



Hernias- Is it a primary defect or a systemic disorder? Role of collagen III in all hernias- A case control study



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HIGHLIGHTS

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- Grade and intensity of staining.

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ABSTRACT

Introduction: The need of this study is to assess the role of collagen III in all hernias which include primary inguinal hernias ventral and recurrent abdominal hernias.

Collagen type III represents the mechanically instable, less cross-linked collagen synthesized during the early days of wound healing. Quantitative assessment of collagen III in scar tissue on transversalis fascia as tissue obtained from cases operated for various hernias and compared to that of patients operated for abdominal surgeries for indications other than hernia was compared.

Materials and methods: In this study we had 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 were included as controls. Size of 1 × 1cm transversalis fascia was taken in both subjects and was sent for quantitative assessment using Immunohistochemistry test. All the above cases were randomized as per age,sex,BMI, co morbidities and materials used for repair.

Results: Results were analysed quantitatively and classified into following groups:Based on intensity of staining into Mild, Intermediate and Well stained and based on Quantity of Collagen III into Grade 0–NIL, Grade 1–1–25%, Grade 2–26–50%, Grade 3–51–75%, Grade 4–76–100% (Table 1). In the case group we had 52.4%,35.7% and 11.9% of the cases in Grade 4, Grade 3 and Grade 2 which proved that there was increased presence of Collagen 3, where as 84.4%,4.4% and 11.1% of patients in the control group were classified as Grade 1, Grade 2, Grade 0. For the quantitative study -Chi square test value –81.279 and the p value < 0.001. For the intensity of staining -Chi square test value –57.64 and p value is < 0.001.

Conclusion: 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.

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1. Discussion

The etiology of hernias are multi factorial, the components of the extracellular matrix, and the importance of collagen which promote a loss of resistance and elasticity of the transversalis fascia

determines the onset and recurrences of hernias should be taken into account in the decision-making process for the surgical repair of inguinal hernias [1]. Collagen type I is characteristic for mature scars or fascial tissues while collagen type III represents the mechanically instable less cross linked collagen synthesized during the early days of wound healing [2,3]. Its already proved that the presence of collagen in adequate amounts and its properties are essential to render the transversalis fascia functional and give it adequate strength [4–6].

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In the study by –Antonio Britto Casanova et al., results showed 17.3% less total collagen in patients with hernias compared with the control group ($P < 0.01$). Type I collagen in patients with indirect inguinal hernias was 23.7% less than the control group ($P < 0.01$), type III collagen was 6.4% less in the controls ($P < 0.01$) [7].

Wagh et al. suggested that diminished collagen, in the sheath of the rectus abdominis muscles of patients causes indirect or direct inguinal hernias [8,9].

KlingeU found out that immunohistochemistry analysis revealed a decrease in the ratio of collagen I/III when compared to controls. The decreased tensile strength of collagen type III play a key role in the development of incisional hernias [10].

Peeters EI, DeHertoghG, JungeK, –2014 they compared the collagen type I/III ratio in patients with primary inguinal, recurrent inguinal, primary incisional and recurrent incisional hernia patients with controls and demonstrated that the ratio was significantly lower in skin and anterior rectus sheath fascia of the cases and also showed that incisional and recurrent inguinal hernia had lower ratio than primary inguinal hernia patients. And the important point noticed was that collagen type I/III ratio in skin was representative for that in abdominal wall fascia [11].

A study by Henriksen NA in which fifty-two patients were included showed that collagen alteration was also dependant on the types of hernias presented like there was more pronounced changes in patients with direct inguinal hernias than in indirect and other hernias. Consistent findings showed a significant increase in the immature collagen type III with relation to the stronger collagen type I in those patients with a hernia, which further resulted in thinner collagen fibres with a decreased biomechanical strength [12]. Rosch r demonstrated that there was a significant lesser amount of collagen and higher amounts of elastic fibres in transversalis fascia from those patients with direct inguinal hernia as compared to those patients with indirect inguinal hernia [13].

Even though genetic factors was thought to be responsible for the elevation of collagen III was not clear, this study conducted in 1993 on “Increases in type III collagen gene expression and protein synthesis in patients with inguinal hernias” in the *Annals of Surgery* was the first attempt which showed the world that individuals with the abnormality in the collagen production have a higher chance for developing hernias. It has been showed that constitutive systemic increase in type III collagen synthesis may result in reduced collagen fibril assembly in the abdominal wall, eventually leading to the development of herniation [14].

In our Case Control study, 45 different types of hernia were operated in our hospital and 45 cases selected as controls were included which satisfied our inclusion and exclusion criteria. A piece of transversalis fascia of size 0.5×0.5 cm was obtained from all the patients with prior permission from the patients before the surgery and was taken in a formalin container to the Central Research Laboratory and was processed and then taken for immunohistochemistry test. Of the 45 patients in cases study, the youngest was 25 years old and the oldest was 60. The mean age was 42.7 years. There were 35males (77.7%) and 10 females (22.2%) among the 45 patients studied in case group. We had a variety of bilateral, unilateral inguinal hernias, lower midline, umbilical and a spigelian hernia.

