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Research Article

Histological Study in Linea Alba of Herniated Patients

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Abstract

Background: several studies were carried out on hernias. The recent studies are concentrated on the architecture of collagen fibers that form the Linea Alba.

Objective: To identify the histological structures and the thickness of collagen fibers in Linea Alba of herniated and non-herniated cases.

Methods: 14 samples from the herniated patients, 10 samples as control. Histological preparation of slides from both samples was stained by Hematoxylin and Eosin (H&E)and Van Gieson stains. Collagen fibers thickness was estimated by digital microscope with camera. According to length and weight, body mass index (BMI) is measured to identify obese patients. Statistical analysis was done by computer SPSS.

Results: Control samples have thick collagen fibers with high density for staining ability and regular arrangement except for some bundles that showed thin collagen fibers in some regions.

All the herniated samples were having thin, irregular collagen fibers with some spaces in between the bundles. More destructive features were seen in herniated samples especially in obese & smoker patients. They had more irregularity in fibers with low staining ability; identified spread of adipose tissue; newly developed blood vessels and some of them were dilated and engorged with blood cells.

Conclusion: We suggest that weak fibers which developed due to different reasons can initiate a hernia later on.

Key words: Hernia. Linea Alba. Histological structure

Introduction

Linea Alba is a fibrous tissue along the midline of the abdomen attaching muscle to bone. It contained collagen fibers that have an important effect on the transmission of force and are formed by the fusion of aponeuroses of abdominal muscles. It is organized into three laminae, anterior, middle, and posterior. Pulei, et al. [1] reported that elastic fibers are more concentrated in the anterior lamina while other researchers noticed that Linea Alba has a special architecture, they demonstrated the presence of two layers of collagen fibers: a transverse layer in the dorsal side and an oblique layer in its ventral side [2].

Tendon is primarily composed of collagen type I, which is organized into fibers Woo [3], Collagen synthesis, maintenance, and repair are done by their tenocyte cells [4].

As a result of different reasons, tissue tearing may occur and a hernia will develop.

Researchers proposed the early biological mechanism for the development of primary and incisional hernia is an abnormality in collagen metabolism. Elastic fibers were fragmented, thickened, and tortuous [5]. Another study noticed high staining scores for both collagen and elastin fibers in a control group as compared with the herniated group [6]. There is an increase in the number of cells, vessels, and disorganization of collagen framework per field in herniated samples as compared with the control group [7].

It has been reported that collagen bundles were thinner, less regular with disrupted architecture in obese patients [8]. It is believed that laparotomy wound healing is impaired in obese animals [9].

In addition; tobacco, alcohol, and diabetes cause remodeling in cremaster muscle and cause inguinal hernia [10] while smoking represents a significant risk factor for incisional hernia [11]. The present study aimed to investigate the histological structure of Linea Alba, the thickness of collagen fibers in herniated cases, and their effect on the onset and recurrence of a hernia.

Patients and Methods

This prospective study was conducted in Basra Teaching Hospital and approved by the local ethics committee. Every patient signed an informed consent after providing a full explanation of the procedure.

Twenty-four patients were operated on between the 1st of December 2018 and the 31st of December 2019.

Twenty-four tissue samples were used in the present study. Linea Alba specimens were obtained from non-herniated patients undergoing laparotomy operated as a control (10 cases) and 14 cases herniated group. A total of twenty-four samples were used in the present study. Slides were examined by two histologists without knowing the source of the sample, whether it was from a patient with a hernia or from a patient who did not complain of a hernia. Those with congenital abnormalities, scars, pregnancy, wounds, tumors, and any other abnormalities in the abdominal wall were excluded from the study.

Specimens were collected directly from patients after operation, tissue samples fixed in 10% neutral formalin for about 12-24 hours, dehydrated in successive increasing ethanol/water concentration 50%, 70%, and 90% then absolute alcohol was used. Tissue cleared with xylene, embedded with melted paraffin . 6-7 µm thick section was cut and mounted over an albumenized glass slide [12]. The sections were stained by H&E for general histological analysis and by Van Gieson stain to visualize more details about collagen fibers.

