The Potential of Transforming Growth Factor-Beta1 (TGFβ1) in Fibrotic Tissue of Leiomyoma Specimens

Marwa Jamal Hussain Al Kinani[1], Ahmed Mahdi Saleh Al-Badri[2]

[1]*Histology, Embryology and Anatomy Branch, College of Medicine, University of Sumer, Iraq*
[2]*Biology Department, College of Science, University of Wasit, Wasit, Iraq*

KEYWORDS
Fibrous Tissue, Uterine Leiomyoma, TGF.

ABSTRACT
Background: Fibrosis can define as the development of excessive fibrous tissue in an organ or tissue. In the situation of the uterus, leiomyoma or scar tissue may involve fibrosis state. The main feature of fibrosis in myoma is the existence of disordered smooth muscle cells and collagen that can be recognized via histological examinations in addition to extracellular component deposition. Leiomyoma is the most public uterine disorder that is mainly consists of smooth muscle cells, myo-fibroblast, and an obvious content of extracellular matrix. The myofibroblast in myoma lesion produced an elevated content of ECM. This paper displays the fibrotic circumstantial in leiomyoma and the mechanisms related to deposition of ECM components.
Methods: this study involved (50) patients of 18-80 years old women diagnosed with benign uterine fibroid. Tumor samples were attained by consent from women undergoing hysterectomy or myomectomy at the Al Karama Teaching Hospitals in Kut, Wasit Province, Iraq between October 2022 to March 2023. samples from normal and fibroid were subjected to routein histological processing and subsequently stained with routein stains for general description, Masson Trichrome stain, Van Gieson's stain to visualize collagen content and histochemical stain (Alcian Blue-Periodic Acid Schiff) to evaluate the profile of mucins secreted via tumor cells.Normal myometrium and myoma tissue expression of TGF β1 growth factor was compared using immunohistochemical staining. Qupath and Image J programs were used to analysis the content of fibrous tissue within normal and myoma tissue.
Results: Microscopical results revealed that myoma have a proliferation of overlap fusiform cells organized in bundles alienated by variable amounts of connective tissue fibers, enclosed peripherally by a pseudo capsule. Special stained sections analysis revealed deposition of extracellular martix components specially collagen fibers that vary within each nodular fibroid (intrafascicular, interfascicular) enclosed myocytes that reflect the state of fibrosis that appears as blue – green colors enclosed in Masson's trichrome stain, while in Van Gieson's stain appears as red color. Proliferative tumor cells were revealed via Alcian Blue-PAS stain in dark blue color. results showed distribution of perivascular fibers in myoma as compared with normal adjacent myometrium, which appears in red color in Van Gieson's stain. Immunostaining recorded the significantly higher intensity of TGF-β in the leiomyoma tissue than in the normal myometrium ($P < 0.05$). The effects of the TGF-β1 inhibitor significantly differed between normal myometrium and leiomyoma tissue, with a greater decrease in cell survival in the leiomyoma tissue ($P < 0.05$).
Conclusion: highly fibrous tissue contents showed within myoma and perivascular than adjacent myometrium, these fibrous tissues were detected via special colours in both Masson Trichrome and Van Gieson's stains. The fibrous tissue within leiomyoma showed higher intensity to TGF than normal myometrium.

1. Introduction

Tissue fibrosis is defined as the excessive or uncontrolled deposition of extracellular matrix ECM proteins [1]. It is frequently associated pathophysiologicly with a wide range of disorders [2]. Due to their excessive invention and deposition, these proteins are abundant in uterine fibroids, which are induced by aberrant proliferation of smooth muscle cells and fibroblasts [3]. Uterine leiomyomas, commonly referred as fibroids or myomas, are the primary non-malignant neoplasms affecting the female reproductive system [4]. The precise etiology of these tumors remains uncertain; however, there is evidence suggesting that steroid hormones and growth factors, along with their receptors, may have to significant impact their pathogenesis [5].

The main key feature of leiomyoma includes single or multiple firm circumscribed masses occupying any site within uterine layers, these masses are identified to have a smooth muscle constituent and often exhibit a significant (ECM), composed of fibroblasts, and purportedly leading to a predominance of collagen fibers [6]. The presence of a fibrous and collagenous component in these tumors justifies the vernacular name "fibroid" that is commonly used [7]. Histologically, leiomyomas consist of fascicles of smooth muscle cells that are intricately intertwined; resulting in whorl-like patterns, the stroma surrounding the embedded smooth muscle cells primarily comprises collagen fibers [8, 9]. The distinguishing features of the uniform cells include eosinophilic fibrillary cytoplasm and cigar-shaped

nuclei [10, 11]. In general fibrosis is promoted and stimulated by a number of different cytokines and growth factors [12, 13] These variables are controlled by steroid hormones, are thought to be the hormones' ultimate effectors, Proteins called growth factors regulate the rate at which cells divide, and this ability confers a wide range of biological effects. Several different kinds of cells secreted it. The majority of these factors' effects on target cells are mediated by interactions with receptors located on the cell surface [14]. One such element is transforming growth factor-ß (TGF-ß), which play a significant role in the etiology of fibrotic disorders via affects numerous cellular processes, including proliferation, differentiation, migration, and apoptosis [15]. Cells of the immune system, blood vessels, and connective tissue all express transforming growth factor; Reliable studies show that TGF- and connective tissue growth factor cooperate to promote chronic fibrosis [16].

