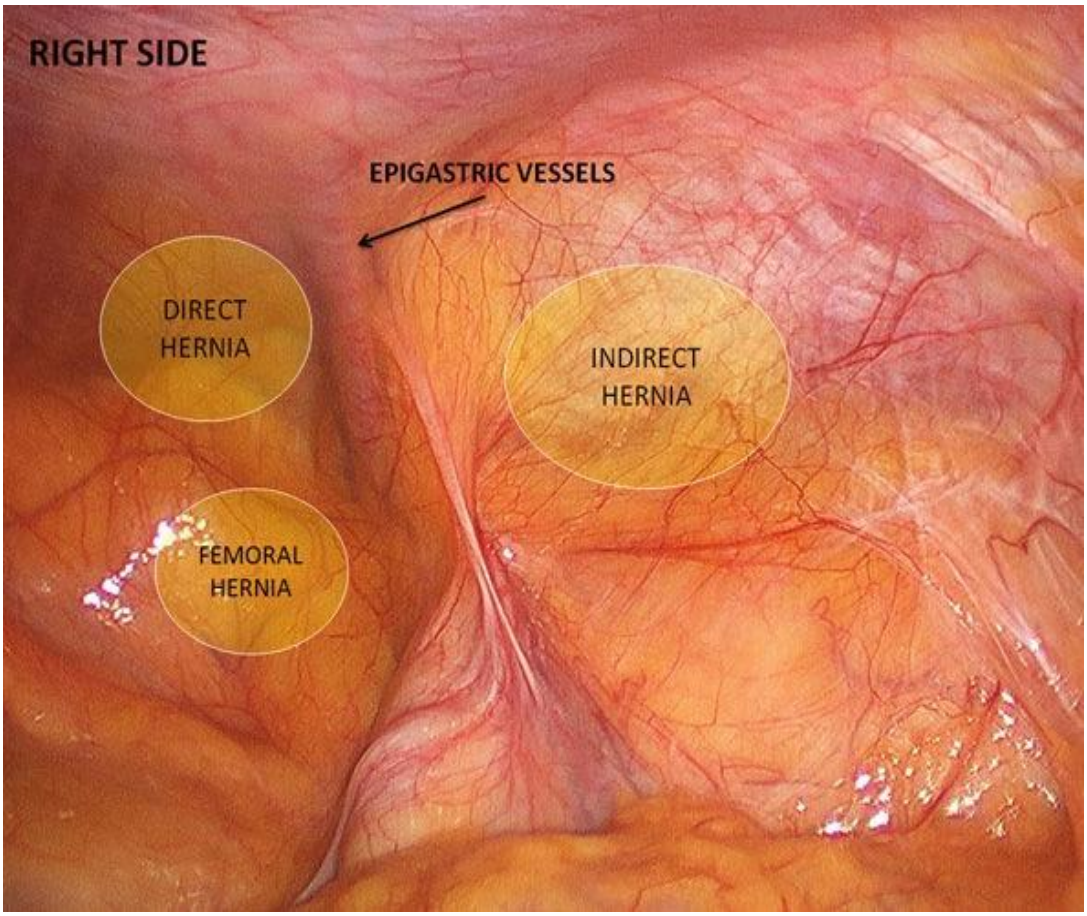


Fig 6: Inguinal hernia: weak areas in inguinocrural region

The area of inguinal weakness is oval-shaped and is limited superiorly by the conjoined tendon (inferior border of the abdominal internal and transverses oblique muscle) and inferiorly by the inguinal ligament.

The Hesselbach's triangle is an anatomic landmark and is bounded by the rectus muscle medially, the inguinal ligament inferiorly, and the inferior epigastric vessels laterally. Indirect inguinal hernias are produced by the area of weakness created by the deep inguinal orifice, in such a way that the hernia sac is directed into the interior of the spermatic cord towards the testicle. Direct inguinal hernias are produced by the area of weakness localized medial to the deep inguinal orifice so that the inguinal canal is only covered by the transversalis fascia.

BIOLOGY OF COLLAGEN

Collagen is the ubiquitous protein of mammals. The term collagen is derived from the Greek word – '*Kolla*' meaning 'glue' and '*gen*' meaning 'to produce'. This is related to the practice of the ancient Greeks who used crude collagen as glue. They obtained this viscous substance by boiling the hides and tendons of horses. Such glue was used to hold together and preserve manuscripts like the Dead sea scrolls.[33]

In Chinese medicine, it is stated for millennia to consume animal cartilage to treat joint disorders.

Within the body of both vertebrates and invertebrates, collagen is the predominant structural biopolymer of extracellular connective tissue matrix. It contributes to 25-35% of the protein content of the whole body. It is produced by fibroblasts and is extracted into the extracellular space. The arrangement of collagen determines the mechanical response to stress. The tensile strength of the tissues depends on the degree of mineralization of the predominant collagen that makes up the tissue. This can result in rigid (bone) to compliant (tendons) tissues.[34]

Collagen occurs in many places throughout the body. Over 90% of the collagen in the human body, however, is Type I.[35]

So far, 29 types of collagen have been identified and described. They can be divided into several groups according to the structure they form:

- Fibrillar (Type I, II, III, V, XI)
- Non-fibrillar
    - FACIT (Fibril Associated Collagens with Interrupted Triple Helices) (Type IX, XII, XIV, XVI, XIX)
    - MACIT (Membrane Associated Collagens with Interrupted Triple Helices) (Type XIII, XVII)
    - Multiplexin (Many Triple Helix domains with breaks) (Type XV, XVIII)
    - Short chain (Type VIII, X)
    - Basement membrane (Type IV)
    - Other (Type VI, VII)
- Type I: Skin, tendon, vascular ligature, connective tissue, bone (main component of the organic part of bone)
- Type II: Cartilage (main collagenous component of cartilage)
- Type III: Reticulate (the main component of reticular fibers), commonly found alongside type I.
- Type IV: Forms basal lamina, the epithelium-secreted layer of the basement membrane.

- Type V: Cell surfaces, hair and placenta
- Type VI: Collagen VI is a form of collagen primarily associated with the extracellular matrix of skeletal muscle.
- Type VII: Forms anchoring fibrils between the basal and reticular laminae of the basement membrane
- Type VIII: Endothelial cells of cornea, keratinocytes, mast cells, microvascular endothelial cells and some tumor cells, sclera, skin and glomerulus (Mesangial cells)
- Type IX: Collagen component of Hyaline cartilage
- Type X: Collagen expressed by chondrocytes during enchondral ossification
- Type XI: Part of the inner ear and the nucleus pulposus, which is the of the vertebrae.
- Type XII: Type XII collagen is found in association with type I collagen, an association that modifies the interactions between collagen I fibrils and the surrounding matrix.
- Type XIII: cell adhesion-associated function in a wide array of cell-matrix junctions – Intercalated discs of heart, Talin, Vinculin.
- Type XIV: embryonic heart, notably within the cardiac interstitium of the developing myocardium.

- Type XV: Expressed in fetal kidney and Lung tissue (especially microvasculature). Also found in the interstitium of adult kidneys.
- Type XVI: Found in keratinocytes, and in smooth muscle and amnion
- Type XVII: It is a transmembrane protein that forms a significant part of hemidesmosomes. It binds keratinocytes to the underlying dermo-epidermal junction and its defect is implicated in epidermolysis bullosa
- Type XVIII: Component of basement membrane. It possesses molecular properties of both collagen and proteoglycan. Chemical cleavage of its C terminal end yields endostatin that has marked anti angiogenic properties
- Type XIX: It is also called cuticle collagen. It forms polymers with fibril forming collagen I and maintains the integrity of extracellular matrix
- Type XX: It is very prevalent in the Corneal epithelium, embryonal skin, notochord, neural retina.
- Type XXI: It is almost exclusively localized to tissue containing Collagen I and forms a part of the extracellular matrix.
- Type XXII: It strengthens skeletal muscles and stabilizes myotendinous junctions.
- Type XXIII: It was discovered from rat prostate carcinoma and its presence is correlated with tumor progression in the human prostate. It can be found

in the developing epidermis and other epithelia such as those in tongue, git, pulmonary parenchyma also in the brain, renal tissue and the cornea.

- Type XXIV: It is a marker of osteoblastic differentiation. It is predominantly a skeletal collagen and is expressed in embryonal trabecular bone and periosteum.
- Type XXV: It is expressed in the cell membrane of brain parenchyma and plays a role in the progression of Alzheimer amyloid plaques.
- Type XXVI: It is made of a cysteine-rich Emilin core and two protein stretches. It is investigated as a cause of aspirin-induced asthma.
- Type XXVII: It plays a major role in the calcification of cartilage and furtherance of cartilage to bone.
- Type XXVIII: It forms a part of von Willebrand protein
- Type XXIX: It is a newly discovered epidermal collagen and being studied as a cause of atopic dermatitis.[37]

Thus the collagen superfamily comprises 29 members in vertebrates numbered with Roman numerals (I–XXVIII). The novel skin is collagen called collagen XXIX, but the COL29A1 gene was demonstrated to be akin to the COL6A5 gene, and the α1(XXIX) chain corresponds to the α5(VI) chain.[38]

The universal structural feature of collagens is the presence of a triple helix that can contribute most of their structure (96% in collagen I) to (less than 10% in

collagen XII). The heterogeneity of the collagen family is further increased by the existence of several α chains, multitudes of molecular isoforms and the presence of supramolecular structures for a single collagen type, and the use of many promoters and alternative splicing.

SUPRAMOLECULAR ASSEMBLY OF COLLAGEN:

When viewed through electron microscopy after rotary shadowing, collagen molecules are seen as rods varying in length from almost 75 nm for collagen XII to 425 nm for collagen VII.[39] The molecular structure of collagen XV removed from tissues haveunusual shapes, many molecules being found in a pretzel, knot,figure-of-eight, configuration.[40,41]Presence of non-collagenous domains causes kinks that are viewed in electron microscopy of nonfibrillar collagen preparations. Electron microscopy can also characterize Non-collagenous domains after rotary shadowing.[42]

FIBRIL-FORMING COLLAGENS

Collagens can be divided into subfamilies based on their supramolecular assemblies: fibrils, beaded filaments, anchoring fibrils, and networks as shown in (Fig. 7). Heterotypic collagen fibrils are the ones that are made of several collagen types. In cartilage collagen fibrils are composed of collagens II, XI, and IX or of collagens II and III[43], collagens I and III in the skin, and of collagens I and V in

cornea.[44]Besides collagen fibrils can be thought of as macromolecular alloys made of collagenous and non-collagenous proteins or proteoglycans. Inarguably, small leucine-rich proteoglycans regulate the formation of fibrils, as do collagens V and XIV[45], and could also influence collagen cross-linking.[46]For the process of fibrillogenesis of collagens I and II, collagens V and XI could act as nucleators and fibronectin and integrins as organizers.[47]

Collagen fibrillogenesis has been extensively studied in tendons, although the site of the initial steps of fibrillogenesis is not clearly defined so far. They may take place in extracellularly where fibril intermediates are clustered and mature fibrils grow through a fusion process of intermediates[48]or they may occur intracellularly, in 28-nm-diameter Golgi-to-membrane carriers with fibrils that are targeted to plasma membrane protrusions called fibripositor.[49]

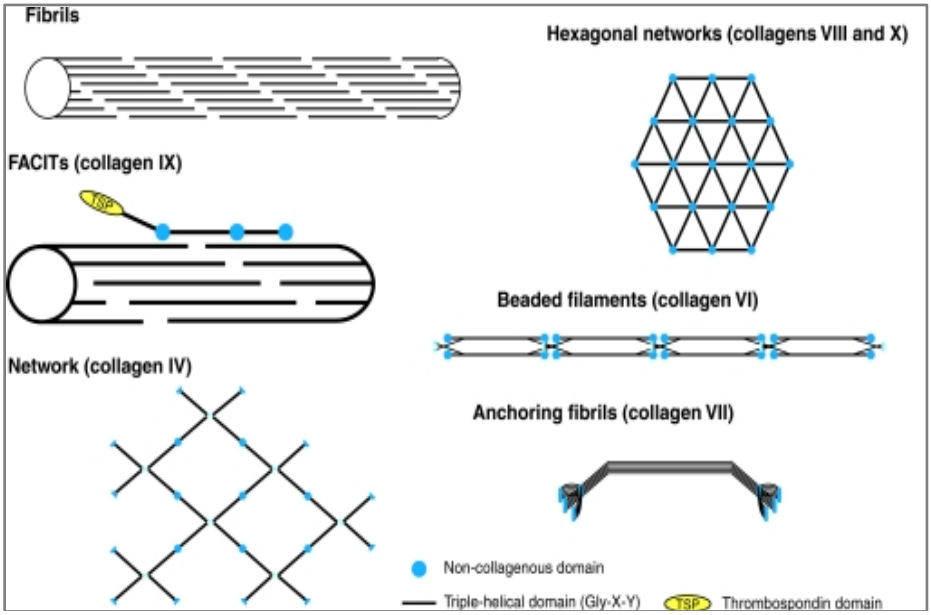


Fig 7: Supramolecular assemblies of collagen

Collagen fibrils exhibit a banding pattern with a periodicity (D) of 64–67 nm[50] and usually collagen molecules are D-staggered within the fibrils. Depending on the tissue collagen fibrils range in diameter from nearly 15 nm up to 500 nm or more.[51,52]The microfibrillar structure of collagen I fibrils has been investigated in situ by X-ray diffraction of rat tail tendons.[53]