Of the 45 patients in control group, the youngest was 22 and oldest was 59. The mean age was 43.7 years. There were 30males (66.6%) and 15 females (33.3%) among the 45 patients studied in control group. Included cases were appendicectomy, intestinal obstruction, abdominoperineal resection, carcinoma stomach, whipples procedure and colostomy closure in the control group and we took a piece of fascia transversalis from all the patients with prior permission and was processed in the same way and taken up for immunohistochemistry (see Figs. 1–5).

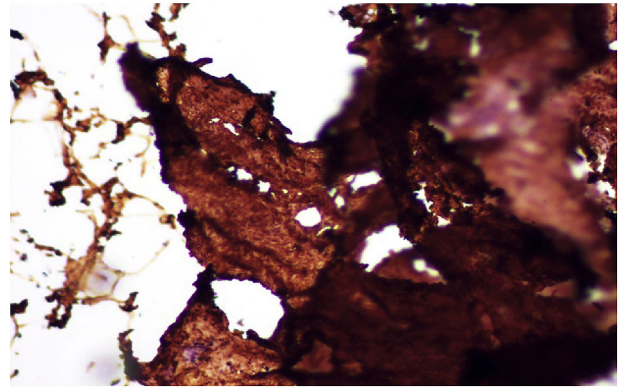


Fig. 1. Grade 4.

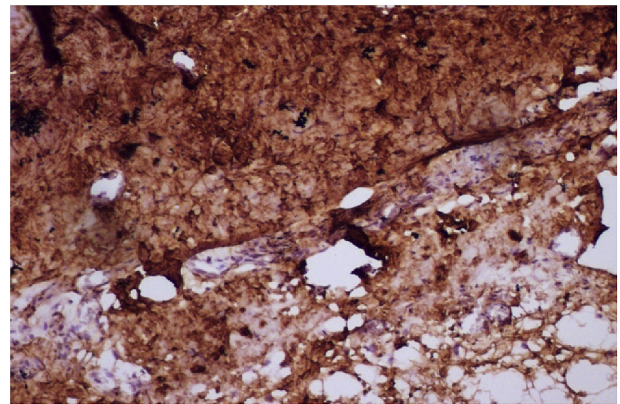


Fig. 2. Grade 3- collagen.

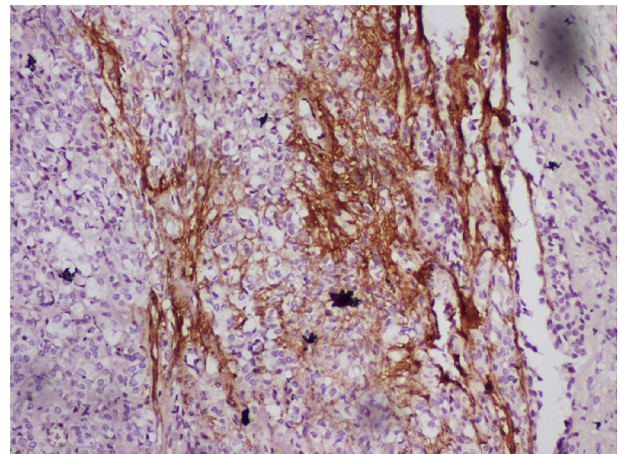


Fig. 3. Grade 2- collagen.

52.4% of the cases in Grade 4 and 35.7% of the cases in Grade 3 in the cases, here we can see the increased amount of collagen and the intensity of staining by the dye used in the immunohistochemistry test. In the control group 84.4% of patients in the control group with Grade 1, 4.4% of the patients with Grade 2 and 11.1% with Grade 0 showed significant amount of decrease in the grading and intensity of staining of collagen. What was different in this study was since we evaluated only collagen III when compared to all other studies cited, a ratio of collagen I: collagen III could not be assessed

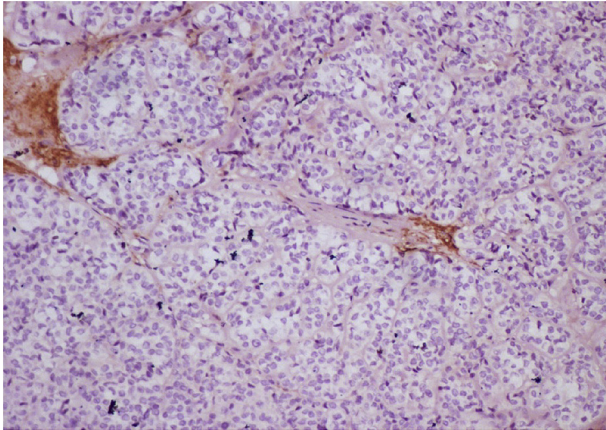


Fig. 4. Grade 1- collagen.