Microscope- Leica Model with a fixed digital camera was used for the demonstration of collagen fibers arrangement and estimation of collagen fibers thickness in micrometers.

Pictures were taken from different fields. Use of body mass index to identify obese patients (BMI > 35).

Statistical analysis was performed by computer-assisted analysis to indicate statistical significance.

Results

Histological interpretation

Control group: Figures 1-4

Tissue sections revealed a high density of fibrous elements that arrange in regular collagen fibers, some of them had a wavy appearance. Tissue sections appeared with a high density for staining ability with both hematoxylin and eosin and Van Gieson stains.

Presences of thick and thin regular fibers that have a range of dimensions 3.68-4.53 micrometers, mean 4.2 µm for female and 4.25µm for male cases (Table 1). A dipose tissue was infiltrated and appeared clearly in the fascia especially in obese cases. Small blood vessels were distributed in between the fibers and large blood vessels were in the loose connective tissue of the fascia.

Cellular elements, tenocytes were prominent at the periphery of collagen fibers, with dark elongated nuclei. Capillaries were seen in between collagen fibers lined by endothelial cells.

Herniated group

Histological findings of the herniated group appeared in some regions having thin, weak, and irregular collagen fibers, (2.29 - 3.2 micrometers) in dimensions as shown in (Figure 5,6) pointed arrow. Other regions appeared normal with a range of dimensions between 3.5 to 4.6 micrometers as in (Figure 7,8) pointed arrows. There was an increase in the spread of blood vessels as compared with figures taken from control cases, some of them engorged with blood cells and dilated as shown in (Figures 9-11) pointed arrows.

There were proliferating cells with large ovoid nuclei and localized in groups, some of them were adjacent to small spaces as shown in (Figure 12) pointed arrow. In addition to interspersed flattened tenocytes in the compact collagenous fibers as in (Figure 13) pointed arrows.

In obese, herniated cases, tissue sections appeared (Figure 14-16) with low density of fibrous elements and disrupted arrangement.

Table 1: Mean of thickness in micrometer of collagen fibers according to sex in the control group

Control	Female	Male
Mean	4.2	4.25
SD	0.483838816	0.588866713

P-value 0.1254808

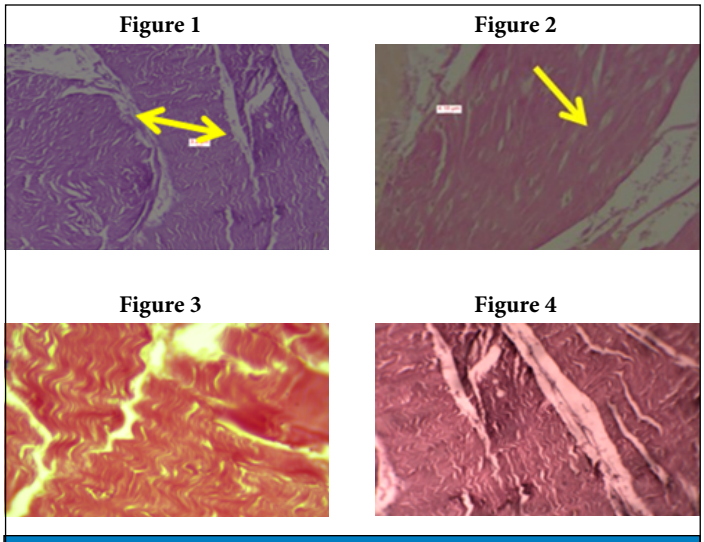


Figure 1: 100X Control sample.

Figure 2: 400X Control sample Collagen bundle is pointed by arrow thick and thin regular fibers High density.

Figure 3: 400X Control sample.

Figure 4:40X Control sample Wavy collagen fibers (VonGeison) weak fibers.