Collagen I forms five molecules of supertwisted fibrils that interdigitate with adjacent microfibrils, leading to the quasi-hexagonal packing of collagen molecules.[53]In contrast, cartilage fibrils are composed of a nexus of four microfibrils (two of collagen II and two of collagen XI) wreathed by a ring of ten microfibrils, In cross-section, each microfibril contains five collagen molecules.[54]

FIBRIL-ASSOCIATED COLLAGENS

The FACITs do not form fibrils by themselves, but they are associated with the surface of collagen fibrils. Collagen IX is covalently linked to the exterior of cartilage collagen fibrils mostly made of collagen I.[55] Collagens XII and XIV are associated to Collagen I-containing fibrils. Collagen XV is associated with basement membrane fibrils and forms a bridge linking large, banded fibrils, likely containing collagens I and III.[56]

NETWORK-FORMING COLLAGENS

Collagen IV forms a network in which four molecules assemble to form tetramers via 7S domain, and two molecules assemble to form dimers via their carboxy-terminal NC1 domain. Because the NC1 domains are trimeric, the NC1 dimer is a hexamer. The three-dimensional structure of the hexameric form of the NC1 domain that plays a major role in collagen IV assembly and in the stabilization of the collagen.[57]

Collagens VIII and X form similar hexagonal networks in Descemet's membrane of cornea and in hypertrophic cartilage, respectively. Collagen VI forms beaded filaments and collagen VII assembles into anchoring fibrils connecting the epidermis to the dermis.[58]

Some collagens participate in distinct molecular assemblies in different tissues. Collagen XVI is a component of microfibrils containing fibrillin-1 in skin, whereas it is incorporated into thin, weakly banded fibrils containing collagens II and XI in cartilage.[59,60] Supramolecular assemblies of many collagens are capable of interaction as shown by the anchoring fibrils that are tightly attached to striated collagen fibrils.[61]

COLLAGEN BIOSYNTHESIS

Collagen biosynthesis has been studied in depth for fibril-forming collagens that are formed as procollagen molecules composed of an amino-terminal propeptide followed by a small, nonhelical, N-telopeptide, a central triple helix, a C-telopeptide and a carboxy-terminal propeptide. Individual proα chains are subject to many posttranslational modifications (hydroxylation of proline and lysine residues, glycosylation of lysine and hydroxylysine residues, sulfation of tyrosine residues,[62]that end with the formation of the triple helix. In the endoplasmic reticulum, the heat shock protein 47 (HSP47) binds to procollagen. It is a specific molecular chaperone of procollagen.[63]More than 20 such HSP47 molecules have to bind per triple helix for the stabilization of procollagen at body temperature.[64] It has been recently suggested that intracellular Secreted Protein Acidic and Rich in Cysteine (SPARC) may be a collagen chaperone because it binds to the triple-helical domain of procollagens and its absence leads to flaws in collagen deposition in tissues.[65]

Propeptides of procollagens are cleaved during the maturation process.[66] The N-propeptide is cleaved by proteinases belonging to the Disintegrin And Metalloproteinase with Thrombospondin motifs (ADAMTS) family, except the N-propeptide of the proα1(V) chain that is cleaved by the procollagen C-proteinase also termed Bone Morphogenetic Protein-1 (BMP-1).[67] BMP-1 cleaves the

carboxy-terminal propeptide of procollagens, except the carboxy-terminal propeptide of the proα1(V) chain, that is processed by furin. The telopeptides contain the sites where cross-linking occurs. This process is initiated by the oxidative deamination of lysyl and hydroxylysyl residues catalyzed by the enzymes of the lysyl oxidase family.[68]

COVALENT CROSS-LINKING OF COLLAGENS

Collagen is thought to be an elastic protein with a resilience of approximately 90%. Collagen fibrils are thus able to deform and form back and their mechanical properties can be studied by force spectroscopy.[69]

Cross-linking is tissue-specific and not collagen-specific. Reducible, bifunctional, cross-links (aldimines and keto-imines) are formed in newly synthesized collagens, and they automatically mature into nonreducible trifunctional cross-links, pyridinoline and deoxypyridinoline in bone and cartilage, pyrrole cross-links in bone, and histidinohydroxylysinonorleucine in skin[73] (Fig. 3). Cross-link maturation provides added resistance to shear stress.

Collagens are long-lived proteins that are altered by glycation.[71-75] Glycation expands with age and several advanced glycation endproducts contribute to the progressive insolubilization and to the increased stiffness of collagens in aged tissues. Two lysine-arginine cross-links, pentosidine (a fluorescent product formed

from ribose), and glucosepane (a nonfluorescent product formed from glucose) have been identified in collagens. Glucosepane, the most abundant cross-link in senescent skin collagen, is able to cross-link one in five collagen molecules in the skin of the elderly.[76]

COLLAGEN DEGRADATION

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases belonging to the metzincin superfamily. They partake in physiological (development and tissue repair) and pathological (tumorigenesis and metastasis) processes. Fibril-forming collagens I, II, and III are cleaved by MMP-1 (interstitial collagenase), MMP-8 (neutrophil collagenase), MMP-13 (collagenase 3). Collagen II is a preferential substrate of MMP-13, whereas collagens I and III are preferentially cleaved by MMP-1 and MMP-8.[77-79]Another group of enzymes, collectively called sheddases[80]releases the ectodomain of membrane collagens as soluble forms.

COLLAGEN AND DISEASE

More than one thousand mutations have been identified in 12 out of more than 20 types of collagen. These mutations can lead to various diseases at the tissue level.[81]

Osteogenesis imperfecta – Caused by a mutation in Type 1 collagen, autosomal dominant disorder, results in brittle bones and irregular connective tissue, some cases can be mild while others can be lethal. Mild cases have lowered levels of collagen while severe cases have structural defects in collagen.[82]

Chondrodysplasias – Skeletal disorder believed to be caused by a mutation in Type 2 collagen; further research is being conducted to confirm this.[83]

Ehlers-Danlos syndrome – Six different types of this disorder, which lead to deformities in connective tissue, are known. Some types can be lethal, leading to the rupture of arteries. Each syndrome is caused by a different mutation, for example, type four of this disorder is caused by a mutation in **collagen type 3**.[84]

HERNIA AND COLLAGEN BIOLOGY:

All techniques employed for hernia repair have to depend on the formation of adequate scar tissue. The scarring process is a form of flawed healing replacing physiological tissues by fibrotic tissues that are rich in fibroblasts and collagens. It forms from a complex network with interactions of many mediators of wound healing and an intensive cross talk between cells, in particular macrophages. Because of the long half-life of the collagens when compared to other growth factors and cytokines, the collagens may reflect best the altered regulation of the scarring process, though changes in further components of the extracellular matrix

have been observed, such as in the expression of the matrix metalloproteinase 2 (MMP-2).[85-90]

Therefore, it is not amazing that a decreased collagen type I/III ratio could be verified in adult patients with groin hernia (and in the scar of patients with recurrent hernia.[91,92] Collagen type I is the hallmark for mature scars or fascial tissue whereas the collagen type III represents the mechanically unstable, sparsely cross-linked collagen formed during the early periods of wound healing.

In patients with recurrent hernias, there seems to be an impaired maturing of their scar tissue, which is not able to close the hernial gap or fix the mesh in place for long. As a result, a recurrence may develop either through a scar or at the border of a synthetic mesh through its defective and flawed fixation.[93] Interestingly, an altered function could be detected even *in vitro* in macrophages' free cultures of fibroblasts from patients with recurrent hernia, indicating a built-in genetic and thereby probably systemic problem.[94]

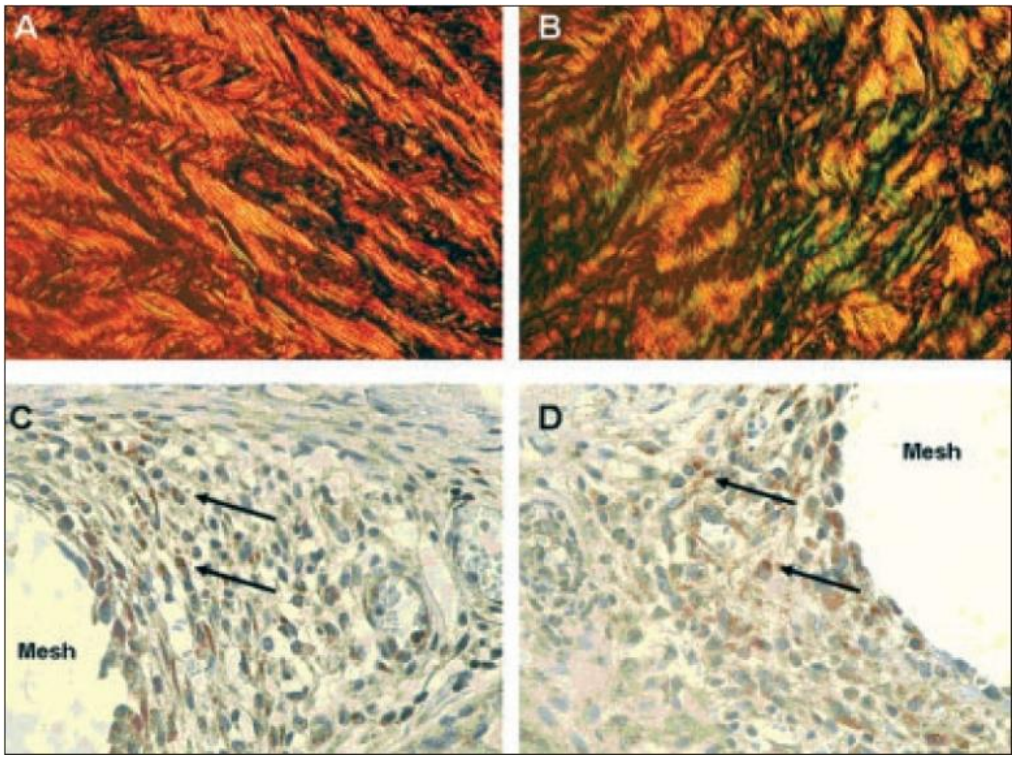


Fig 8: Cross polarization microscopical (CPM) and immunohistochemical features of human fascial tissue. A- Tissue with normal Collagen I/III ratio. B – Scar tissue with reduced Collagen I/III ratio. C,D – Positive cytoplasmic expression of MMP-2. Cells marked with arrows

The low quality of the scar of patients with recurrences explain the outcome curves [Fig 9]. exogenous factors such as smoking could be identified as major risk factors. Likewise, it clarifies the high frequency of incisional hernia in patients with abdominal aortic aneurysm and their demonstrated defect of the collagen metabolism. It explains the frequent development of recurrences, if not the entire scar, was reinforced and that the best technique sometimes fails even in the hands of experts.

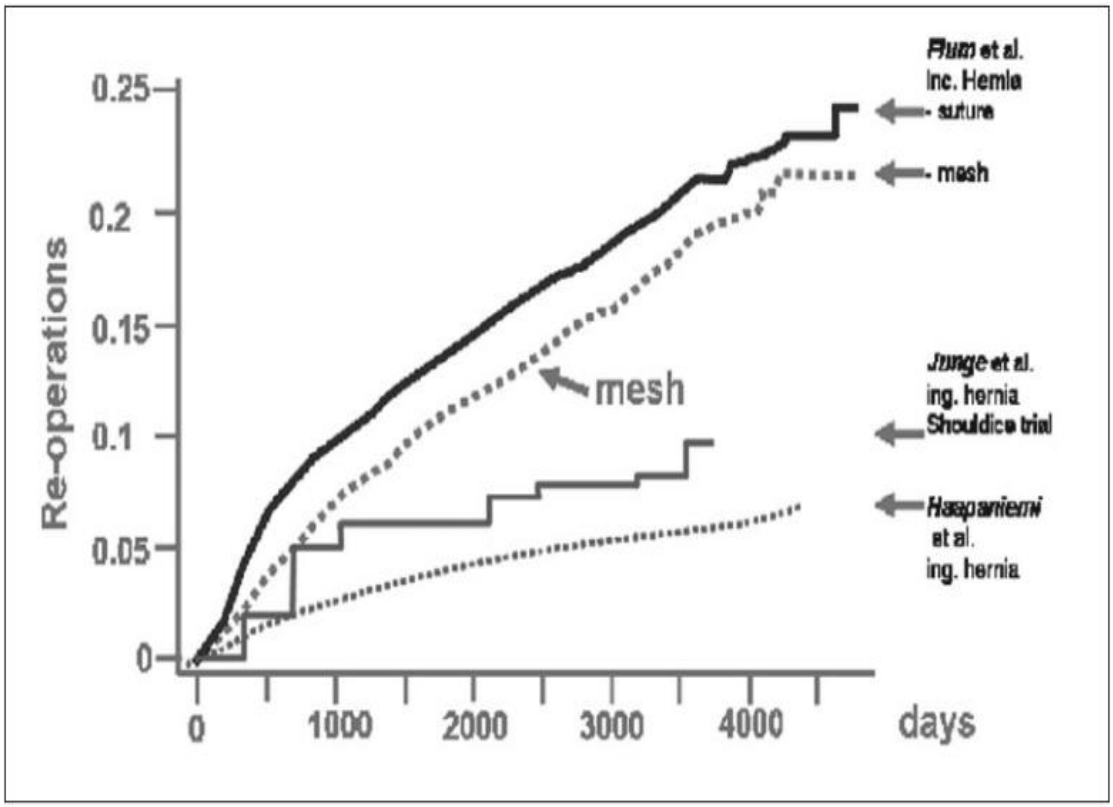


Fig 9: Cumulative incidences of recurrences after incisional and inguinal hernia repair[95,96]

Patients with hernia and in particular those with an incisional hernia are likely predisposed for recurrent hernia formation. Unfortunately, until now we do not have any predicting markers to identify those with an impaired wound healing and scar formation. The most substantial factor still is a patient's history of hernia repair with markedly elevated re-recurrence rates.

