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Uterus and myoma histomorphology

İ. Ceylan[1], T. Peker[2], N. Coşkun[3], S. Ömeroğlu[3], A. Poyraz[5]

[1] Ankara University, Institute of Health, Neuroscience Department, Sihhiye, Ankara
[2] Department of Anatomy, Faculty of Medicine, Gazi University, Beşevler, Ankara
[3] Department of Histology and Embryology, Faculty of Medicine, Gazi University, Beşevler, Ankara
[4] Department of Pathology, Faculty of Medicine, Gazi University, Beşevler, Ankara (Turkey)

Summary

Objective: Uterine fibroids, or leiomyomas are common benign neoplasms of the myometrium. These neoplasms are composed of large amounts of extracellular matrix and disarrayed smooth muscle tissue. The aim of this study was to examine the histomorphological differences between myoma uteri and uterus. *Materials and Methods:* Thickness of muscle fascicles and collagen fibers, and vascular and histological structures were evaluated by using morphometric, histomorphologic and immunohistochemical analyses, and findings represented by using three-dimensional (3D) modeling. The authors used a light microscope and photos were captured using a specific program for analysis. Light micrographs were assembled into 3D images. *Results and Conclusion:* Histological and 3D findings demonstrated that the muscle fiber is a vital part of the myometrium and loss of its contractility indicates a significant deviation from the uterine structure.

Key words: Histomorphology; Immunohistochemistry; Three-dimensional modeling; Uterine myoma; Uterus; Leiomyomas.

Introduction

Uterine leiomyomas (fibroids or myomas) are benign clonal tumors that arise from the smooth muscle cells of the uterus. They appear clinically in approximately 25% of women, although with the application of new imaging techniques, the actual clinical prevalence may be higher [1, 2].

At the microscopic level, myomas consist of whorled, anastomosing fascicles of uniform, spindle-shaped smooth muscle cells. The cells have indistinct borders and eosinophilic cytoplasm; the nuclei are elongated and exhibit finely dispersed chromatin. Myomas may show areas of hemorrhage as well as cystic degeneration and microcalcification in some cases [3].

Deviations of uterine myomas from the normal anatomic structure have been described elsewhere [4, 5]. It is difficult, however, to demonstrate these deviations in three-dimensional (3D) form. Computers can generate true 3D models and several 3D visualization techniques have been developed to enable one to visualize not only a flat (horizontal) representation of a 3D object, but also an approximation of its Z-axis [6].

It is useful to demonstrate microscopic structures and related pathologies using 3D images or animations, especially for medical education. Use of web-based 3D models for anatomy training enhances education, but such 3D models must be used as part of an integrated training package that includes other material including video clips, text book descriptions, and self-assessment tools [7].

The authors investigated the histomorphology of normal myometrium and uterine myoma using histochemical and immunohistochemical techniques. Light microscopy was used for image analysis. In addition, two-dimensional light micrographs were assembled into 3D images that may be useful for medical education, medical research, and clinical studies.

Materials and Methods

Leiomyoma (n=8) and normal myometrium (n=8) tissue were collected by consent from women undergoing laparoscopic myomectomy from the Department of Gynecology and Obstetrics (Gazi University Faculty of Medicine). The present study was approved by the Ethics Committee of Gazi University Faculty of Medicine.

Uterine tissues were fixed in 10% neutral buffered formalin and embedded in paraffin after routine histological procedures were performed. Then, four-µm sections were obtained from each paraffin block and stained with haematoxylin and eosin (H&E), Masson trichrome, and Van Gieson [8].

The avidin-biotin peroxidase method was used for the immunohistochemical studies to investigate α-smooth muscle actin, desmin, S-100, PGP 9.5, and CD56 activities [9]. The activity of these antibodies was assessed semiquantitatively. Histological and immunohistochemical analysis was performed using a light microscope and photos were captured by using the Leica Q Win 3 program.

Light micrographs of myometrium and fibroid tissues were reconstructed into 3D images using Cinema 4D Release 15 3D modeling and animation software. The 3D modelling process included sculpting, texturing, lightning, and image editing, respectively. Sculpting of 3D histological structures was created

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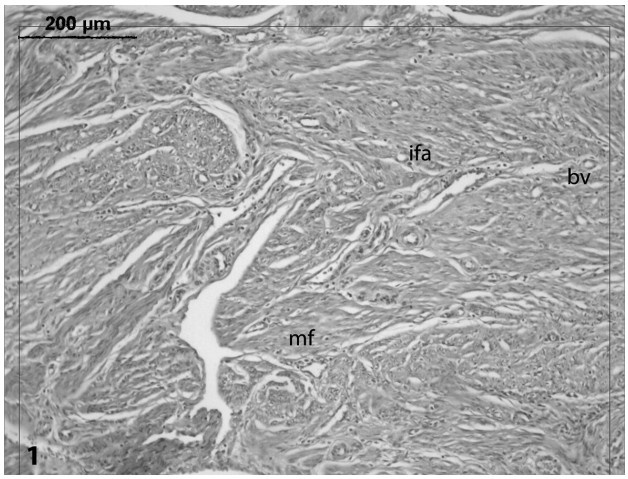

Figure 1. Normal myometrium. ifa: interfascicular area; bv: blood vessel; mf: muscle fascicle (original magnification H&E ×10).