Adipose tissue was infiltrated to occupy regions in the sections. Thin collagen bundles were present in an irregular manner and separated by spaces. In addition to other histological features that appeared in the field, there were a large number of blood vessels, some were dilated and engorged with blood cells.

The histological findings in the herniated cases from smoker patients show the low density of fibers, less staining ability, more disrupted collagen fibers that have many spaces as in (Figure 17-19).

About collagen fibers thickness, there were no statistically significant differences between the control and herniated cases.

Discussion

The present study revealed that some control cases got variability

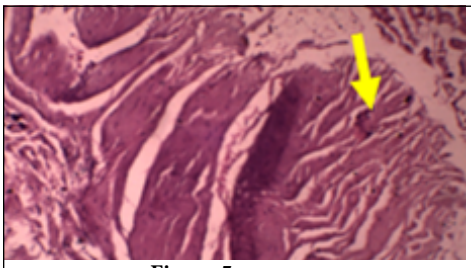
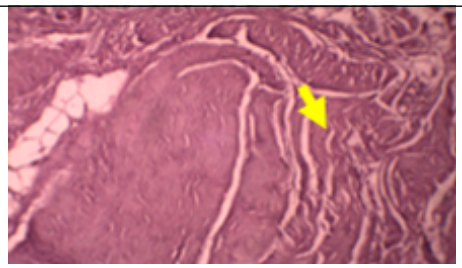


Figure 5



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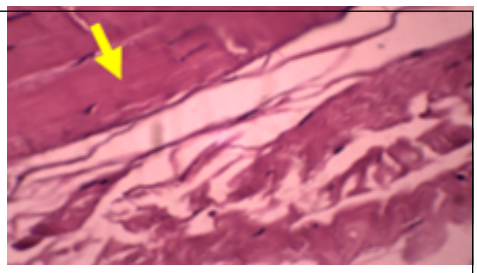

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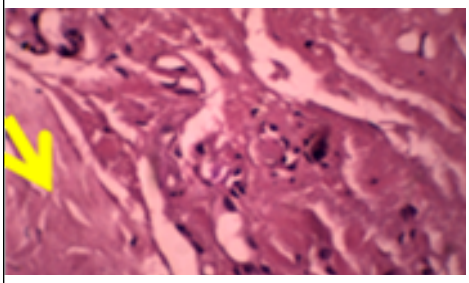

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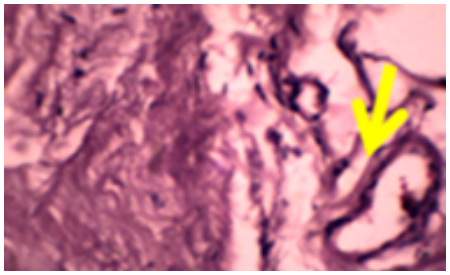

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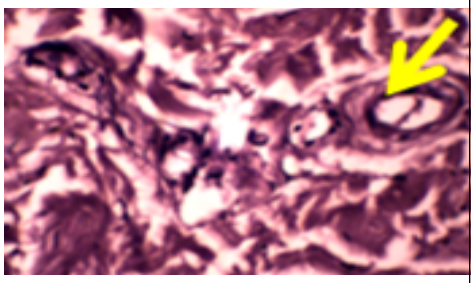

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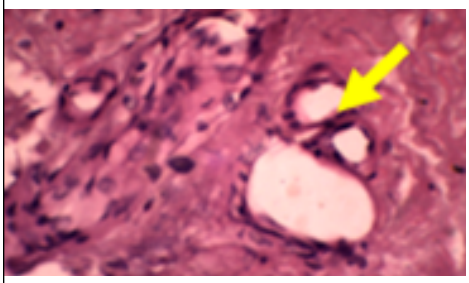