THE BLADE, BIOLOGY OR BOTH:

While recurrent and incisional hernias following suture repair are most likely caused by a faulty biology, the recurrence subsequent to mesh repair may be regarded as a technical fault. In reflection of the tensile strength of existing mesh materials; it is the extent of overlap, which defines whether and when a recurrence may appear. Almost all recurrences manifest at the border of a dislocated, shrunken or undersized mesh, almost never through a mesh itself. Thus, it really should be possible to prevent recurrences by mesh repair.

It is the possibility of an insufficient scar formation, that requires a supplementary reinforcement with non-absorbable alloplastic nets as flat meshes with an extensive overlap. Taking into consideration all patients with primary hernia, the experiences of the past decades with suture repairs indicate that we should expect 15–20% to develop a recurrent hernia. It will depend on the long-term biocompatibility of mesh materials whether it is justified to apply a mesh repair to all of the patients or to restrict it to selected patients at risk. Future perspective may provide further possibilities to improve scar quality itself, e.g., by biological active meshes.

The correlation between connective tissue and hernia began with a significant discovery in 1964. **Zolton T. Wirtschafter and J. Peter Bentley**[97] did a study providing a lathyritic diet to young and old Long-Evans rats. The ingestion of seeds of Lathyrus odoratus caused generalized connective tissue disorder especially osteolathyrism in mammals. The authors extracted collagen from the Skin and peritoneum of Lathyrus fed rats and concluded that the rates of occurrence of hernia was greater in this group compared to controls. Abnormal fibrinogenesis and defective collagen formation were postulated to cause diminished tensile strength of fascia

In a study by **MA Ajabnoor et al**[98] Fibroblastic cell cultures were derived from the hernial sac and the surrounding muscles especially internal oblique of 130 Saudi patients with different types of hernias, and from 21 control subjects. The tissues were subject to explantation and subcultures. Three variables were studied 1. Cell proliferation 2. Rates of incorporation of ^{14}C proline 3.Rates of collagenase activity. The rates of cell proliferation were studied for 39 days. Results implied decreased rates of proliferation of cells derived from patients compared to controls. *In vitro* studies of the rates of incorporation of ^{14}C proline into the muscle biopsies revealed decreased rates of label incorporation in the samples derived from patients compared to controls. However, no differences were detected between rates of collagenase activities of the biopsies obtained from patients compared to those of

controls. These findings suggested that collagen synthesis is probably defective in the patients with hernia.

Similarly, in an article next year (1993), it wasdetermined[99] if alterations in fibrillar collagen synthesis were associated with the development of inguinal hernias. This study was undertaken to study collagen synthesis in patients with inguinal hernia in the absence of any other connective tissue disease. Trypsin-chymotrypsin-resistant type I and III collagens were isolated and analyzed. The study concluded that a constitutive and systemic increase in type III collagen synthesis might result in reduced collagen fibril assembly in the abdominal wall, eventually leading to the development of herniation. Although it is not yet clear what genetic factors are responsible for the elevation in type III collagen synthesis in patients with hernias. This study was a first attempt to define individuals with an abnormality in collagen production that may be specifically related to herniation.

JM Bellon et al[100] aimed to examine the transversalis fascia of patients with direct and indirect hernia in an effort to identify the differences between each type of hernia. They analyzed the ultrastructure of the fascia surrounding the hernial lesions, the proline and lysine hydroxylation in the tissue, the type I–type III collagen ratio and the presence of metalloproteinases. They did not detect ultrastructural differences in the collagen fibrils from fascia in direct and indirect hernias. The interfibrillar matrix was more abundant in direct hernias, showing

abundant electron-dense particles. No differences in proline hydroxylation were observed between each type of hernia. A small decrease in lysine hydroxylation was detected in patients with direct hernia. As opposed to our study this one evaluated collagen I/III ratio based on Enzyme-linked immunosorbent assays (ELISAs). This showed no statistically significant differences in the type I–type III collagen absorbance ratios. Immunohistochemistry revealed no differences in the expression of matrix metalloproteinase-1. FT from patients presenting direct hernia showed a very strong staining vs. metalloproteinase-2 when compared with that observed in indirect hernia.

The relation between Collagen I and III is regulated by several collagenases, mainly matrix metalloproteinases-1 and -13[101] whereas fibronectin plays a key role in the adherence of cells within the extracellular matrix. The aim of this study was to investigate whether an alteration in type I and type III collagen synthesis, amounts of MMP-1 and MMP-13 and the expression of fibronectin were associated with the development of inguinal hernia. They analyzed the hernial sac of patients with indirect and direct inguinal hernias and peritoneum in controls by immunohistochemistry and Western blot analysis. The results showed that the ratio of I/III collagen was markedly decreased in patients with either indirect or direct hernias as compared with controls with a concomitant increase in type III collagen synthesis. MMP-13 was expressed neither in the hernial sac nor in the peritoneum

of the controls, but the positive reactions of MMP-1 were found in the surface of the subserosa of the hernial sac in patients with indirect or direct hernia without any difference compared to controls. In regard to the known alterations of the collagen metabolism in fascia and skin of hernia patients the changed collagen I/III ratio with its increase of type III collagen in hernial sacs support the presence of a systemic disturbance of collagen metabolism. The absence of changes in the expression of collagenases (MMP-1, MMP-13) and the constant levels of fibronectin underline the central role of collagen synthesis for the development of indirect or direct hernias.

Alain Pans et al[102]suggested that a defect in collagen fiber structure may play a role in inguinal hernia formation. They performed a biochemical investigation of the collagen in the transversalis fascia and rectus sheath. The samples were collected from 40 adult patients with uni- or bilateral hernias and from 20 control subjects without hernia (autopsies and organ donors). A constant area of tissue was taken by using a calibrator. The wet and dry weights per 100 mm^2 were determined and the total collagen concentration as well as its sequential extractability in NaCl, acetic acid, and pepsin was measured. The ratios of type I/III collagen were assessed by polyacrylamide gel electrophoresis.The significant increase of collagen extractability with pepsin in the Direct Hernia fasciae suggests that molecular alterations of collagen could be involved in the genesis of groin

hernias. This connective tissue pathology is expressed only in the inguinal region, since they observed no major difference between the rectus sheaths of controls and those of patients. The qualitative study I/III collagen ratio showed no difference between the fascia groups.

The study by **Pablo Bórquez M**[103] is solely dedicated to the quality of skin in patients with and without hernia. Skin from the surgical wound was obtained from 23 patients with and 23 patients without inguinal hernia. Patients without hernia had compact collagen tracts homogenously distributed towards the deep dermis. In contrast, patients with hernia had zones in the dermis with thinner and disaggregated collagen tracts. Connective tissue had a lax aspect in these patients. Collagen fiber density was 52% lower in patients with hernia, compared to subjects without hernia. No differences in elastic fiber density or distribution was observed between groups. Thus collagen fiber quality was distorted in the skin of patients with hernia.

Rodrigues Jr AJet al[104]demonstrated structural and quantitative age-related changes of the elastic fibers in transversalis fascia, which may play a role in inguinal hernia formation. Transversalis fascia fragments were acquired during surgical intervention and underwent histological quantitative analysis of collagen by colorimetry and analysis of elastic fibers by histomorphometry. They demonstrated significantly lower amounts of collagen and higher amounts of

elastic fibers in transversalis fascia from patients with direct inguinal hernia compared to indirect inguinal hernia patients. The transversalis fascia from direct inguinal hernia patients exhibited structural changes of the mature elastic fibers, which are accountable for elasticity, and lower density of oxytalan elastic fibers, which are responsible for resistance. These variations encouraged loss of resiliency of the transversalis fascia.

Raphael Rosch[105]analyzed type I and type III procollagen messenger ribonucleic acid (mRNA) and MMP-1 and MMP-13 mRNA in fibroblasts from the skin of patients with and without hernia (controls) by reverse transcription polymerase chain reaction (RT-PCR) and Northern Blot. The results indicated that the ratio of type I to type III procollagen mRNA was decreased in patients with primary hernia, showing significant differences as compared to controls ($p = 0.01$). This decrease like the previous study was mainly due to the increase of type III procollagen mRNA. Furthermore, RT-PCR analysis revealed that the expression of MMP-1 mRNA in patients with primary hernia is equivalent to that of controls. In addition, MMP-13 mRNA is expressed neither in patients with primary hernia nor in controls.

When studying recurrent inguinal hernia **Zheng H**[106]extracted RNA from skin fibroblasts of three groups (control group I = healthy skin; control group II = plain skin scar; recurrent inguinal hernia group = skin of recurrent inguinal hernias; each n = 5). Reverse transcription-polymerase chain reaction (RT-PCR) and Northern blot analysis were used to investigate the expression of procollagen type I/- III. Both ratios of procollagen types I to III mRNAs and collagen types I to III were decreased in the recurrent hernia group compared to those of both control groups. Significant differences were caused by the increase of both procollagen type III mRNA and collagen type III protein synthesis. The data of this study strongly suggest recurrent inguinal hernias to be a disease of the collagen matrix.

With regards to prosthesis, **Karsten Junge**[107]studied Seventy-eight prostheses (Prolene, Atrium, Marlex, Vypro, Mersilene, Gore-Tex) implanted for inguinal and incisional hernia repair that was explanted because of recurrence, chronic pain or infection. The mean implantation period was 17.9±11.2 (range 0.5–48) months. Collagen formation was investigated quantitatively (collagen–protein ratio) and qualitatively (collagen type I/III ratio). Results were related to clinical data that included gender, age, implantation period, indication for implantation/explantation, type and location of the prosthesis. Samples explanted for recurring hernias exhibited a significantly decreased ratio compared to samples explanted because of pain or infection. The Multivariate analysis excluded

independent effects of age, gender, indication for implantation of prostheses, location and implantation period on collagen type I/III ratio.

This group also studied gentamicin-supplemented polyvinylidenfluoride mesh materials. Gentamicin mesh[108] induced a significantly decreased expression of MMP-8 and MMP-13 at the interface after implantation compared to the other groups. The quality of collagen formation conveyed by the collagen type I/III ratio showed significantly higher ratios around the Gentamicin mesh 21 and 90 days after implantation. A 5.3-fold expression of type I alpha 1 collagen mRNA was found.

ALM Meyer et al[109]did a Qualitative and Quantitative analysis of collagen types in inguinal hernia patients. The inclusion criteria were similar to this study but the controls were 24 corpses which had passed away less than 8 hours. Collagen I was visualized as thicker fibers that were strongly birefringent while Collagen III was thin, sparse fibers that were stained with Picosirius stain.

A statistically meaningful difference (P = 0.788) was not found in the amount of collagen type I in the fascia of the patients and of the controls. On the other hand, a greater amount of collagen type III was found in the patients, which showed a statistically meaningful difference (P = 0.0220) as seen in Figure 14.