2. Methodology

Study Cases

A total of 50 cases involving women aged 18 to 80 years were included in this study. Fibroid samples were obtained with consent from women undergoing hysterectomy or myomectomy at the Al Karama Teaching Hospitals in Kut, Wasit Province, Iraq during October 2022 to March 2023. Relevant details, such as patients' ages, parities, as well as menstrual histories, were documented from the departments of gynecology and pathology. The uterine samples underwent thorough evaluation by hospital pathologists who utilized established histopathologic criteria for diagnosis. The specimens were visually documented through, capturing details such as the numbers, shape, and location of the tumors. All the gathered specimens were transported to the pathology laboratory in a solution containing 10% formalin for the purpose of conducting routine and specialized histological preparations.

Tissue Processing

After immersion in a 10% buffered formalin solution. A total of six myoma sections were extracted from tumors that exhibited adequate dimensions, with four of these sections obtained from the outer region of the tumor and two from the inner region. Additionally, samples of myometrium and endometrium were collected from a significant number of patients. With four of these sections obtained from the region of the tumor and two from the inner region. The immobilized tissues underwent standard processing procedures, being embedded in paraffin, sectioned at a thickness of 4-5 micrometers, and subsequently stained with routine hematoxylin and eosin stain. Special stains, including Masson trichrome stain, Van Gieson's stain, were used to visualize collagen content, and a histochemical stain (Alcian Blue-Periodic Acid Schiff) was applied to evaluate the mucin produced by tumor cells.

Immunohistochemical staining

Dewax and hydrate the paraffinzed section, section incubated with E-IR-R217C (3% H_2O_2) for 10 min to eliminate endogenous peroxidase activity. Wash with PBS or TBS, 2 min×3 times. Then add E-IR-R217A (Normal Goat Blocking Buffer, incubate at 37°C for 30 min. Shake off any excess liquid. Section were incubated with TGFβ1 primary monoclonal antibody (TGF β1 Mouse (5D2): E-AB-22215) at 4c overnight then washed with PBS and dried. Secondary antibody E-IR-R217B (Polyperoxidase-anti-Mouse/Rabbit IgG), were added and incubated at room temperature then Washed in PBS three times. Prepare DAB Working Solution DAB (E-IR-R217D). Washed the sections with deionized water dismiss the chromogenic reaction, then counterstaining, dehydrated, clearing and covering.

TGFβ scoring

TGFβ staining was designated semi quantitatively, where score 0 presented less than 1% of cells as negative; score 1: 1-12% of cells; score 2: 12-27% of cells; score 3: 27-50% of cells; score 4: 51-76% of cells; and score 5: more than 76% of cells. The intensity of staining was classified as strong, moderate and poor. In this manner, TGF expression data compared between myoma and normal

myometrium [17].

Statistical analysis: computerized software statistical for the social sciences program (SPSS version 24) was used to analyze the data. By using the Pearson Chi-square test, assessment of TGF immune expression and pathological parameters had been calculated. A P value of ≤ 0.05 is considered as statistically significant.

3. Results and discussion

Microscopic Examination

Microscopically myoma exhibit a proliferation of fusiform cells organized in bundles that overlap, separated by variable amounts of fibrous connective tissue, and peripherally enclosed by the presence of a pseudo capsule formed through the compression of adjacent tissues as shown in figure 1 (a, b, c, d). myometrium in Leiomyoma appears as a well differentiated bundle of smooth muscle interlacing within the tumor mass contrasting with the adjacent myometrium where the bundles run coarsely parallel to the long axis of the body of the uterus.The myometrium samples in their natural state exhibited ECM and smooth muscle bundles that retained their integral structural composition, The myometrium next to the unaffected tissue in the uterus had a comparatively less ordered structure while myoma appeared as nodular structures formed by collagen fibers islands within the ECM and discrete clusters of smooth muscle bundles lacking a consistent orientation as showed in figure 4.

Sections with Masson's trichrome and Van Gieson's stains were employed to examine uterine fibroids and neighboring myometrium. The analysis revealed different concentration of collagen fibers in myoma reflecting the state of fibrosis and appearing as blue color while myocytes appeared in red color in Masson's trichrome stain as showed in figure 1 (b), In sections stained with Van Gieson's stain, collagen fibers appeared as red in color, as depicted in figure 1 (c).These collagen fibers produced by a proliferative tumor cells were demonstrated via Alcian Blue-Periodic Acid Schiff stain figure 1 (d). Fibroid nodules showed different amount of collagen fibers deposition between myocytes. These myocytes appeared irregular shape with long nuclei as seen in figure 2. These nodules demonstrating variable degrees of collagenous stroma appearing as little, medium or heavy collagenous content stained blue in Masson's trichrome stain as shown in figure 2 (a), while appearing red in Van Gieson's stains, as depicted in figure 3 (b).

The degree of fibrosis was determined by observing at the amount of extracellular fibrous matrix within tumor area that reflects both collagenous matrixes around blood vessels and between muscle fascicles figures 3. The tumors' collagen deposition could be intrafascicular, interfascicular, or a mix of the two as showed in (figures 3, 4) that show deposition of collagen fibers appear in blue-green colors within intrafascicular, interfascicular figure 4. These fibers arranged in different orientation (loss of regularity form) and enclosed myocytes with various concentrations and varied fibrous contents which appear in figure analysis image J (figures 5, 6). According to figure.7 , section of Leiomyoma contain a number of blood vessels within ECM and these vessels enclosed by variable amount of collagenous stroma that appears in red color in Van Gieson's stain as demonstrated in figure7.