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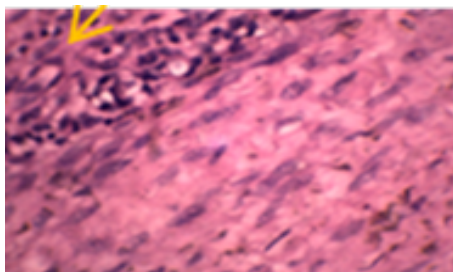

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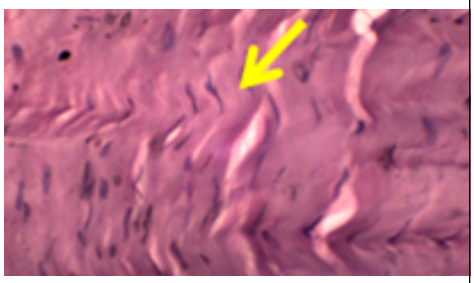

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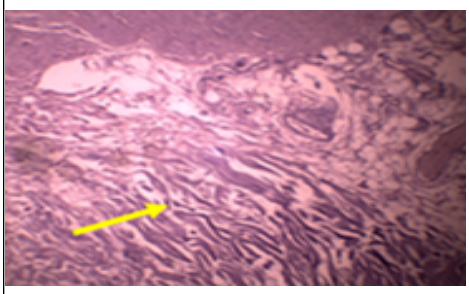

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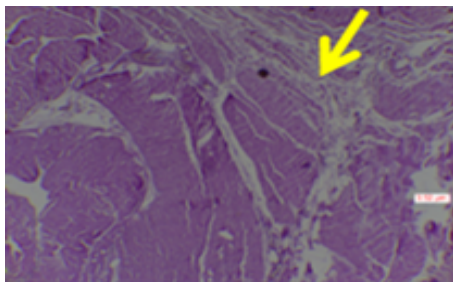

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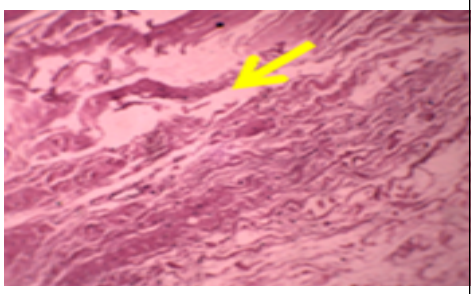

Figure 16

Figure 5: 40X Hernia sample.

Figure 6: 40X Hernia sample Thin fibers (2.29- 3.2 micrometers) weak, and irregular fibers.

Figure 7: 100X Hernia sample.

Figure 8: 100X Hernia sample Fiber dimension 3.5 micrometer Fiber dimension 4.6 micrometers.

Figure 9: 400X Hernia sample.

Figure 10: 400X Hernia sample Increase of blood vessels Vessels engorged with blood cells.

Figure 11:400X Hernia sample.

Figure 12: 400X Hernia sample Dilated vessels cells with large ovoid nuclei in groups.

Figure 13: 400X Hernia sample .

Figure 14:100X Hernia sample (obesity) Flattened tenocytes in the disorganized low density fibers compact collagenous fibers

Figure 15: 40X Hernia sample (obesity).

Figure 16: 40X Hernia sample (obesity) Infiltration of adipose tissue irregular, thin collagen bundles separated by spaces

in the thickness of collagen fibers in various regions with a range of dimensions between 3.68 to 4.53 micrometers (Table-1). Histologically it was identified as a change in the morphology of collagen fibers that appeared as thin weak fibers in some bundles. According to our results, there was no significant difference in the thickness of collagen fibers between control and herniated cases in both sexes. We propose a kind of relationship between these thin collagen fibers with the possibility of hernia occurrence if it is associated with other reasons (genetic, diabetes, smoking, alcohol, or malnutrition) [10,13,14].

Our finding confirmed others who had evidence about significant correlation between thickness and density of collagen fibers in Linea Alba with its tensile strength which mentioned by, Korenkov , et al. [15].