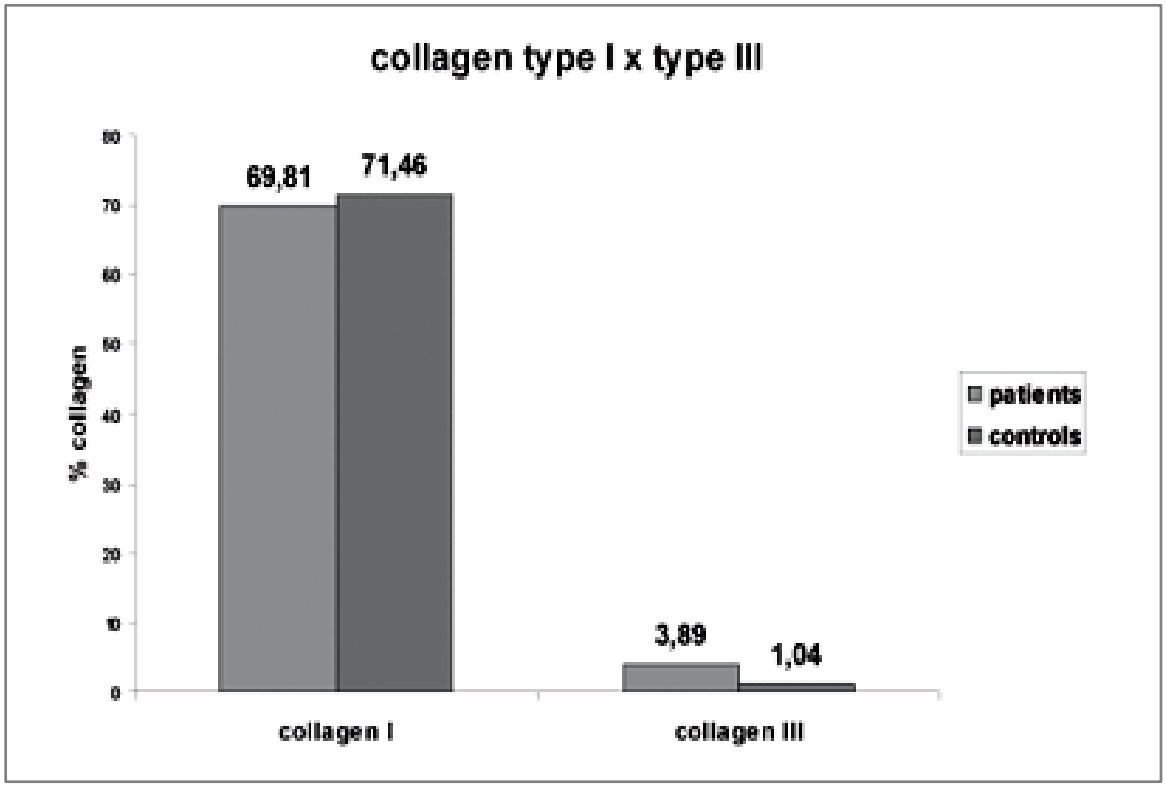


Fig 10: Collagen I and III – Difference between cases and controls

The compared the amount of collagen type III superior to 5% - in the sample field to the Nyhus classification for inguinal hernia. The patients classified as Nyhus IIIa had a greater amount of collagen type III with a statistical meaningful difference ($P = 0.033$).

The second part of this study uses skin as a marker of collagen composition of hernia defects. A similar study is by **Peeters E.**[110]Collagenorganization was examined in Haematoxylin-Eosin sections of anterior rectus sheath fascia, and collagen type I/III ratio. The study concluded that in both skin and abdominal wall fascia of hernia patients, collagen type I/III ratio was lower compared to control patients, with more pronounced abnormalities in incisional and recurrent inguinal hernia patients. Importantly, collagen type I/III ratio in skin was representative for that in abdominal wall fascia.

A more recent study is that of **Lazarenko**[111] of Russia. The trial included 141 patients for the period 2012-2015. Group I of patients without ventral hernias was divided into subgroup AI - primary operation and BI. Group II consisted patients with ventral hernias. There were significant differences between collagen type I/III ratio in skin and aponeurosis. In patients with ventral hernias collagen type I/III ratio in skin is 2.54 times lower than in patients without hernias. Significant correlation of collagen types in skin and aponeurosis allows predicting the risk of postoperative ventral hernias on basis of skin fragment.

In a study very similar to ours **Koruth S**[112]included a total of 90 patients, of which 45 patients underwent mesh repair for the various hernias and 45 patients who underwent laparotomies for various reasons. This study signifies that ventral,

recurrent and primary inguinal hernias are not just caused because of a primary defect but an acquired disorder with respect to collagen distribution.

One another recent study carried out in a similar fashion as ours was that of **Gonçalves O**.[113]Samples of the transverse fascia and of the anterior sheath of the rectus abdominis muscle were collected from 40 men aged between 20 and 60 years with type II and IIIA Nyhus inguinal hernia and from 10 fresh male cadavers (controls) without hernia in the same age range. The staining technique was immunohistochemistry for collagen I, collagen III and elastic fibers; quantification of fibrillar components was performed with an image analysis processing software. But no statistically significant differences were found in the amount of elastic fibers, collagen I and collagen III, and the ratio of collagen I / III among cases and controls.

The importance of collagen in herniology is stressed in the Danish paper by **Burcharth J and Rosenberg J**.[114] Patient groups with reduced type-I/III collagen ratio and consequently increased risk of herniation include patients with Ehlers-Danlos, Marfan's syndrome, osteogenesis imperfecta, cutis laxa, and patients with abdominal aortic aneurysms, colonic diverticula or stress urinary incontinence. The future perspective may be individualization of the operative technique for patients with a hernia, depending on their collagen profile.

Franz MG[115] while studying the biology of hernia formation reinforced that all abdominal wall hernias occur when tissue structure and function are lost at the load-bearing muscle, tendon, and fascial layer. The basic biologic mechanisms are primary fascial pathology or surgical wound failure. In both cases, cellular and extracellular molecular matrix defects occur. Primary abdominal wall hernias are associated with extracellular matrix diseases. Incisional hernias and recurrent inguinal hernias involve a combination of technical and biologic curbs.

Oğuzkurt P[116]investigated and compared the distribution and intensity of staining of extracellular matrix proteins--laminin, fibronectin, and types 1 and 4 collagen--in various congenital inguinoscrotal abnormalities and the peritoneum through immunohistochemical staining. The sacs associated with undescended testis (n = 28), hydrocele (n = 29), inguinal hernia (n = 31), and parietal peritoneum (n = 28) were stained with antibodies for laminin, fibronectin, and types 1 and 4 collagen. The peritoneum served as the control group. Type 1 collagen was intensely expressed in the sacs obtained from the hydroceles compared with the other groups and the peritoneum. Expression of type 4 collagen was significantly increased in the sacs associated with hydrocele and inguinal hernia compared with the peritoneum.

Ozdogan M et al[117]searched for changes in collagen and elastic fiber contents of the skin, rectus sheath, transversalis fascia and peritoneum in primary

inguinal hernia patients. This study was similar to the current one except that wide variety of tissues were used and the staining was different. Twenty patients operated on for inguinal hernia included in the study (11 direct and 9 indirect). Nine patients underwent open cholecystectomy served as the control group. A 0.5 x 1 cm. tissue was sampled from skin, rectus sheath, transversalis fascia and peritoneum in HR group. Skin, rectus sheath and peritoneum samples were taken from the patients in Control group. The sections of those samples were submitted to two different staining methods: "Masson's trichrome" for collagen and "van Gieson" for elastin fibers and graded with light microscopy.

The rectus sheath samples of Control had higher staining scores for both collagen and elastin fibers in comparison with hernia group. The control group had a significantly higher score for collagen in peritoneum samples. There were no statistically significant differences between the patients with direct and indirect inguinal hernias for collagen or elastin fibers scores in skin, rectus sheath, transversalis fascia and peritoneum samples.

Taniguchi Set al[118] studied the Impact of collagen subtype proportions in peritoneal tissues on inguinal hernia formation in adults and infants.They correlated the ratios of collagen type I to type III between adults and infants with and without inguinal hernia, in an attempt to clarify the pathogenesis of this disorder. They extracted collagen from the hernial sacs of patients with an inguinal

hernia, and from the normal peritoneum of patients without an inguinal hernia. After separation by electrophoresis, the collagen bands were quantified and we compared the ratios of collagen type I to type III between the cases and controls. The ratio of collagen type I to type III was significantly lower in the adults with an inguinal hernia than in those without the disorder. Whereas the ratios were similar in infants with and without an inguinal hernia confirming that a simple herniotomy is an adequate treatment.

One another study carried out in infants by **Hosgor et al**[119]aim to investigate whether an alteration in type I and type III collagen synthesis was associated with the development of childhood inguinoscrotal pathologies. The expression pattern of type I and IIIcollagen did not differ among sacs obtained from patients with inguinal hernia, hydrocele, and undescended testis when compared with that of controls. However, strong expression of type III collagen was observed in the hernial sacs of right-sided male inguinal hernia compared with the left side.

Casanova AB[120] studied specifically the Collagen in the transversalis fascia of patients with indirect inguinal hernia. Biopsy samples from 26 patients and 26 cadavers were analyzed. The results showed 17.3% less total collagen in patients with hernias compared with the control group. Type I collagen in patients with indirect inguinal hernias was 23.7% less than the control group, type III collagen was 6.4% less in the controls.

To conclude we would like to quote the comprehensive paper published by **Henriksen NA**[121] where Fifty-two papers were included. Collagen alteration depended on the type of hernia; there were more pronounced changes in patients with a direct inguinal hernia than in those with an indirect inguinal hernia. A constant finding was a significant increase in immature type III collagen relative to the stronger type I collagen in patients with a hernia. This resulted in thinner collagen fibers with lessened biomechanical strength. It was also suggested that these alterations are due to variation in the synthesis, maturation or degradation of collagen by matrix metalloproteinases.

Robert Bendavid from Israel propounded the **Unified theory of hernia formation**[122]whereinhe states that we have progressed from the simple concept of increased intra-abdominal pressure overwhelming a weak abdominal wall to the complex malady that calls upon several basic sciences to clarify the countless facets, though one final common pathway, of its pathophysiology. The target organ of all the known injurious stimuli is the collagen matrix. One day, classification may re-assign hernias under ‘‘Inborn errors of Metabolism’’ and ‘‘Diseases of Malnutrition.’

MATERIALS AND METHODS

This is a prospective case-control study. The study period was from November 2015 to January 2017. Those patients admitted with nonrecurrent inguinal hernia and undergoing laparoscopic or open hernioplasty were included in our study.

The exclusion criteria were the following:

Age less than 18yrs and more than 75 yrs

Patients on steroid medications

Smokers Patients who had undergone previous infraumbilical abdominal procedures Recurrent hernia

Patients with known connective tissue disorders Non consenting patients

The study population was divided into two groups:

1. Group A (Hernia /Case group); This group included patients with non recurrent inguinal hernia (unilateral /bilateral)

2. Group B (Control group); This included patients undergoing intra-abdominal procedures (laparotomy /laparoscopy for causes other than any hernia of the abdominal wall such as femoral hernia, umbilical or paraumbilical hernia and incisional hernia)

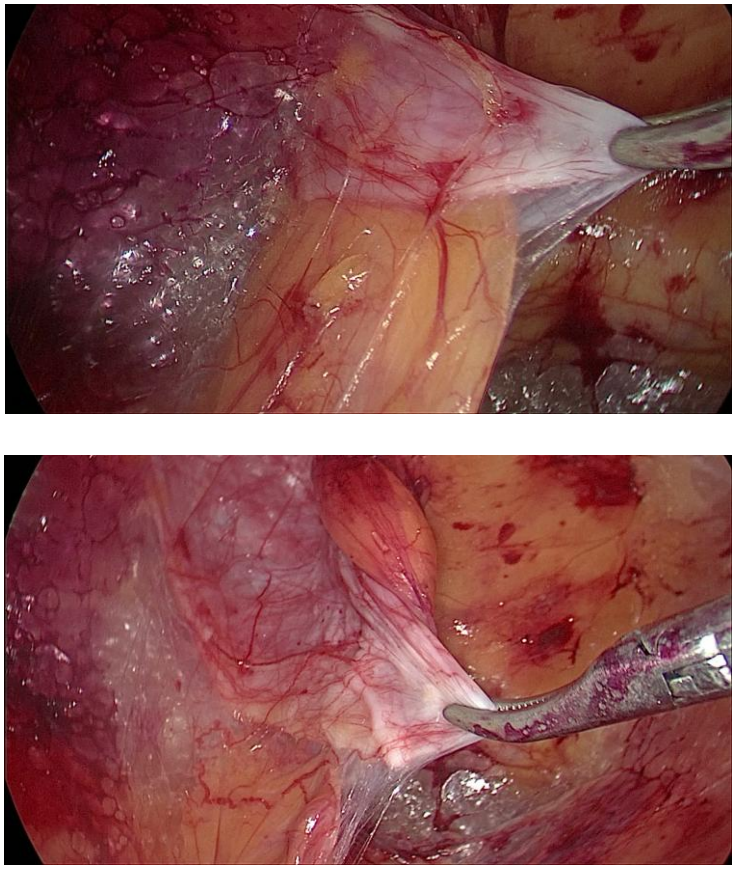


Fig 11: Transversalis fascia specimen taken from the pseudosac

Sample size in each group:

Group A: 30

Group B: 30

Biopsy specimen of size 0.5cm x 0.5cm - skin and transversalis fascia from 30 patients of hernia group and 30 patients of control group were taken from the operation theatre complex in formalin containers. They were received in the pathology laboratory of PSGIMS&R. These specimen were allotted a specific reference number, which aids in their later identification from the archives. These

specimen were processed and submitted for hematoxylin and eosin (H&E) stain. As our study included the use of antibodies to detect Collagen I and Collagen III antigens, the tissues were also processed separately in Poly- L- Lysine coated slides. The slides stained with hematoxylin and eosin were used for routine histological examination. Massons trichrome stain was used to confirm the presence of collagen fibres and Immunohistochemical stains to separately stain Collagen I and III and quantify them. The slides coated with Poly-L-Lysine, underwent immunohisto-chemistry staining for detection of Collagen I and III in the fibroblasts of skin and transversalis fascia.

HEMATOXYLIN AND EOSIN STAIN

These are the most widely used stain in a histopathology laboratory. The advantage of H&E lies in its simplicity and ability to clearly demonstrate enormous number of different tissue structures.