ICH staining with TGF documented high to moderate expression in leiomyoma lesion compared with normal myometrium (figure8). Myometrium sections with leiomyoma showed variable intensity as low, moderate and strong (figure 9) which reflect the effect of TGF on deposition and differentiation of extracellular matrix (ECM) cells especially smooth muscle cells, fibroblasts and myofibroblasts (figure 10). The content of ECM components in myomal stroma related to the level of fibrosis state.

At intensity level varied in myoma tissue, 20 (66.6%) of 30 myoma sample showed strong intensity to TGF, 7 (23.3%) of 30 showed moderate intensity while 3 only (10%) showed low intensity. As compared with normal myometrium TGF expression, the highest intensity recorded in 5 (25%) of 20 samples, 2 (10%), 13 (65%) as moderate and low intensity respectively (Table 1). IHC data analysis showed that all myometrium samples recorded positive reaction to TGF transformation growth marker 50 (100%) of 50 samples distributed between normal and myoma lesion (table 1).

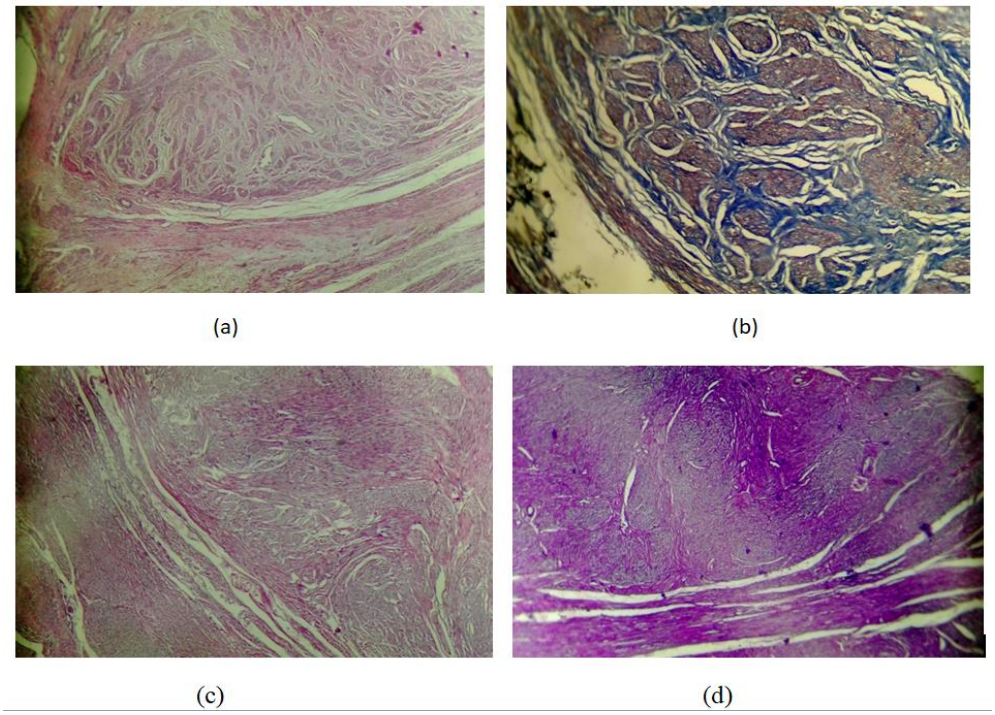


Figure 1: microscopic sections of uterine showed leiomyoma enclosed by psudocapsule separated normal myometrium and well-differentiate myoma organized as a bundle of smooth muscle cells interlacing in the tumor mass H&E stain (a), Masson's trichrome stain (b), Van Gieson's stain (c), Alcian Blue-Periodic Acid Schiff stain (d) 4x.