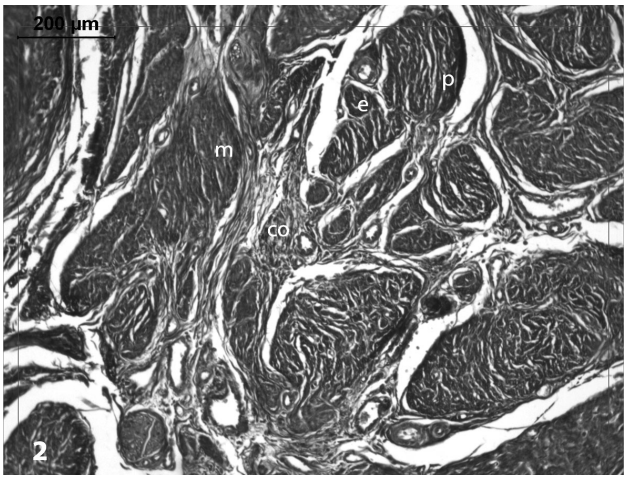

Figure 2. — Normal myometrium. Myocytes (m) are dark red and collagen fibers (co) are blue. Endomysium (e) surrounds a single muscle fiber and perimysium (p) surrounds muscle bundles (original magnification Masson trichrome ×10).

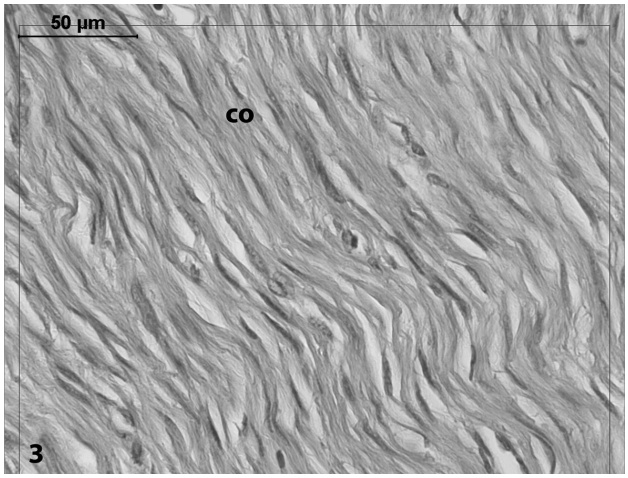

Figure 3. — Myoma tissue. Dense collagen fiber bundles (co) are visible (original magnification H&E ×40).

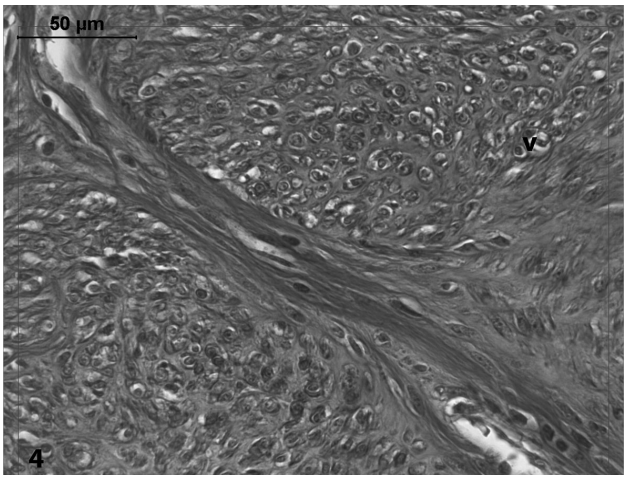

Figure 4. — Myoma tissue. Vacuolization (v) owing to atrophy of myocytes can be seen (original magnification Van Gieson ×40).