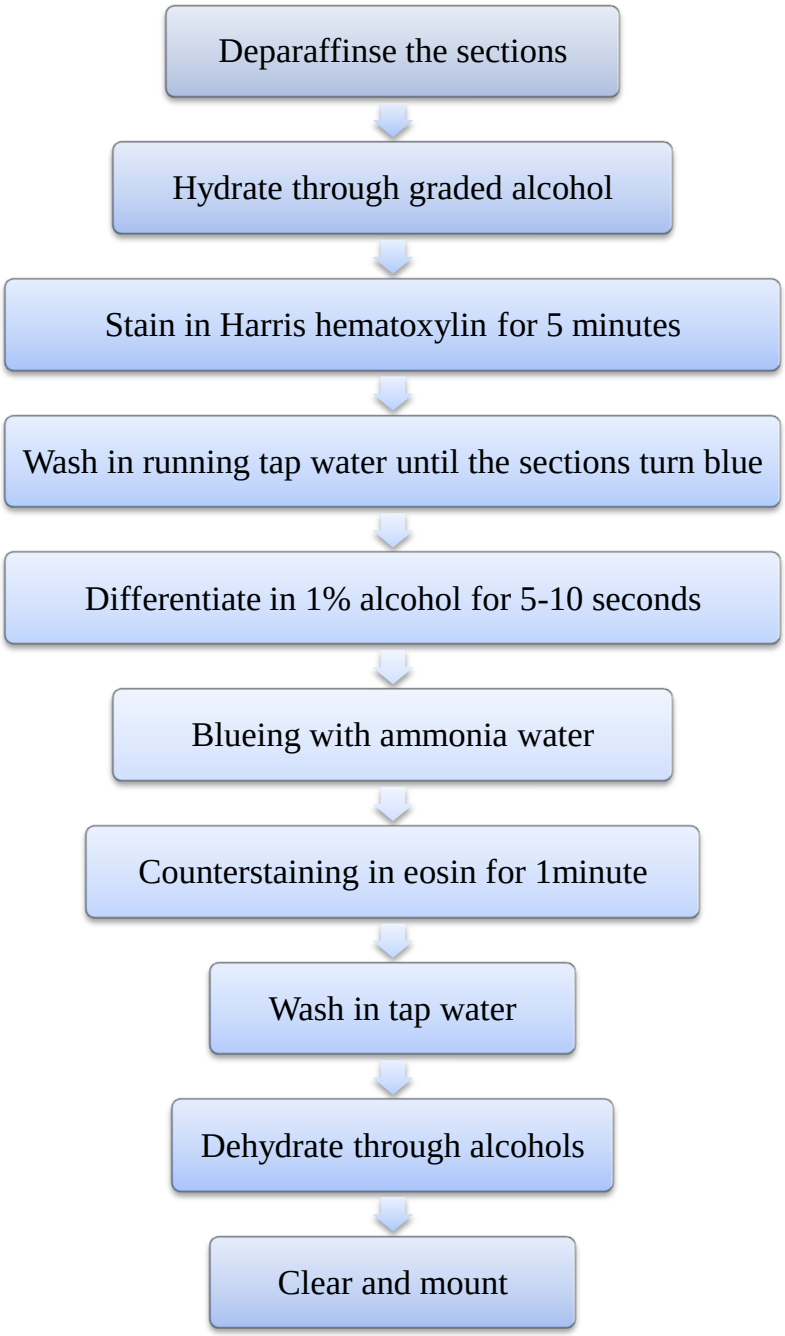
The hematoxylin component stain the nuclei blue, while the eosin component stains the cytoplasm and most of the connective tissue fibres. There are various methods of performing the H&E staining, we commonly use the Harris Hematoxylin method. This is a type of alum hematoxylin. It is chemically oxidised with mercuric oxide. As mercuric oxide is toxic, we commonly use sodium or potassium iodate as a substrate for oxidation. The process is enumerated in order below and the end results are

Nuclei – Blue

Cytoplasm – pink

Red cells – orange

Fibrin; deep pink



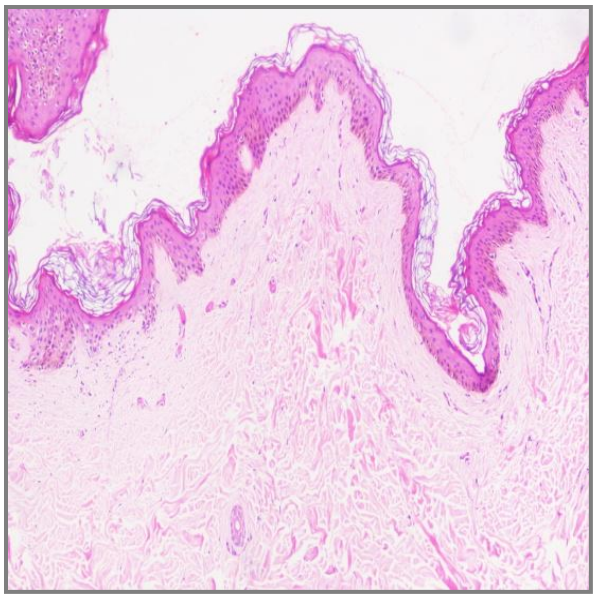


FIG 12: H&E SECTION OF SKIN

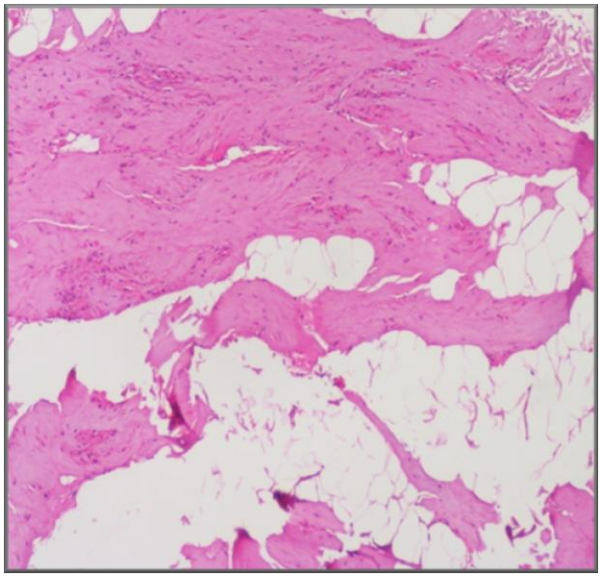


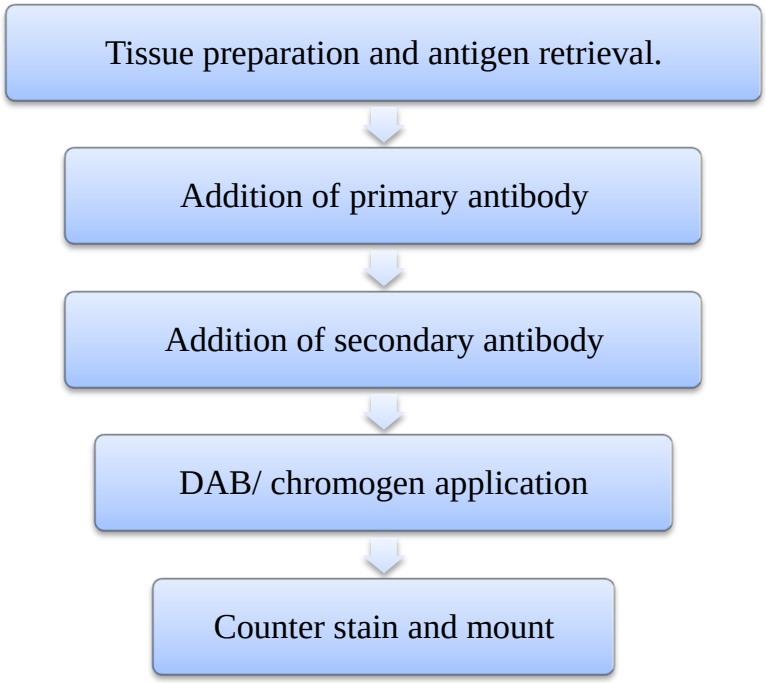
FIG 13: H&E SECTION OF TRANSVERSALIS FASCIA

Staining for Collagen I and Collagen III:

The sections are cut at a thickness of about 4 micrometer. These are then floated on to Poly-L - Lysine coated slides and incubated at 37 degree for one day and further incubated at 58 degree overnight.

Precautions to be taken:

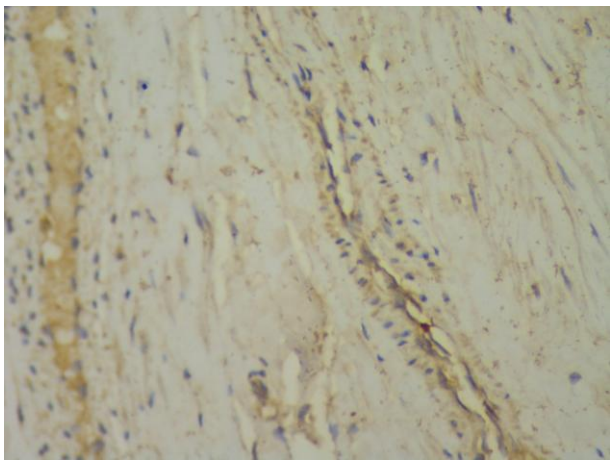
The sections are not allowed to dry at any stage of the procedure. The steps of incubation with antibody are carried out at a temperature of 37 degrees. Adequate controls for each antibody tested are to be used. The steps of antibody staining are:



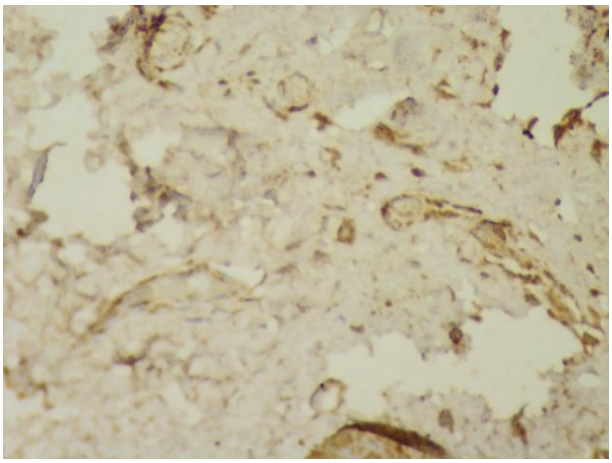
10 x 10x View of skin showing varying grades of intensity of staining for collagen immune histochemistry are shown in the following pages

The Grading is as follows

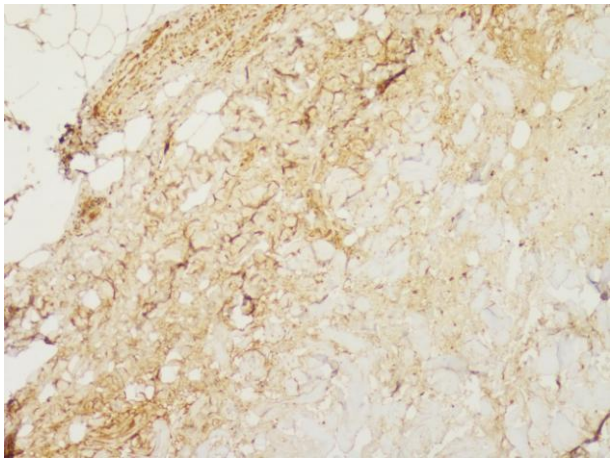
Grades	Percentage of Positivity
Grade I	0% - 25%
Grade II	26% - 50%
Grade III	51% - 75%
Grade IV	76% - 100%