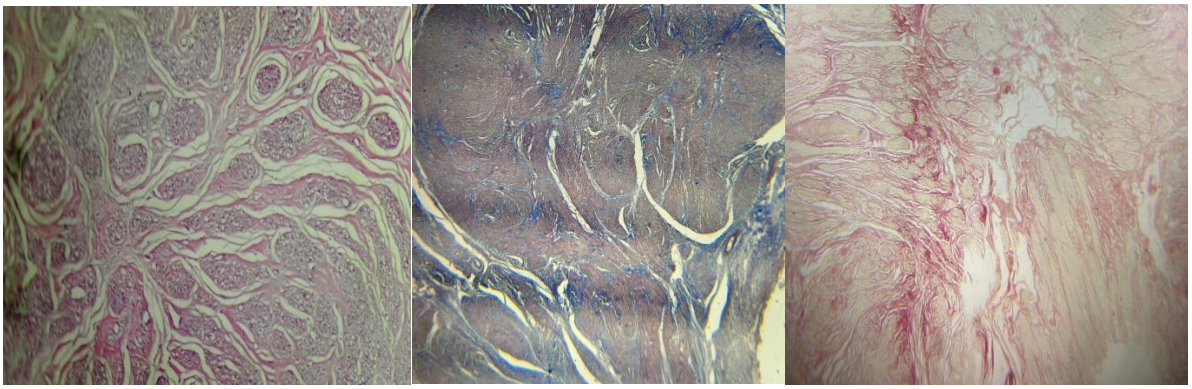
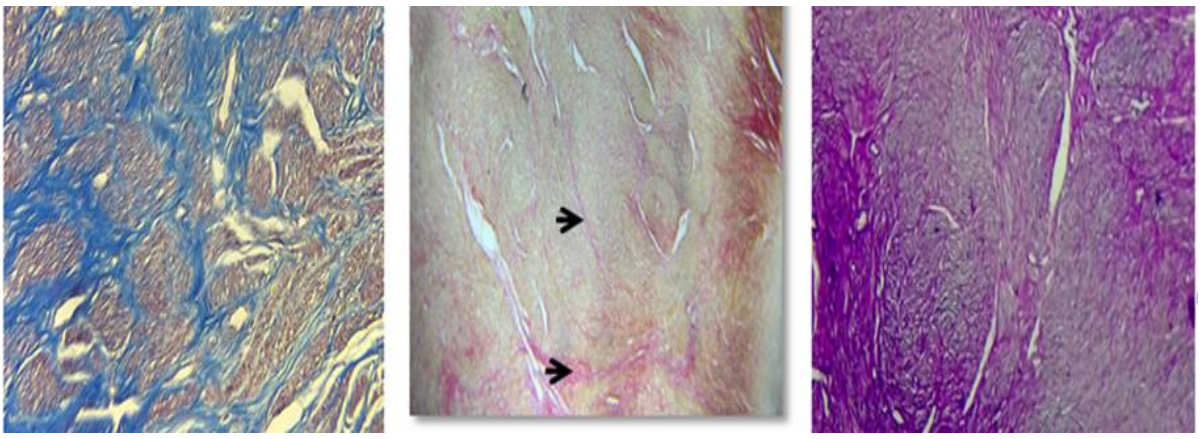
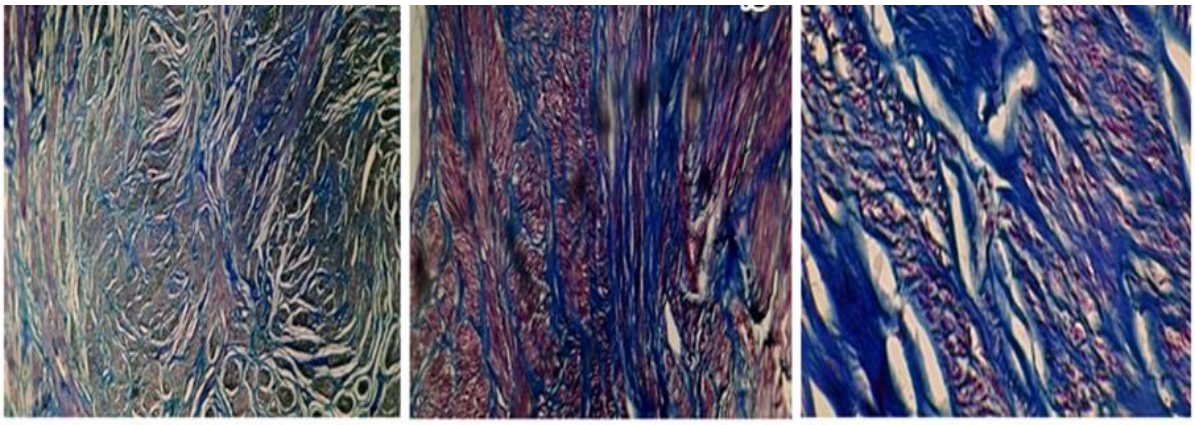


Figure 2: microscopic sections of leiomyoma stained with three stains show fascicles of smooth muscle bundle interlacing with collagen fibers within tumor mass as multi nodular myoma in H&E stain (a), Masson's trichrome stain (b), Van Gieson's stain (c) 10x.



(a) (b) (c)

Figure 3: microscopic sections of myoma show red color myocytes within intrafascicular, interfascicular collagen fibers in blue colour stained in Masson's trichrome stain (a) , red color in Van Gieson's stain (b), Alcian Blue-Periodic Acid Schiff stain (c), 100x.



(a) (b) (c)

Figure 4: microscopic section of leiomyoma stained in Masson's trichrome stain showed varies level of collagen deposition within ECM appears in mild -moderate blue-green colours around red colour myocytes (a), intensely blue colour fibres (b) (c), 10x.

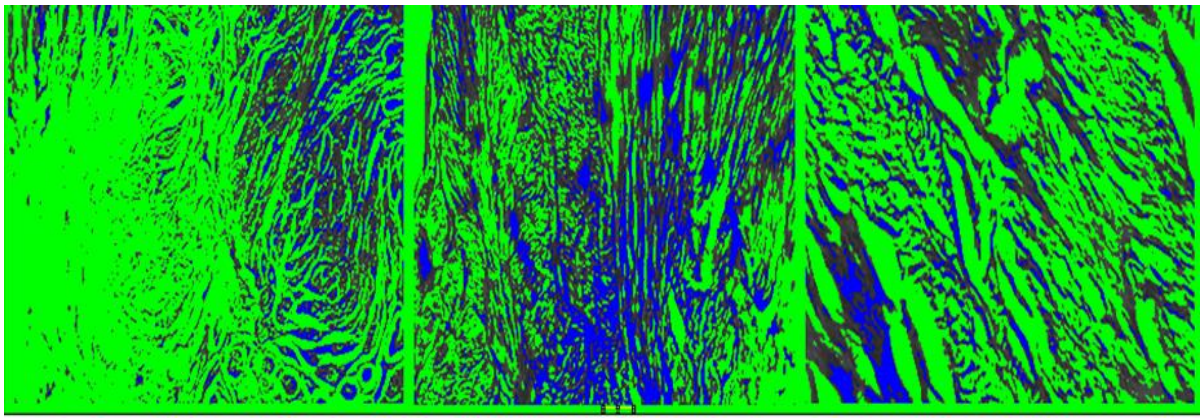


Figure 5: microscopic section of myoma showed various levels of collagen fibers deposition within ECM appears in green colour by using (image J).