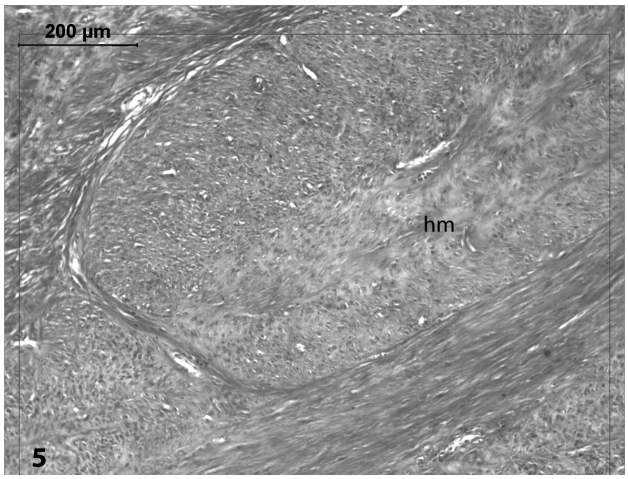

Figure 5. — Myoma tissue. Note hyalinized matrix (hm) (original magnification Van Gieson ×10).

according to histological sections. Three-dimensional images were rendered using HDRI in tiff format at 800×600 dpi. This allows preservation of details that may be lost due to limiting contrast ratios. Histological 3D models benefit from this as it creates more realistic scenes than with the more simplistic lighting models used.

Masson trichrome stained slides were measured for muscle fascicle and the collagen fiber quantity in the connective tissue. Likewise Van Gieson stained sections were counted blood vessels for analysis of vascular structures. All these analyzes were performed using a specific program and ten areas were randomly selected in six cross-sections from myoma uteri and uterus tissue. Statistical analyses were performed using SPSS statistical software. All data are expressed as means ± SD. Data obtained from the counts in binary groups were evaluated using the Mann Whitney U test; *p* values less than 0.05 were accepted as statistically significant [9].