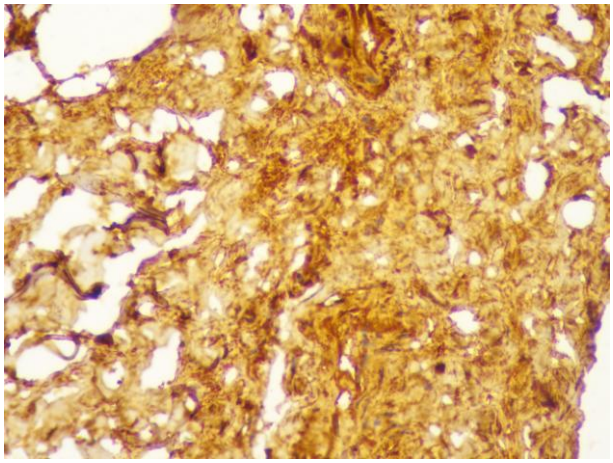
Transversalis Fascia – Grade I



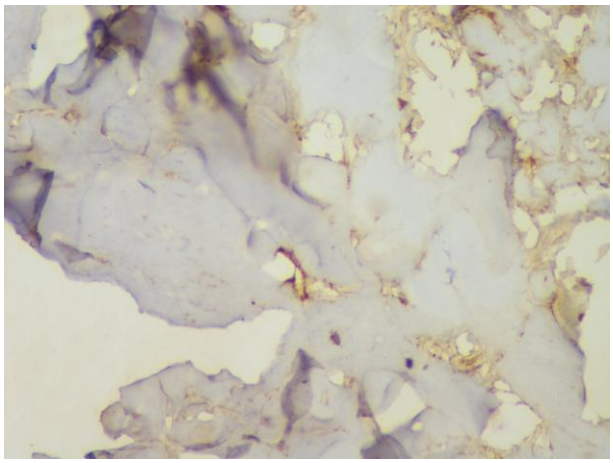
Transversalis Fascia – Grade IIs



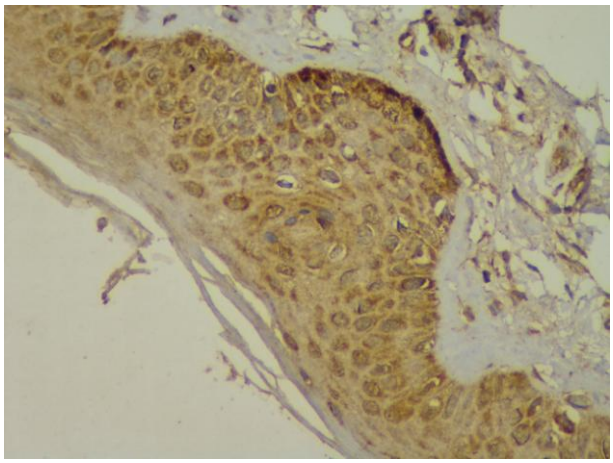
Transversalis Fascia – Grade III



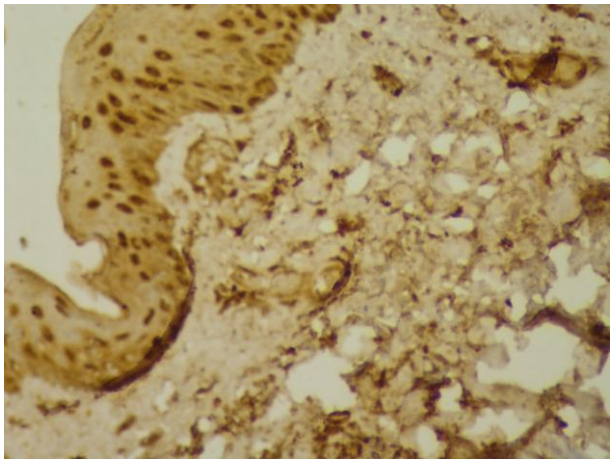
Transversalis Fascia – Grade IV



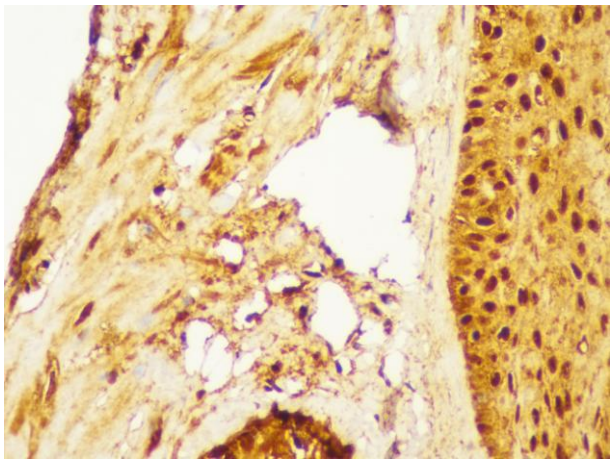
Skin Grade I



Skin Grade II



Skin Grade III



Skin Grade IV

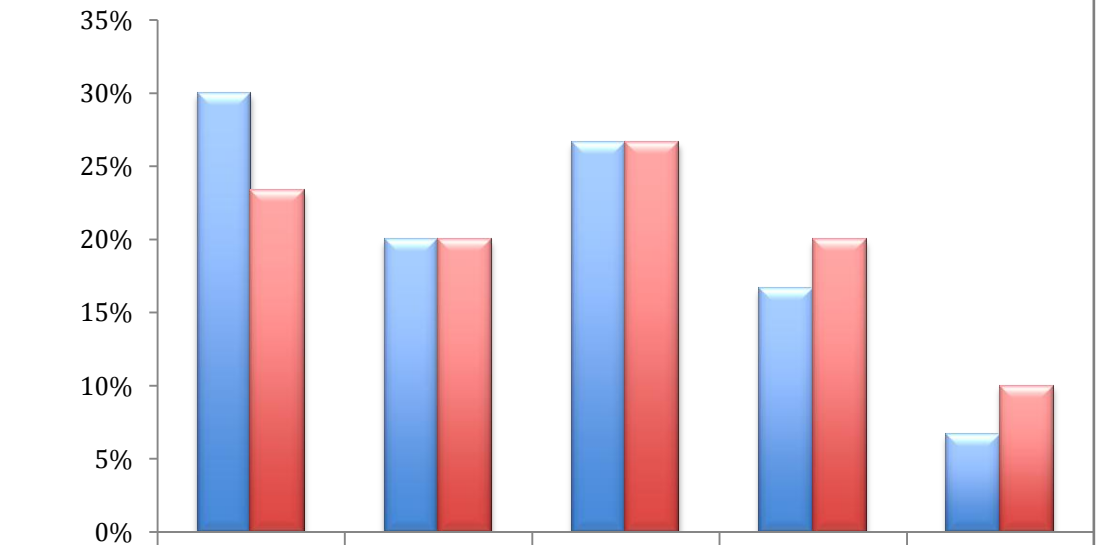
OBSERVATION AND RESULTS

For the purpose of this study 30 biopsy specimen of skin and transversalis fascia of patients with inguinal hernia, and 30 biopsy specimen from skin and transversalis fascia of matched control population were received in the Department of Pathology in formalin containers. Each specimen was 0.5cm x 0.5cm. A complete histopathological examination with H&E followed by comparison of Collagen I and Collagen III immunohistochemical staining pattern among the hernia cases and controls was carried out. Within the result, we have included the following:

- Age-wise distribution of cases and controls included in the study
- Age wise distribution of Collagen types in skin and transversalis fascia among cases and controls
- A Comparison of Collagen I, Collagen III and Collagen I and III ratio in skin of cases and controls.
- A Comparison of Collagen I, Collagen III and Collagen I and III ratio in transversalis fascia of cases and controls.
- A Comparison of mean of Collagen types in skin and transversalis fascia in Cases.
- A Comparison of mean of Collagen types in the skin and transversalis fascia in Controls.

Table 1: AGE DISTRIBUTION				
AGE	**CONTROL**	**(%)**	**CASES**	**(%)**
< 30	9	30%	7	23%
31 - 40	6	20%	6	20%
41 - 50	8	27%	8	27%
51 - 60	5	17%	6	20%
> 60	2	7%	3	10%
Total	**30**		**30**	

Fig 14: Age Distribution [N=60][p>0.05]



	< 30	31 - 40	41 - 50	51 - 60	> 60
CONTROL	30%	20%	27%	17%	7%
CASES	23%	20%	27%	20%	10%

Table 2: Mean Age of study Groups								
AGE	**Mean**	**SD**	**Std. Error**	**95% CI for Mean**		**Minimum**	**Maximum**	**sig**
				Lower	**Upper**			
CONTROL	41.57	12.907	2.357	36.75	46.39	24	72	>0.05
CASES	42.23	14.567	2.659	36.79	47.67	21	78	

There was no significant difference in the distribution of patients among cases and controls with respect to age – cases and controls were age matched

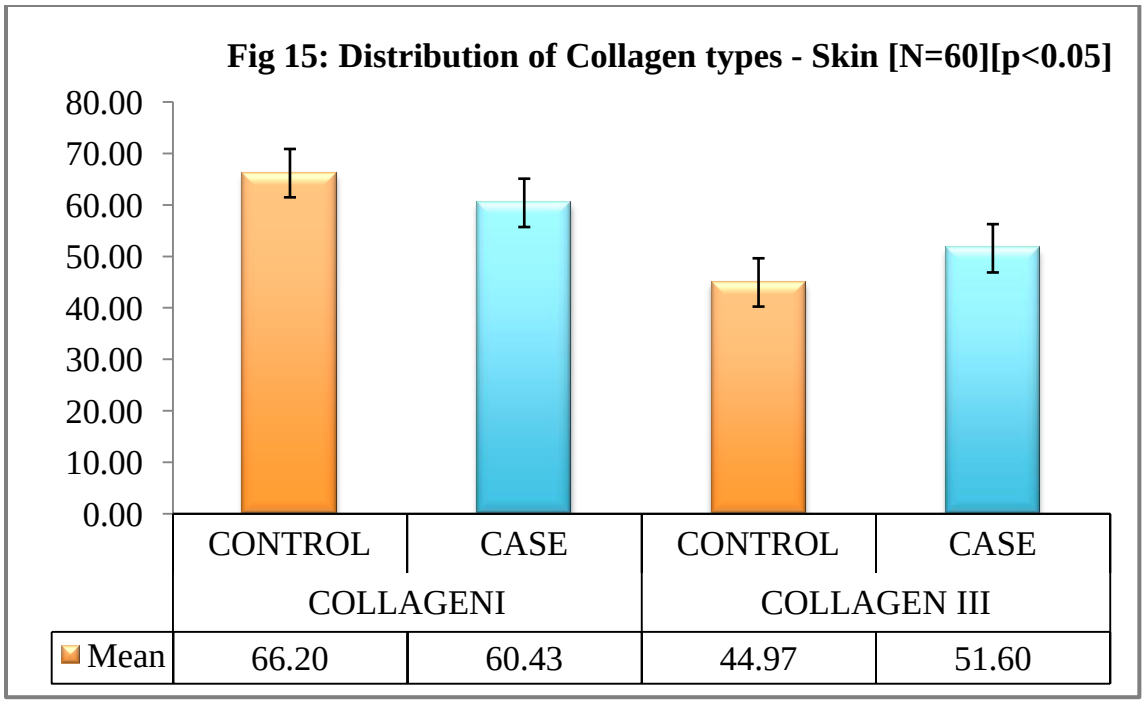
Table 3 a: Age Distribution of Collagen				95% CI for Mean					
Type	**Study**	**Mean**	**SD**	**Lower**	**Lower**	**Upper**	**Min**	**Max**	**sig**
Skin Collagen I	<30	71.29	8.281	3.13	63.63	78.94	56	80	<0.05
	31 - 40	63	10.64	4.344	51.83	74.17	53	82	
	41 - 50	60.75	11.399	4.03	51.22	70.28	39	71	
	51 - 60	50	16.075	6.563	33.13	66.87	31	71	
	> 60	50	17.776	10.263	5.84	94.16	30	64	
Skin Collagen III	<30	60.29	10.996	4.156	50.12	70.46	40	71	>0.05
	31 - 40	55.83	11.479	4.686	43.79	67.88	45	78	
	41 - 50	49.38	9.87	3.489	41.12	57.63	30	61	
	51 - 60	43	19.95	8.145	22.06	63.94	20	69	
	> 60	46	25.515	14.731	-17.38	109.38	20	71	
Skin RATIO	<30	1.2	0.15535	0.05872	1.0563	1.3437	1.06	1.44	>0.05
	31 - 40	1.135	0.07842	0.03202	1.0527	1.2173	1.02	1.2	
	41 - 50	1.235	0.09008	0.03185	1.1597	1.3103	1.14	1.34	
	51 - 60	1.2567	0.32684	0.13343	0.9137	1.5997	1.02	1.9	
	> 60	1.1967	0.30006	0.17324	0.4513	1.942	0.9	1.5	

Table 3 b: Age Distribution of Collagen			95% CI for Mean						
Type	**Study**	**Mean**	**SD**	**Lower**	**Lower**	**Upper**	**Min**	**Max**	**sig**
Transversalis fascia Collagen I	<30	62.14	7.581	2.865	55.13	69.15	54	74	>0.05
	31 - 40	60	6.387	2.608	53.3	66.7	51	71	
	41 - 50	60.62	12.489	4.416	50.18	71.07	41	80	
	51 - 60	47.83	9.988	4.078	37.35	58.32	37	62	
	> 60	58	10.536	6.083	31.83	84.17	47	68	
Transversalis fascia Collagen III	<30	49.29	10.404	3.932	39.66	58.91	32	61	>0.05
	31 - 40	52.17	7.195	2.937	44.62	59.72	45	64	
	41 - 50	49.88	11.495	4.064	40.27	59.48	34	67	
	51 - 60	36.83	9.786	3.995	26.56	47.1	23	46	
	> 60	48.33	7.024	4.055	30.89	65.78	41	55	
Transversalis fascia RATIO	<30	1.2914	0.20684	0.07818	1.1001	1.4827	1.06	1.68	>0.05
	31 - 40	1.155	0.0855	0.0349	1.0653	1.2447	1.03	1.28	
	41 - 50	1.2213	0.07434	0.02628	1.1591	1.2834	1.15	1.33	
	51 - 60	1.3233	0.16367	0.06682	1.1516	1.4951	1.15	1.61	
	> 60	1.1967	0.04509	0.02603	1.0847	1.3087	1.15	1.24	

There was no significant difference in the percentage of collagen staining in the various age groups of both cases and controls.