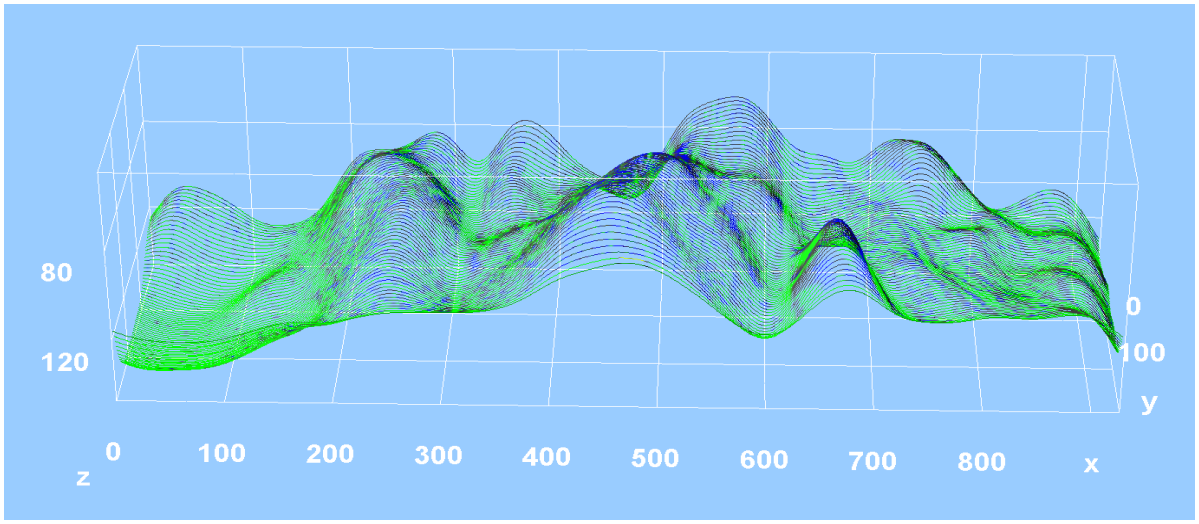
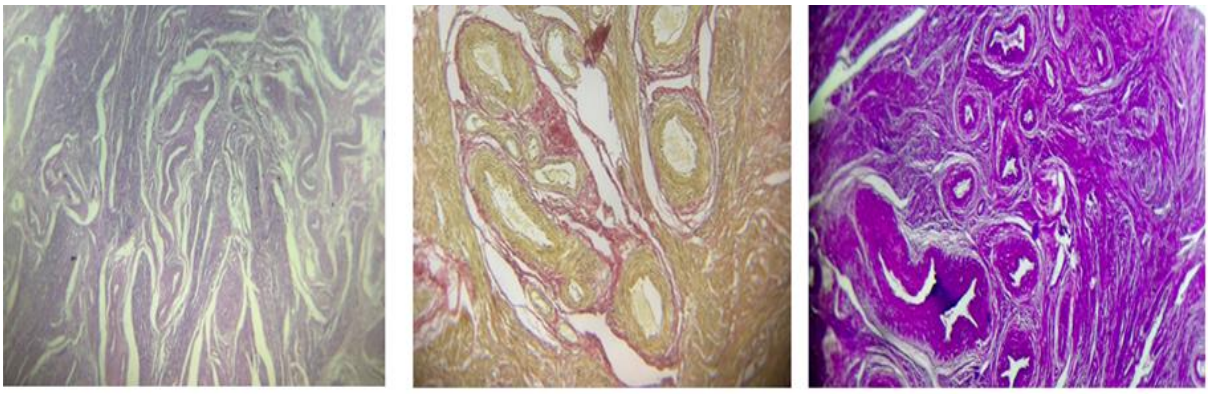
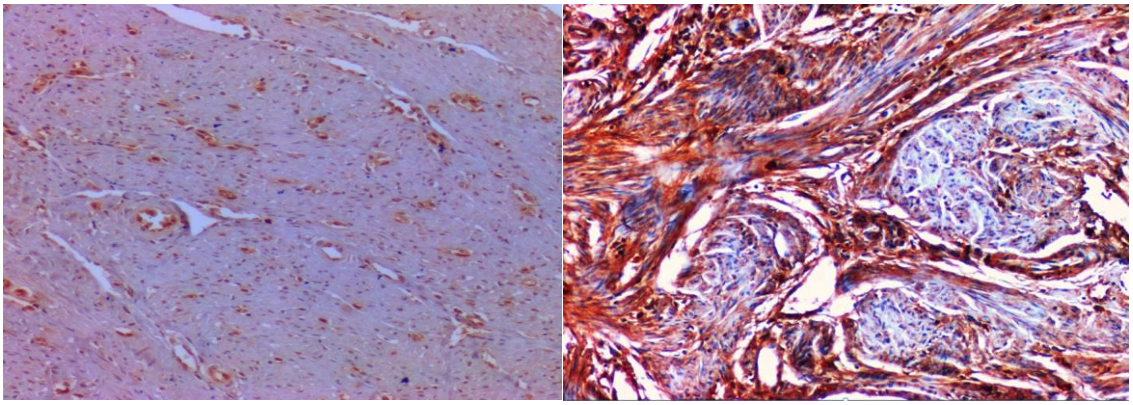


Figure 6: surface plot show sections of myometrium with Leiomyoma showed various levels of collagen fibres deposition within ECM appears in green colour by using (image J).