All the herniated cases revealed regions with a low density of collagen fibers as in figures 5, 6. They were disorganized fibers with thin, weak morphology and 2.29-3.2 micrometers in dimension. These findings agree with previous studies, Pans et al[7]. Also, a thin, disorganized network of collagen fibers was identified in obese patients (Figure 14-16); that confirmed the findings of Liyu Xing et al. & Szczesny W. et al [8,9].

Another histological feature was the presence of spaces in between collagen fibers which were seen in figures 8 and 10. We propose the development of these spaces resulted from the formation of immature collagen fibers that haven't the ability to form bundles or as a result of the degenerative process in tissue as a result of traumatic action. These findings confirm other studies, Liyx Xing et al [9]

In figure 12, we identified numbers of proliferating cells and small adjacent spaces, these cells were tenoblasts, which appeared in clusters and away from the collagen fibers. The small spaces are the newly developed vessels that formed as a result of the tissue repair process, our finding is in agreement with Franchi et al [16].

Our results are in agreement with Pans et al [7] about the increase in cells and vessel number and disorganization of collagen fibers in the herniated group. Tendon healing can occur intrinsically by the proliferation of tenocytes, Sharma & Mafulli [17]. Neovascularization is critical to the repair of tissue and healing of wounds,

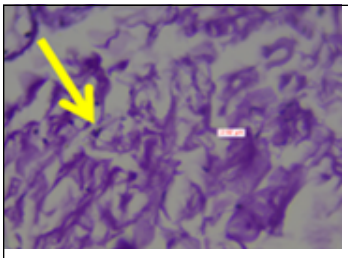

Figure 17

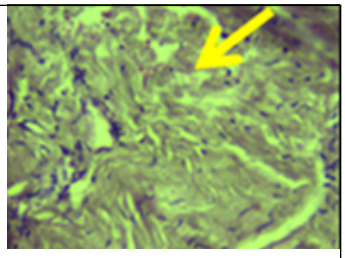

Figure 18

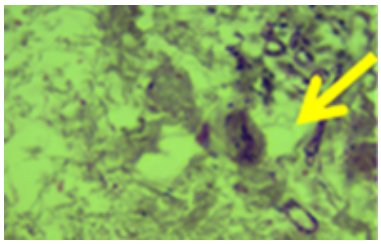

Figure 19

Figure 17: 400X Hernia sample.

Figure 18: 400X Hernia sample (smoking) Low density of fibers, less staining ability disrupted collagen fibers.

Figure 19: 400X Hernia sample (smoking) Many spaces between fibers.

while tendons have poor regenerative capacity [16,18-20].

Adipose tissue was infiltrated in fascia in samples from obese patients. In these samples, collagen bundles of Linea Alba are identified, with a low density of fibrous elements, less regularity, and widespread of newly developed blood vessels. Our findings are in agreement with Szczesny, et al [8].

Obesity is a risk factor for the incidence and recurrence of the hernia [21,4]. Other researchers confirmed the same findings histologically by the presence of a small number of collagen fibers in Linea Alba above the umbilical region at obese patients [22,23].

The smoker herniated cases are identified in Figures 17-19. Tissue response was seen by newly developed fibers that are immature and fail to form bundles. These fibers had low staining ability with hematoxylin and eosin stains. They were disorganized with many spaces. These results confirm other studies about the effect of tobacco on remodeling in collagen fibers of cremaster muscle and causing hernia [10].

The morphological findings in both obese and smoker patients are identified by their disrupted architecture of collagen fibers. They

failed to develop bundles with the presence of newly developed vessels and infiltration with the adipose tissue, especially in obese patients.

Conclusion

Thin collagen fibers were restricted in some regions of control cases and identified with more features in herniated cases. We proposed these weak fibers were developed as a result of many reasons and could be a cause for the development of hernias in the future. Obese and smoker herniated patients had more disrupted irregular, weak collagen fibers that give more chance for recurrence of a hernia.

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