Table 4: Distribution of Collagen types in skin - Study Group									
				95% CI for Mean					
Type	**Study**	**Mean**	**SD**	**Lower**	**Lower**	**Upper**	**Min**	**Max**	**sig**
Collagen I	Control	66.20	10.52	1.92	62.27	70.13	40	79	
	Case	60.43	13.97	2.55	55.22	65.65	30	82	>0.05
Collagen III	Control	44.97	9.28	1.69	41.50	48.43	20	60	
	Case	51.60	15.05	2.75	45.98	57.22	20	78	<0.05
RATIO	Control	1.51	0.27	0.05	1.41	1.61	1.03	2.19	
	Case	1.21	0.19	0.03	1.14	1.28	0.9	1.9	<0.001

While studying the skin of both cases and controls the difference in intensity of staining of Collagen I was not significant between cases and controls (P>0.05). But the intensity of staining of collagen III was significantly higher (P<0.05) in cases than controls as shown in the figure below



There was a very significant (P<0.001) reduction in ratio of Collagen I to III in cases when compared to controls as shown in the figure below

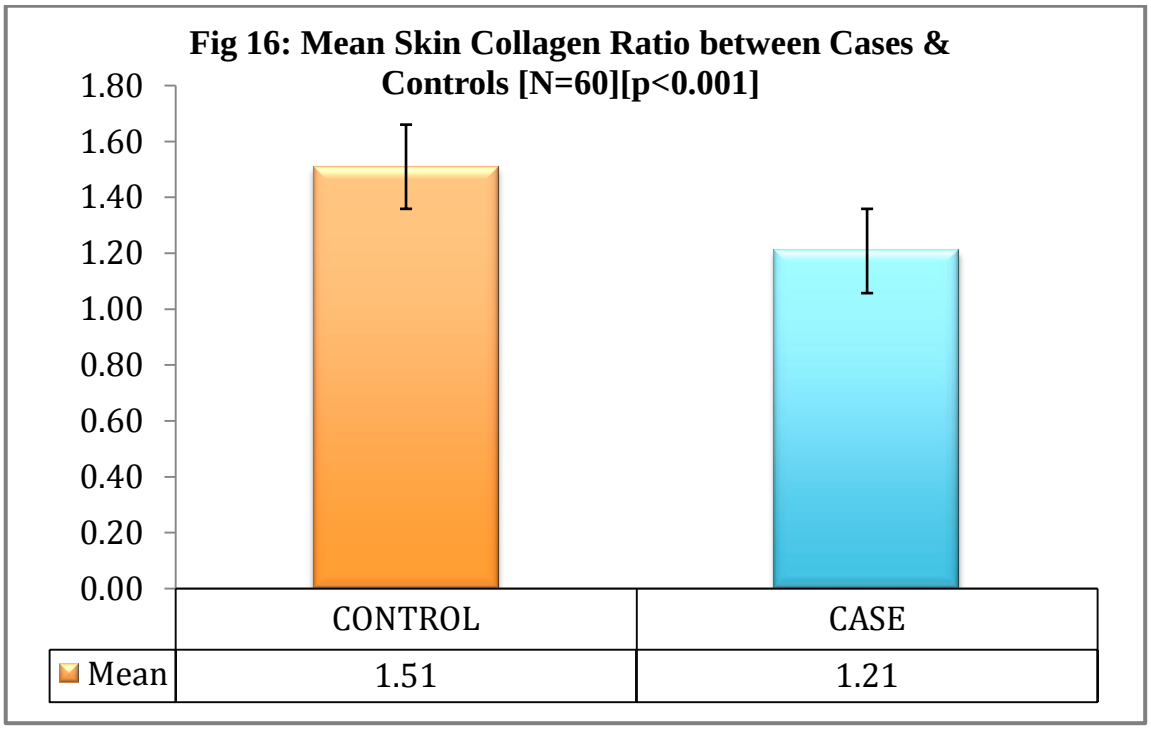
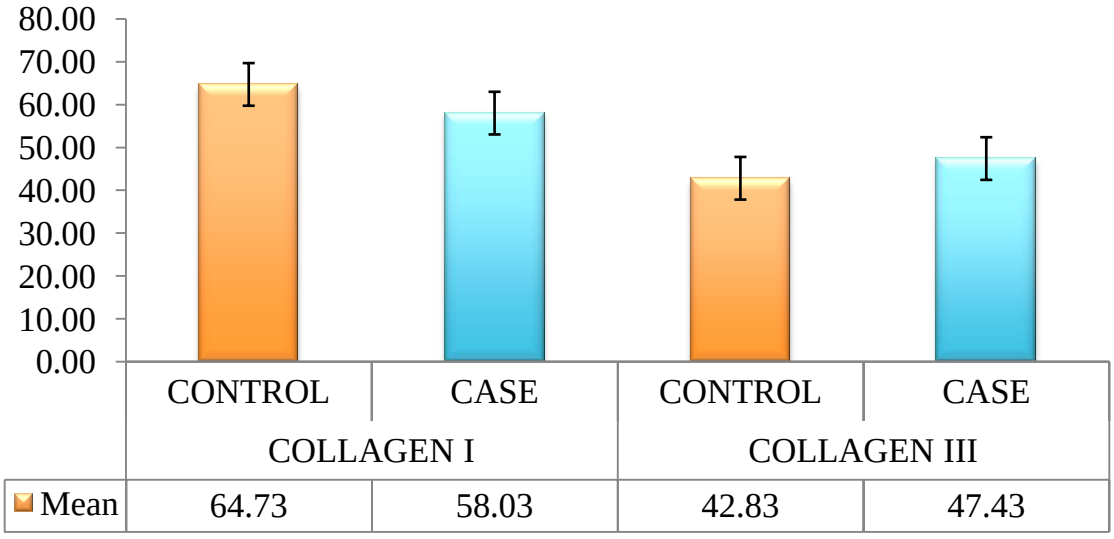


Table 5: Distribution of Collagen types in Transversalis fascia - Study Group									
				95% CI for Mean					
Type	**Study**	**Mean**	**SD**	**Lower**	**Lower**	**Upper**	**Min**	**Max**	**sig**
Collagen I	Control	64.73	8.24	1.50	61.66	67.81	43	78	
	Case	58.03	10.47	1.91	54.12	61.94	37	80	<0.05
Collagen III	Control	42.83	9.10	1.66	39.43	46.23	24	59	
	Case	47.43	10.66	1.95	43.45	51.41	23	67	>0.05
Ratio	Control	1.56	0.33	0.06	1.44	1.69	1.08	2.41	
	Case	1.24	0.14	0.03	1.19	1.30	1.03	1.68	<0.001

While studying the transversalis fascia of both cases and controls the difference in intensity of staining of Collagen I was significant between cases and controls ($P<0.05$). But the intensity of staining of collagen III was not significantly ($P>0.05$) higher in cases than controls.

Fig 17: : Distribution of Collagen types - Transversalis fascia [N=60][p<0.05]



This was unlike the skin study. But similar to the skin study there was a reduction in ratio of Collagen I to III and this difference was very significant (P<0.001)

Fig 18: Mean Transversalis fascia Collagen Ratio between Cases & Controls [N=60][p<0.001]

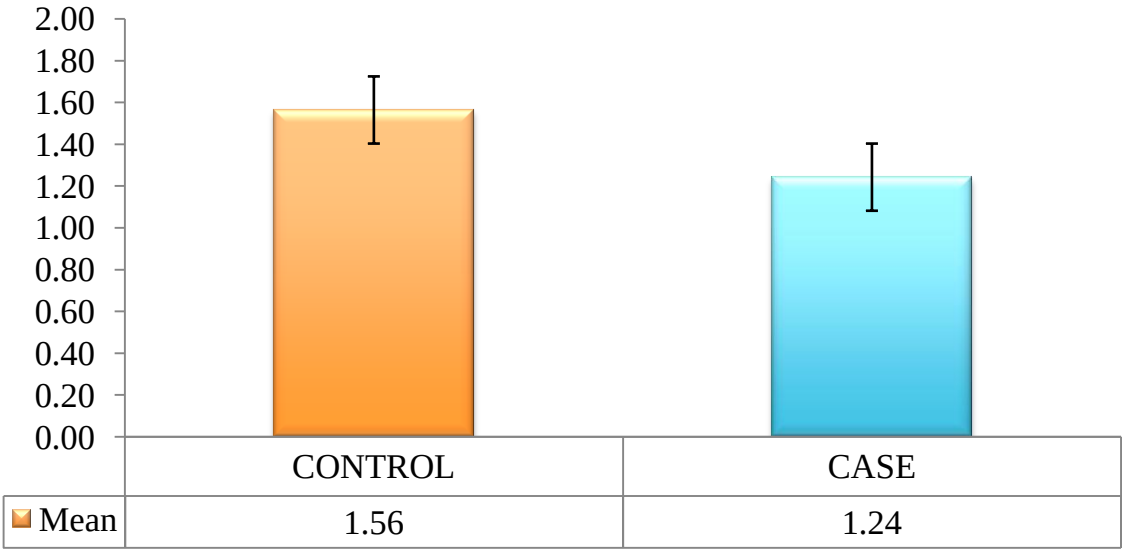


Table 6: Comparison of Mean of Collagens Types between skin and Transversalis Fascia in Control Groups									
Type	**Study**	**Mean**	**SD**	**95% CI for Mean**			**Min**	**Max**	**sig**
				Lower	**Lower**	**Upper**			
Collagen I	SKIN	66.20	10.52	1.92	62.27	70.13	40	79	
	TF	64.73	8.24	1.50	61.66	67.81	43	78	<0.05
Collagen III	SKIN	44.97	9.28	1.69	41.50	48.43	20	60	
	TF	42.83	9.10	1.66	39.43	46.23	24	59	<0.05
Ratio	SKIN	1.51	0.27	0.05	1.41	1.61	1.03	2.19	
	TF	1.56	0.33	0.06	1.44	1.69	1.08	2.41	<0.05

Table 7: Comparison of Mean of Collagens Types between Skin and Transversalis Fascia in Case Groups									
Type	**Study**	**Mean**	**SD**	**95% CI for Mean**			**Min**	**Max**	**Sig**
				Lower	**Lower**	**Upper**			
Collagen I	SKIN	60.43	13.97	2.55	55.22	65.65	30	82	
	TF	58.03	10.47	1.91	54.12	61.94	37	80	>0.05
Collagen III	SKIN	51.60	15.05	2.75	45.98	57.22	20	78	
	TF	47.43	10.66	1.95	43.45	51.41	23	67	>0.05
Ratio	SKIN	1.21	0.19	0.03	1.14	1.28	0.9	1.9	
	TF	1.24	0.14	0.03	1.19	1.30	1.03	1.68	>0.05

Comparing the ratio of collagen I to III in skin and transversalis fascia it was found that the difference was significant (P>0.05) between the two tissues in the control group whereas the difference was not significant in the case group. So in patients with hernia the skin ratio was representative of transversalis fascia ratio.

Statistical analysis:

The data are reported as the mean +/- SD or the median, depending on their distribution. Frequencies are expressed in percentages.

The differences in quantitative variables between groups were assessed by means of the unpaired t-test. Comparison between groups was made by the Non-parametric Mann – Whitney test. ANOVA was used to assess the variables.

The chi-square test was used to assess differences in categoric variables between groups.

A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analyzed with a statistical software package (SPSS, version 16.0 for windows)

DISCUSSION

The field of herniology has progressed from the simple concept of increased intra-abdominal pressure overwhelming an already weak abdominal wall to understanding hernia as a complex disease that calls upon several basic sciences to explain its pathophysiology. The fundamental mechanism of hernia formation is the loss of structural integrity at the musculo-tendinous layer.Many factors predisposing to hernias have been proposed which includes; an open processus vaginalis, increased intraabdominal pressure, familial predisposition, malnutrition, iatrogenic factors, impaired collagen metabolism and increased secretion of matrix metalloproteinases. The target organ of all injurious stimuli is the collagen matrix.

There is an excessive degradation of collagen in the transversalis fascia of patients with inguinal hernia. Understanding the role of protease-antiprotease imbalance in inguinal hernia has now shed some light on the reasons for failure of hernia repairs. A high recurrence rate observed in elderly patients following hernia repairs is related to fibroconnective tissue weakness and impaired collagen metabolism. Increased physical activity can be considered only a triggering or secondary cause in the development of inguinal hernias.

An age-matched comparison of Collagen I, III and their ratio in inguinal hernia patients and their controls were made in our study as depicted in tables 5 and 6

This study uses the same inclusion criteria as the study conducted by ALM Meyer et al.[109] but the results were different. In the Meyer study there was no statistically significant difference in the distribution of Collagen I among cases and controls but there was a significant rise in Collagen III. This may be due to the different Picosirius stains used. Fresh cadavers were used as controls. The sample size of our study is larger than this study and Immunohistochemical staining is more sensitive and specific than Picosirius staining. In this study, it was found that in skin samples the distribution of Collagen I in cases was the same as in controls. Collagen III distribution was higher in cases then controls.

The use of cadavers as controls may alter the results as shown by the study by Goncalves et al.[113] Though the staining method was similar to ours the controls were cadavers. No statistically significant difference was found in the transversalis fascia. Rectus sheath samples were used instead of skin and this too did not yield any significant result.

A similar study with live controls but with wide variety of tissues was that of Ozdogan et al.[117] Massons trichrome was used to stain the skin, transversalis

fascia, rectus sheath and peritoneum. In contrast to our study statistically significant differences were not obtained in skin and transversalis fascia.

Sensitive methods like ELISA (JM Bellon et al[100]) and Polyacrylamide gel electrophoresis were (Alain Pans et al[102]) were used to study the Collagen I to III ratio but similar to the previous study no statistically significant differences were obtained among cases and controls.

The picture in Transversalis fascia in our study was – Controls had significantly higher Collagen I. The cases had more Collagen III staining than controls but the difference was not statistically significant.