(a) (b) (c)

Figure 7: microscopic sections of uterine leiomyoma showed different numbers and size of blood vessels within the stroma in H&E stain (a) Van Gieson's stain, 100X. (b) Alcian Blue-Periodic acid Schiff stain (c), 100X



(a) (b)

Figure 8: Microscopic section of TGFβ1 immunostaining in (a) normal myometrium showed low TGF expression. (b) leiomyoma showed moderate TGF expression, 100x.

Table1: Intensity of TGF-β staining in normal myometrium compared to leiomyoma lesions

cases	N.	Negative expression	Poor intensity	Moderate intensity	Strong intensity	*P*-valu
Normal myometrium	20	0 (100%)	13 (65%)	2 (10%)	5 (25%)	0.001
Myoma lesion	30	0 (100%)	3(10%)	7 (23.3)	20 (66.6%)	

Discussion

This study presents evidence for the presence of fibrous tissue within myometrium with Leiomyoma foci that appears as nodules that contain from irregular myocytes within fibrous ECM, Identification was based on both microscopically and immunohistochemical criteria. Microscopically, In normal myometrium, the myocytes existing within few or little amount of fibrous ECM as compared with adjacent and leiomyoma foci myometrium that showed a bundle of smooth muscle interlacing in tumor mass with excessive construction of ECM or of collagen fibers deposition which consider the main feature of uterine fibroid, this description was agreed with Aleksandrovych *et al*., [18] which he described the Leiomyoma as a production of bundle fusiform cells separated by variable aggregates of fibrous connective tissue also he refers that the ECM formed islands in leiomyoma tissue samples, and the smooth muscle bundles were arranged in asymmetrical groupings, the majority of spindle cells had

elongated nuclei. Muscle bundles create nodular forms in leiomyoma foci. Tissue samples taken from healthy myometrium showed intact structures supported by smooth muscle bundles. The surrounding myometrium was less structured structurally than the unaltered tissue (from the "healthy" uterus) and this was agreed with the findings of this study.

The result of both Masson's trichrome and Van Gieson's stains showed variable amount of collagenous matrix within myoctyes and also around the blood vessels, these collagen fibers appear as blue color in Masson's trichrome stain while in red color in Van Gieson's stain with different orientation which confirm previous studies showing that the finding of Giuliani *et al* [19] which he refers that leiomyoma have an abnormal collagen fibril construction and orientation, suggesting that its essential to study the interaction of collagen fibers and the variations in fibrous ECM may play a role in the pathogenesis of leiomyomas. Thus, the study of specific collagen parameters, such as collagen bundles size and orientation, turns out to be particularly interesting because its morphometry should conclude specific cellular performances [20] As a result, the amount of collagenous ECM deposition within fascicular mass and around blood vessels determine the degree of fibrosis which is agree with the detailed explanation of Flake, [21] which he classified the fibrosis in uterine Leiomyoma according to the amount of collagenous deposition.

IHC TGF result was accepted with Bhat et al. [22] which they confirm the high expression of leiomyoma tissue to TGFβ1 than normal myometrium. The explantation for the results include some reasons like the profound impact of estrogen and progesterone on leiomyoma growth is primarily linked to their capacity to stimulate the mRNA production of various growth-promoting substances. Additionally, activated estrogen and progesterone receptors engage with cytoplasmic elements involved in cell proliferation and viability pathways, thereby triggering their activation, the inflammation in the uterus due to infection, menstruation, mechanical injuries and oxidative stress enhance the EMT pathway to form myofibroblasts that produce ECM as a response to the effect of several cytokines and growth factors. In normal circumstances, this process can repair tissues damaged. However, it can also lead to fibrosis if there is no control or the deregulation of the controlling pathways is too high [23].

Typically, the process of fibrosis progression comprises of three distinct stages. Commencing with an organ injury, there is a subsequent activation of fibroblast into myofibroblast. Following this, the myofibroblast releases an excessive amount of (ECM) components, consequently enhancing the size and rigidity of the tumor tissue, Ultimately, the stiff nature of the ECM provides a conducive environment for the proliferation of fibroid cells [24].

The uterine fibroid primarily consists of benign smooth muscle cells which are distributed within a significant amount of extracellular matrix (ECM). This ECM, in return, contains numerous activated fibroblast cells, also known as myofibroblast cells, In a non-injured state, fibroblasts remain in a dormant condition. However, under acute or chronic stress conditions, these fibroblasts become activated and transform into myofibroblasts, ultimately leading to the synthesis of ECM components. In cases of chronic inflammation, myofibroblast cells are continuously stimulated by cytokines and inflammatory mediators such as TGF. In non-malignant cells, TGF-β inhibits cell proliferation and promotes programmed cell death [25].

Transforming growth factor-beta (TGF-β) serves as a cytokine with potent chemoattractant properties. This particular factor, which plays a role in recruiting and activating fibroblasts, is widely recognized as a potent profibrogenic cytokine implicated in fibrosis across various organs [26]. TGF is initially secreted in a latent complex form, necessitating extracellular activation for its signaling and activity. Due to its tissue-specific expression and interactions with receptors and regulatory molecules, this ligand elicits a diverse array of cellular responses [27]. The presence of chronic inflammation is a critical factor in promoting the synthesis and release of TGF-β, while estrogen and progesterone hormones are also acknowledged for their ability to enhance TGF-β production in fibroblasts [28]. In leiomyoma tissue, the expression of TGF-β1 was reported to be fivefold higher. The activation of the

phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway in fibroblast cells by TGF-β is primarily responsible for the transition of fibroblasts to myofibroblasts. This transformation leads to the excessive production and accumulation of extracellular matrix (ECM) components.This explains the higher levels of TGF-β1 in myoma cells compared to healthy cells, and thus the appearance of fibrosis in our lliomyoma samples. Numerous investigations have demonstrated that TGF-β not only induces proliferation of smooth muscle cells but also plays a role in the development of uterine leiomyoma [14, 29].