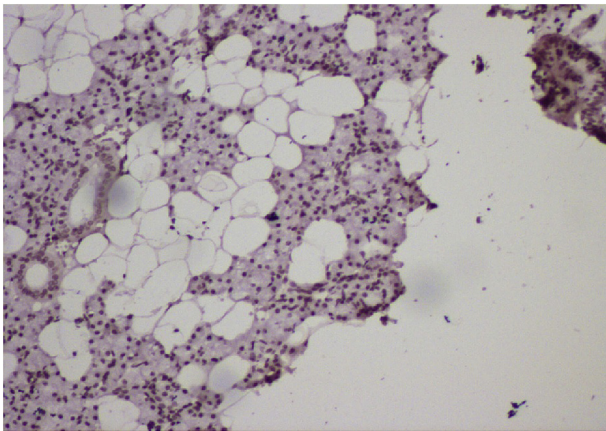


Fig. 5. Grade 0.

but the results and analysis showed a significant increase in collagen III in hernia patients when compared to the controls.

Limitation of this milestone concept it's a quantitative analysis and not a standardized way of calculating the amount of collagen keeping in mind the human errors.

2. Results

A total of 90 patients of which 45 represented cases and 45 represented controls were recruited into our study based on the inclusion and exclusion criteria.

All slides were assessed and mounted on high power and low power microscope.

Results were analysed quantitatively and classified into following groups:

3. Quantity of collagen III (see Table 1)

Grade 0	NIL
Grade 1	1–25%
Grade 2	26–50%
Grade 3	51–75%
Grade 4	76–100%

3.1. Statistical analysis of the grading of staining Table 2

Assessing the results in the case group we had 52.4% of the cases in Grade 4 and 35.7% of the cases in Grade 3 and 11.9% cases in Grade 2 which signifies that there is an increased presence of Collagen 3 in the patients which we have included in our study where as we had an 84.4% of patients in the control group with Grade 1, 4.4% of the patients with Grade 2 and 11.1% with Grade 0. *Chi square test value is 81.279 and the p value < 0.001 which is significant.*

4. Intensity of staining

They were classified into Mild, Intermediate and Well stained groups.

Out of the 22 slides(in cases) included in Grade 4, 20 of them were included in the group of well stained and 2 of them was included in intermediate group. 15 of them included in Grade 3 had 7 with well stained slides and 8 with intermediate stain. 5 slides were included as mild stain.

Out of the 38 slides included in Grade 1 (in Controls) most of them were included in mild stain with 2 of the slides included in intermediate stain.

4.1. Statistical analysis of intensity of staining Table 3

Assessing the intensity of staining 64.3% of the cases were included in well stained group, 23.8% were included in the intermediate group and 11.9% were included in the mild group.

In the control group 95% of the slides were included in the mild group and 5% were not stained. *Chi square test value was 57.64 and p value is < 0.001 which was significant.*

5. Conclusion

Despite sincere efforts made to optimize the patients and after taking all the precautionary measures prior to elective surgical procedure incisional hernias, recurrent hernias still remains a

Table 1
Results.

45 cases	45 Controls
22 Slides- Grade 4	38 slides-Grade 1
15 slides- Grade 3	2 Slides-Grade2
5 slides – Grade 2	5 Slides- Grade 0
3 slides – Improper staining	

Table 2
Statistical analysis.

Grade	Cases	Controls	Total
0	0 0.0%	5 11.1%	5 5.7%
1	0 0.0%	38 84.4%	38 43.7%
2	5 11.9%	2 4.4%	7 8.0%
3	15 35.7%	0 0.0%	15 17.2%
4	22 52.4%	0 0.0%	22 25.3%
Total	42 100.0%	45 100.0%	87 100.0%

Chi-square = 81.279, p < 0.001.

Table 3
Intensity of staining.

Intensity	Cases	Controls	Total
Well Stained	27 64.3%	0 0.0%	27 32.9%
Intermediate	10 23.8%	2 5.0%	12 14.6%
Mild	5 11.9%	38 95.0%	43 52.4%
Total	42 100.0%	40 100.0%	82 100.0%

Chi-square = 57.64, $p < 0.001$.

challenge to all surgeons. Presence of high quantity of collagen III in the tissue obtained from cases operated for hernia and poor staining in those operated for other than hernia indicates that there is an intrinsic and inherent weakness in the tissue which makes the individual more prone for developing hernia that is primary, incisional or recurrent hernias. The staining and quantitative assessment has proved 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.

Ethical approval

By MS Ramaiah Medical College, Ethical Committee.

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Personal.

Author contribution

Both authors equally helped in collecting data and gathering information about the study.

Conflicts of interest

No.

Guarantor

DR. Sam Koruth.

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