Our results in the transversalis fascia are similar to the study conducted by Casanova et al.[120] In this study the percentage of Collagen I in cases was significantly lower than controls. But, only cases of indirect inguinal hernia were included. The type of hernia was not used as a separate variable in our study.

In both skin and transversalis fascia the Collagen I/III ratio was very significantly ($P<0.001$) reduced in cases than in controls. This was unlike any of the previous studies mentioned.

We used skin as a marker of collagen biology similar to the study by Pablo Bórquez et al.[103] This group found significant distortions in the arrangement of

collagen fibers in the skin of patients with inguinal hernia. This study was solely dedicated to skin biopsies.

This led us to compare not only cases and controls but to compare the two different tissues – skin and transversalis fascia separately among cases and controls. This was done to see if skin was representative of the collagen distribution in transversalis fascia.

A study by Peeters et al.[110] had looked at the collagen distribution in anterior rectus sheath, instead of transversalis fascia, and had found that skin had an identical districution.

In our study there was no significant difference in the Collagen distribution and ratio between skin and transversalis fascia in cases. So the skin ratio was representative of transversalis fascia ratio in patients with inguinal hernia., and it would, therefore, imply that a skin biopsy would suffice to identify a patient with unfavourable collagen distribution

To summarize, this study compares cases with primary inguinal hernia with age matched live controls. Sensitive immunohistochemical staining was used and a statistically significant difference was found in Collagen I staining (higher in controls) in transversalis fascia and Collagen III staining (higher in cases) in skin.

A very significant reduction in collagen I to III ratio was seen in cases compared to controls in both skin and transversalis fascia.

Comparing the two different tissues it was found that in cases skin collagen distribution was representative of transversalis fascia collagen distribution making skin an apt specimen to detect chances of recurrence of hernia.

This study is limited by its size. Further studies on recurrent hernias and ventral hernias will establish the role of altered collagen biology in hernias.

SUMMARY AND CONCLUSION

- Our study was a prospective case control study, which included 30 cases of inguinal hernia and 30 controls.
- Inguinal hernia patients showed a considerable increase in the staining of Collagen III compared to their control group when skin samples were studied
- In transversalis fascia Cases had significantly less Collagen I.
- This proves the hypothesis that inguinal hernia is a local manifestation of a systemic disease rather than a mere local mechanical defect and alsoemphasizes on the role of Collagens in the pathophysiology of hernias.
- Further, the Ratio of Collagen I and III was comparable in skin and trasversalis fascia. Since skin ratio is representative of transversalis fascia a larger population-based study is required to establish skin samples as a marker of hernia occurrence and recurrence

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PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

To
Dr K Siva Prasanna
Postgraduate
Department of General Surgery
Guides: Dr Vimalkumar Govindan / Dr S Shanthakumari
PSG IMS & R
Coimbatore

Ref: Project No.15/331

Date: November 13, 2015

Dear Dr Siva Prasanna,

Institutional Human Ethics Committee, PSG IMS&R reviewed and discussed your application dated 23.10.2015 to conduct the research study entitled *"Role of collagen composition in the aetio-pathogenesis of inguinal hernia"* during the IHEC meeting held on 13.11.2015.

The following documents were reviewed and approved:

1. Project Submission form
2. Study protocol (Version 1 dated 23.10.2015)
3. Informed consent form (Version1 dated 23.10.2015)
4. Data collection tool (Version 1 dated 23.10.2015)
5. Permission letter from concerned Head of the Department
6. Current CVs of Principal investigator, Co-investigators
7. Budget

The following members of the Institutional Human Ethics Committee (IHEC) were present at the meeting held on 13.11.2015 at IHEC Secretariat, PSG IMS & R between 10.00 am and 11.00 am:

Sl. No.	Name of the Member of IHEC	Qualification	Area of Expertise	Gender	Affiliation to the Institution Yes/No	Present at the meeting Yes/No
1	Mr. R. Nandakumar	BA., BL	Legal Expert, Chairperson	Male	No	Yes
2	Dr. S. Bhuvaneshwari (Member-Secretary, IHEC)	MD	Clinical Pharmacology	Female	Yes	Yes
3	Dr. S. Shanthakumari	MD	Pathology, Ethicist	Female	Yes	Yes
4	Dr Sudha Ramalingam	MD	Epidemiologist Alt. Member - Secretary	Female	Yes	Yes
5	Dr D Vijaya	M Sc., Ph D	Basic Medical Sciences (Biochemistry)	Female	Yes	Yes

The study is approved in its presented form. The decision was arrived at through consensus. Neither PI nor any of proposed study team members were present during the decision making of the IHEC. The IHEC functions in accordance with the ICH-GCP/ICMR/Schedule Y guidelines. The approval is valid until one year from the date of sanction. You may make a written request for renewal / extension of the validity, along with the submission of status report as decided by the IHEC.

PSG Institute of Medical Sciences & Research
Institutional Human Ethics Committee

Recognized by The Strategic Initiative for Developing Capacity in Ethical Review (SIDCER)
POST BOX NO. 1674, PEELAMEDU, COIMBATORE 641 004, TAMIL NADU, INDIA
Phone : 91 422 - 2598822, 2570170, Fax : 91 422 - 2594400, Email : ihec@psgimsr.ac.in

Following points must be noted:

1. IHEC should be informed of the date of initiation of the study
2. Status report of the study should be submitted to the IHEC every 12 months
3. PI and other investigators should co-operate fully with IHEC, who will monitor the trial from time to time
4. At the time of PI's retirement/intention to leave the institute, study responsibility should be transferred to a colleague after obtaining clearance from HOD, Status report, including accounts details should be submitted to IHEC and extramural sponsors
5. In case of any new information or any SAE, which could affect any study, must be informed to IHEC and sponsors. The PI should report SAEs occurred for IHEC approved studies within 7 days of the occurrence of the SAE. If the SAE is 'Death', the IHEC Secretariat will receive the SAE reporting form within 24 hours of the occurrence
6. In the event of any protocol amendments, IHEC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. Alteration in the budgetary status should be clearly indicated and the revised budget form should be submitted
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval
   d. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented
   e. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IHEC and only then can they be implemented
   f. Any deviation-Violation/waiver in the protocol must be informed to the IHEC within the stipulated period for review
7. Final report along with summary of findings and presentations/publications if any on closure of the study should be submitted to IHEC

Kindly note this approval is subject to ratification in the forthcoming full board review meeting of the IHEC.

Thanking You,

Yours Sincerely,

13.11.

SECRETARY
PSG IMS&R
COIMBATORE-641004.
INSTITUTIONAL HUMAN ETHICS COMMITTEE

Dr S Bhuvaneshwari
Member - Secretary
Institutional Human Ethics Committee

Informed Consent

I , Siva Prasanna K am carrying out a study on the topic

“ *The role of collagen composition in the aetio-pathogenesis of inguinal hernia*” as a part of my research project being carried out under the aegis of the Department of General Surgery

My research guide is : Dr. Vimal Kumar Govindan M.S.,F.R.C.S.,

Co-guide is : Dr. Shanthakumari MD

*The **Justification** for this study is:*

The susceptibility to hernia is based on the presence of a congenital sac and the failure of transversalis fascia. But anatomical factors alone are not sufficient to explain the development of all inguinal hernias.

All groin herniae arise in the Myopectineal orifice of Fruchaud which is mainly closed off by the transversalis fascia. Transverasalis fascia is structurally dependent on its collagen composition arranged in the framework to support tissue tension forces. The results of earlier studies indicate that inguinal herniation could be related to abnormal structural changes of collagen in fascia and skin of patients.

*The **Objective** of this study is:*

To compare the composition of collagen in the tissues from the abdominal wall of patients with inguinal hernia and matched controls with no hernia

***Sample size**:* 60

Study participants: Patients getting admitted for non recurrent inguinal hernia repair in the general surgery ward

Location: PSG IMS&R

We request you to kindly co operate with us in this study. We propose to collect background information and other relevant details related to this study. We will be carrying out:

Initial interview: 15 minutes

Data collected will be stored for a period of 1 ½ year. We will use the data as a part of another study.

Tissue sample collection*:* 0.5cm x 0.5cm tissue will be collected

No. of sites it will be collected from*:* 1. Skin 2. Fascia (from the site of operation)

Whether tissue sample collection is part of routine procedure or for research (study) purpose*:* Research purpose

Specify purpose, ***discomfort*** *likely to be felt and* ***side effects****, if any:* No significant side effects are anticipated

Whether tissue sample collected will be ***stored*** *after study period :* No, it will be destroyed

Whether tissue sample collected will be ***sold****:* No

Whether tissue samples collected will be ***shared*** *with persons from* ***another*** *institution:* No

Benefits *from this study:* Increase the knowledge of occurrence of hernia and will therefore benefit other patients

Risks *involved in participating in this study:* Nil

How the ***results*** *will be used:* The results will be used to establish collagen composition defects as an important cause of inguinal hernia

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, you have the right to withdraw from the interview / study at anytime. You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at

any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor

would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will NOT be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to

participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: 94866 00845

Contact number of Ethics Committee Office: 0422 2570170

Extn.: 5818

DATA COLLECTION SHEET

Name:

IP. No:

Diagnosis:

Date of sampling:

Comorbidities:

Duration of symptoms:

Family history:

History of Chronic cough:

History of Frequent vomiting:

History of Constipation:

History of Difficulty in micturition:

History of Lifting heavy weights:

Procedure:

H&E Staining (Skin):

Grade:

H&E Staining (Transversalis fascia):

Grade:

IHC Staining (Skin):

Collagen I:

Collagen III:

Collagen I/Collagen III:

IHC Staining (Transversalis fascia):

Collagen I:

Collagen III:

Collagen I/Collagen III:

Master Table - Controls

Control	Age	Sex	Skin		Ratio	Trans Versalis Fascia		Ratio
			Collagen I	Collagen III		Collagen I	Collagen III	
1	43	M	70	47	1.49	74	45	1.64
2	31	M	78	40	1.95	75	38	1.97
3	24	M	70	56	1.25	68	51	1.33
4	26	M	79	55	1.44	75	50	1.5
5	58	M	68	50	1.36	62	42	1.48
6	47	M	47	40	1.17	43	24	1.79
7	30	M	71	50	1.42	78	47	1.66
8	54	M	40	20	2	45	35	1.28
9	72	M	42	35	1.2	49	43	1.14
10	38	M	73	56	1.3	70	57	1.23
11	28	M	58	38	1.53	67	32	2.09
12	53	M	78	54	1.44	64	43	1.49
13	30	M	63	42	1.5	69	52	1.33
14	28	M	79	54	1.46	70	40	1.75
15	50	M	72	55	1.31	64	59	1.08
16	25	M	74	49	1.51	65	35	1.86
17	58	M	55	53	1.03	60	32	1.88
18	37	M	67	40	1.67	60	35	1.71
19	40	M	62	33	1.88	56	45	1.24
20	30	M	76	52	1.46	70	48	1.46
21	48	M	78	48	1.62	68	57	1.19
22	25	M	68	45	1.51	61	51	1.2
23	66	M	58	42	1.38	63	43	1.46
24	42	M	59	45	1.31	60	37	1.62
25	43	M	65	45	1.44	70	34	2.05
26	48	M	69	43	1.6	65	39	1.67
27	47	M	57	26	2.19	70	29	2.41
28	54	M	65	38	1.71	64	35	1.83
29	32	M	75	38	1.97	67	55	1.22
30	40	M	70	60	1.16	70	52	1.35

Master Table – Cases

Control	Age	Sex	Skin		Ratio	Trans Versalis Fascia		Ratio
			Collagen I	Collagen III		Collagen I	Collagen III	
1	46	M	67	50	1.34	60	45	1.33
2	32	M	54	45	1.2	51	45	1.13
3	21	M	64	60	1.06	55	52	1.06
4	24	M	74	65	1.13	70	61	1.15
5	56	M	62	57	1.09	52	45	1.15
6	46	M	39	30	1.3	41	34	1.2
7	32	M	65	55	1.18	60	50	1.2
8	56	M	31	25	1.24	39	30	1.3
9	78	M	30	20	1.5	47	41	1.15
10	32	M	82	78	1.05	71	64	1.11
11	25	M	56	40	1.4	54	32	1.68
12	54	M	71	69	1.02	62	45	1.38
13	32	M	59	50	1.18	60	51	1.18
14	29	M	75	70	1.07	58	43	1.35
15	49	M	71	61	1.16	80	67	1.19
16	22	M	75	64	1.17	60	43	1.39
17	57	M	59	55	1.07	55	46	1.19
18	35	M	53	52	1.02	59	46	1.28
19	42	M	63	47	1.34	60	48	1.25
20	27	M	80	71	1.13	74	59	1.25
21	42	M	71	55	1.29	75	64	1.17
22	26	M	75	52	1.44	64	55	1.16
23	65	M	56	47	1.19	59	49	1.2
24	43	M	57	50	1.14	54	47	1.15
25	46	M	50	43	1.16	52	39	1.33
26	65	M	64	71	0.9	68	55	1.24
27	57	M	38	20	1.9	37	23	1.61
28	52	M	39	32	1.22	42	32	1.31
29	34	M	65	55	1.18	59	57	1.03
30	42	M	68	59	1.15	63	55	1.15