Immunohistochemical staining results of Jung and his team, Bhat et al., [22] showed significant higher intensity of TGF-β in the leiomyoma tissue than in the normal myometrium. Chegini *et al*. [30] presented TGF-β receptors as signal transducers of TGF-β in leiomyoma and normal adjacent myometrium, which are prominently expressed in leiomyoma. Lee and his team [31] offered that TGF-β3 was increased in leiomyoma in comparison to normal myometrium. Dragnei, 2013 also refers that immunoreactivity of leiomyomas samples to TGF- β 1 was higher than in normal myometrium. another articles showed a higher level of TGF-β in leiomyoma than in the myometrium according to patient menstrual cycle. Azam *et al*., (2021) refers that TGF-β1 reaction was more obviously in healthy myometrium with high intensity features compared to myoma.

4. Conclusion and future scope

The extracellular matrix deposition, particularly the interstitial collagen fibers, exerts fibrosis in uterine leiomyoma samples that show a disordered pattern which increased compared to the adjacent myometrium. Due to three types of special dyes, the intensity of the coloring of these fibers varied and reflected the severity of fibrosis.

Acknowledgements

We extend our sincere thanks to the staff at Al Karama Teaching Hospital in Kut, Wasit Province, especially the Gynecology and pathology Department, as well as the staff in histology laboratory for facilitating the collection of the samples studied in this work, as well as the pathologists.

Reference

[1] M. Malik, J. M. Norian, D. McCarthy-Keith, J. Britten, and W. H. Catherino, “Why leiomyomas are called fibroids: The central role of extracellular matrix in symptomatic women,” Seminars in Reproductive Medicine, vol. 28, no. 03, pp. 169–179, Apr. 2010, doi: 10.1055/s-0030-1251475.

[2] N. G. Frangogiannis, “Transforming growth factor–β in tissue fibrosis,” Journal of Experimental Medicine, vol. 217, no. 3, Feb. 2020, doi: 10.1084/jem.20190103.

[3] I. Szydłowska, M. Grabowska, J. Nawrocka-Rutkowska, M. Piasecka, and A. Starczewski, “Markers of cellular proliferation, apoptosis, Estrogen/Progesterone receptor expression and fibrosis in selective progesterone receptor modulator (Ulipristal Acetate)-Treated uterine fibroids,” Journal of Clinical Medicine, vol. 10, no. 4, p. 562, Feb. 2021, doi: 10.3390/jcm10040562.

[4] S. Okolo, “Incidence, aetiology and epidemiology of uterine fibroids,” Best Practice & Research in Clinical Obstetrics & Gynaecology, vol. 22, no. 4, pp. 571–588, Aug. 2008, doi: 10.1016/j.bpobgyn.2008.04.002.

[5] E. E. Marsh and S. E. Bulun, “Steroid hormones and leiomyomas,” Obstetrics and Gynecology Clinics of North America, vol. 33, no. 1, pp. 59–67, Mar. 2006, doi: 10.1016/j.ogc.2005.12.001.

[6] [A. B. Moore et al., “Human uterine leiomyoma-derived fibroblasts stimulate uterine leiomyoma cell proliferation and collagen type I production, and activate RTKs and TGF beta receptor signaling in coculture,” Cell Communication and Signaling, vol. 8, no. 1, Jun. 2010, doi: 10.1186/1478-811x-8-10.

[7] A. Östman, “PDGF receptors-mediators of autocrine tumor growth and regulators of tumor vasculature and stroma,” Cytokine & Growth Factor Reviews, vol. 15, no. 4, pp. 275–286, Aug. 2004, doi: 10.1016/j.cytogfr.2004.03.002.

[8] X. Li and N. Chegini, “The expression and potential regulatory function of MicroRNAs in the pathogenesis of leiomyoma,” Seminars in Reproductive Medicine, vol. 26, no. 06, pp. 500–514, Oct. 2008, doi: 10.1055/s-0028-1096130.

[9] A. Di Lieto, M. De Falco, G. Mansueto, G. De Rosa, F. Pollio, and S. Staibano, “Preoperative administration of GnRH-a plus tibolone to premenopausal women with uterine fibroids: evaluation of the clinical response, the immunohistochemical

expression of PDGF, bFGF and VEGF and the vascular pattern," Steroids, vol. 70, no. 2, pp. 95–102, Feb. 2005, doi: 10.1016/j.steroids.2004.10.008.

[10] P. Rubisz et al., "The Usefulness of Immunohistochemistry in the Differential Diagnosis of Lesions Originating from the Myometrium," International Journal of Molecular Sciences, vol. 20, no. 5, p. 1136, Mar. 2019, doi: 10.3390/ijms20051136.

[11] D. Dixon et al., "Cell proliferation and apoptosis in human uterine leiomyomas and myometria," Virchows Archiv, vol. 441, no. 1, pp. 53–62, Mar. 2002, doi: 10.1007/s00428-001-0568-7.

[12] S. D. Quinn, J. Vedelago, E. Kashef, W. Gedroyc, and L. Regan, "Measurement of uterine fibroid volume: a comparative accuracy and validation of methods study," European Journal of Obstetrics & Gynecology and Reproductive Biology, vol. 171, no. 1, pp. 161–165, Nov. 2013, doi: 10.1016/j.ejogrb.2013.08.036.

[13] Antar, S.A.; Ashour, N.A.; Marawan, M.E. and Al-Karmalawy, A.A. (2023): Fibrosis: Types, Effects, Markers, Mechanisms for Disease Progression, and Its Relation with Oxidative Stress, Immunity, and Inflammation. *Int. J. Mol. Sci.*, 24, 4004.

[14] Ciarmela P, Islam MS, Reis FM, et al: Growth factors and myometrium: biological effects in uterine fibroid and possible clinical implications. Hum Reprod Update 17: 772-790, 2011.

[15] Xiongm S.; Cheng, J.; Klausen, C.; Zhao, J.; Leung, P. (2016): TGF-β1 stimulates migration of type II endometrial cancer cells by down-regulating PTEN via activation of SMAD and ERK1/2 signaling pathways. *Oncotarget*, Vol. 7, No. 38.

[16] Valle-Tenney, R.; Rebolledo, D.L.; Lipson, K.E. and Brandan, E. (2020): Role of hypoxia in skeletal muscle fibrosis: Synergism between hypoxia and TGF-signaling upregulates CCN2/CTGF expression specifically in muscle fibers. *Matrix Biol*., 87, 48–65.

[17] Faraji A, Shamsadinimoghadam R, Jahromi MA, Namazi N. TGF-β1 role in uterine leiomyoma and endometrial polyp: an insight to drug-based treatment instead of surgical techniques. Obstet Gynecol Sci. 2021 Jan;64(1):107-113. doi: 10.5468/ogs.20191. Epub 2020 Nov 26. PMID: 33238661; PMCID: PMC7834754.

[18] V. Aleksandrovych, A. Pasternak, and K. Gil, "Telocytes in the architecture of uterine fibroids.," PubMed, vol. 59, no. 4, pp. 33–44, Jan. 2019, doi: 10.24425/fmc.2019.131378.

[19] A. Giuliani et al., "Advanced 3D imaging of uterine leiomyoma's morphology by propagation-based Phase-Contrast microtomography," Scientific Reports, vol. 9, no. 1, Jul. 2019, doi: 10.1038/s41598-019-47048-0.

[20] O. Yokosuka et al., "Decreased pregnancy rate is linked to abnormal uterine peristalsis caused by intramural fibroids," Human Reproduction, vol. 25, no. 10, pp. 2475–2479, Aug. 2010, doi: 10.1093/humrep/deq222.

[21] G. P. Flake et al., "The Natural History of uterine leiomyomas: light and electron microscopic studies of fibroid phases, interstitial ischemia, inanosis, and reclamation," Obstetrics and Gynecology International, vol. 2013, pp. 1–20, Jan. 2013, doi: 10.1155/2013/528376.

[22] Bhat AS, Singh NA, Rymbai E, Birendra S, Jayaram S, Selvaraj D. Importance of Fibrosis in the Pathogenesis of Uterine Leiomyoma and the Promising Anti-fibrotic Effects of Dipeptidyl Peptidase-4 and Fibroblast Activation Protein Inhibitors in the Treatment of Uterine Leiomyoma. Reprod Sci. 2023 May;30(5):1383-1398. doi: 10.1007/s43032-022-01064-0. Epub 2022 Aug 15. PMID: 35969363.

[23] Ciebiera MWM, Wrzosek M, Męczekalski B, Nowicka G, Łukaszuk K, Ciebiera M, et al. Role of transforming growth fac tor β in uterine fibroid biology. Int J Mol Sci. 2017;18(11):2435.

[24] Foster DS, Jones RE, Ransom RC, Longaker MT, Norton JA. The evolving relationship of wound healing and tumor stroma. JCI Insight. 2018;3(18):e99911

[25] Vallée A, Lecarpentier Y. TGF-β in fibrosis by acting as a con ductor for contractile properties of myofibroblasts. Cell Biosci. 2019;9(1):98

[26] Meng XM, Tang PM, Li J, Lan HY. TGF-beta/Smad signaling in renal f ibrosis. Front Physiol 6: 82, 2015.

[27] Ciebiera MWM, Wrzosek M, Męczekalski B, Nowicka G, Łukaszuk K, Ciebiera M, et al. Role of transforming growth fac tor β in uterine fibroid biology. Int J Mol Sci. 2017;18(11):2435.

[28] Luo N, Guan Q, Zheng L, Qu X, Dai H, Cheng Z. Estrogen mediated activation of fibroblasts and its effects on the fibroid cell proliferation. Transl Res. 2014;163(3):232–41.

[29] Islam, M. S., Akhtar, M. M. & Segars, J. H. Vitamin D deficiency and uterine fibroids: an opportunity for treatment or prevention? Fertil. Steril. 115, 1175–1176 (2021).

[30] Chegini N, Luo X, Ding L, Ripley D. The expression of Smads and transforming growth factor beta receptors in leiomyoma and myometrium and the effect of gonadotropin releasing hormone analogue therapy. Mol Cell Endocrinol. 2003; 209:9–16.

[31] Lee BS, Nowak RA. Human leiomyoma smooth muscle cells show increased expression of transforming growth factor-β 3 (TGF β 3) and altered responses to the anti-proliferative effects of TGF β. J Clin Endocrinol Metab 2001; 86:913-20.