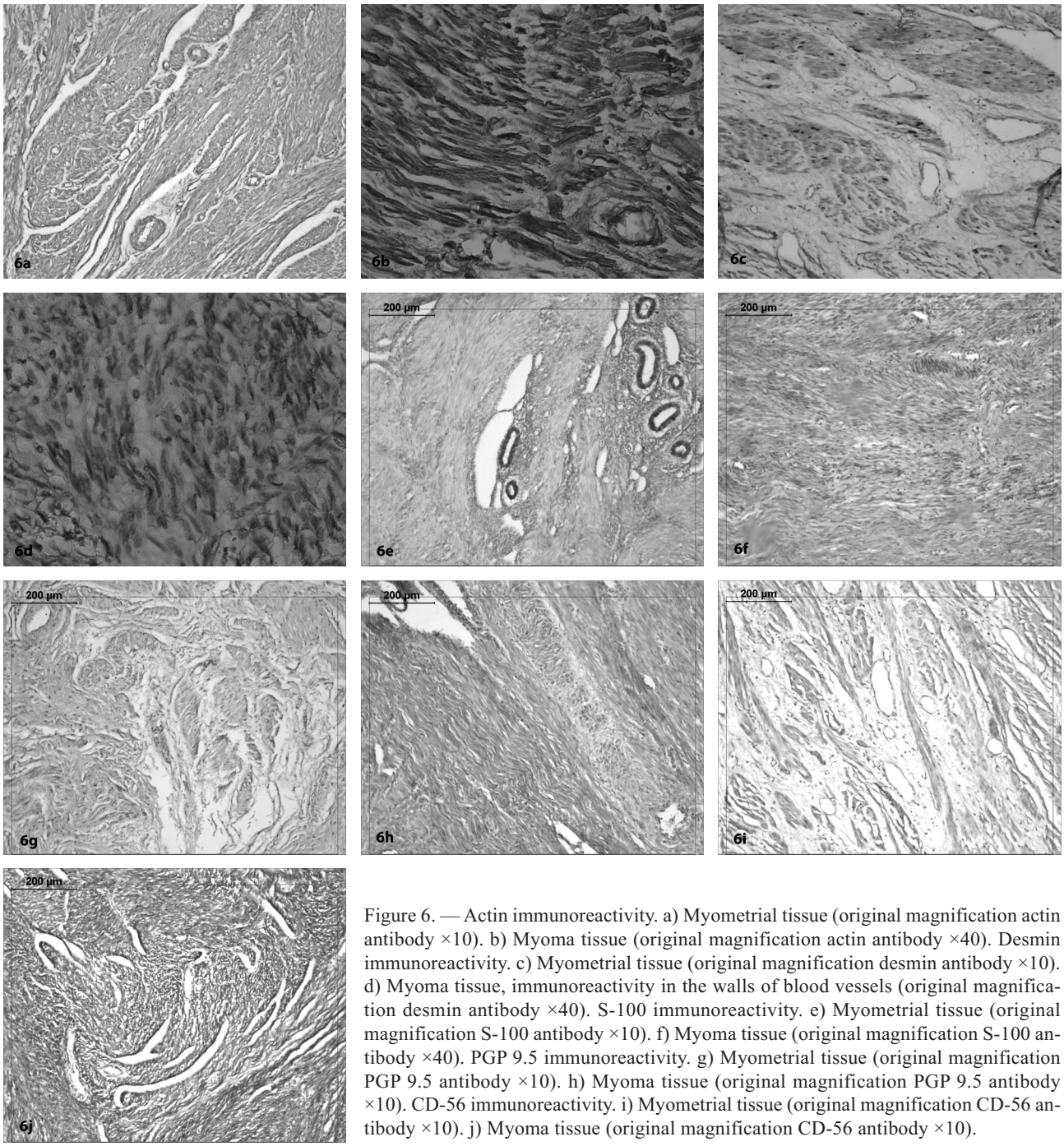

Figure 6. — Actin immunoreactivity. a) Myometrial tissue (original magnification actin antibody ×10). b) Myoma tissue (original magnification actin antibody ×40). Desmin immunoreactivity. c) Myometrial tissue (original magnification desmin antibody ×10). d) Myoma tissue, immunoreactivity in the walls of blood vessels (original magnification desmin antibody ×40). S-100 immunoreactivity. e) Myometrial tissue (original magnification S-100 antibody ×10). f) Myoma tissue (original magnification S-100 antibody ×40). PGP 9.5 immunoreactivity. g) Myometrial tissue (original magnification PGP 9.5 antibody ×10). h) Myoma tissue (original magnification PGP 9.5 antibody ×10). CD-56 immunoreactivity. i) Myometrial tissue (original magnification CD-56 antibody ×10). j) Myoma tissue (original magnification CD-56 antibody ×10).

Results

Histological sections of normal uterine myometrium layer have fusiform, smooth, and tightly packed muscle fibers in to the fascicles. The authors found collagen fibers in interfascicular areas together with a few blood vessels of various diameters (Figure 1). Also, after Masson's trichrome and H&E staining, muscle fibers seen red and collagen fibers seen blue. Most of the collagen fibers located between the muscle fascicles. Interfascicular collagenous stroma was not abundant and smooth muscle fibers were in parallel to each other in the linear orientation. Also myocytes were fusiform, and nuclei were located in the center and oval-shaped. (Figure 2). In the sections that stained with van Gieson, blood vessels were clearly seen in the interfascicular area.

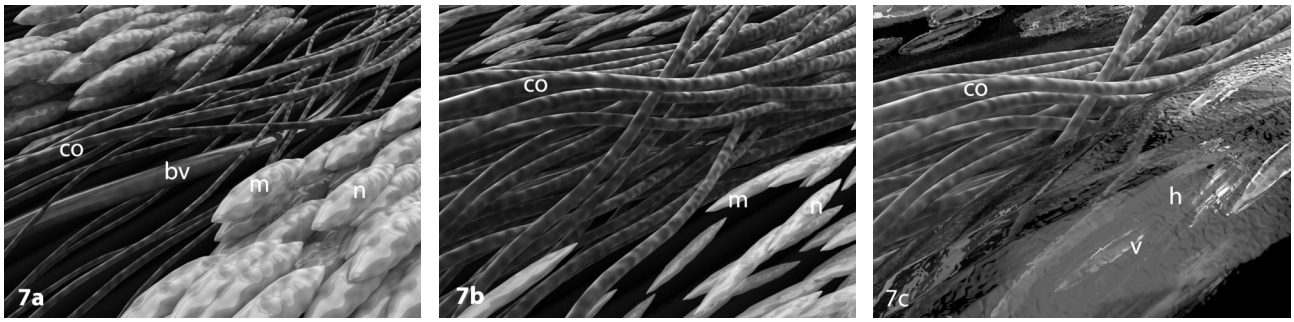

Figure 7. — Three dimensional models. a) Myometrium. b) Myoma. c) Hyalinized matrix. co: collagen; m: myocyte; bv: blood vessel; n: nucleus; h: hyalinization; v: vacuolization. 3D models designed by Tuncay Peker.

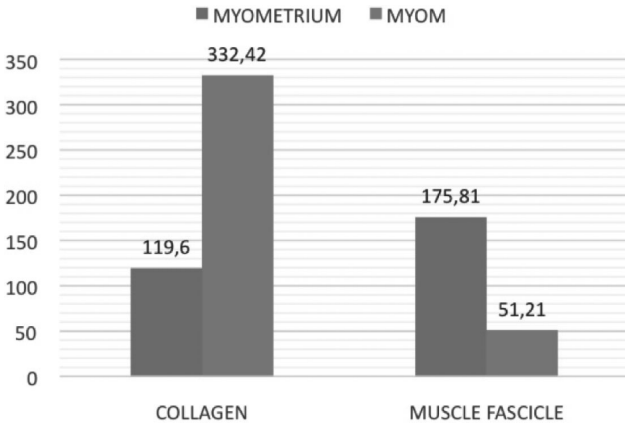

Figure 8. — Measurements of diameter in collagen fibers and muscle fascicles.

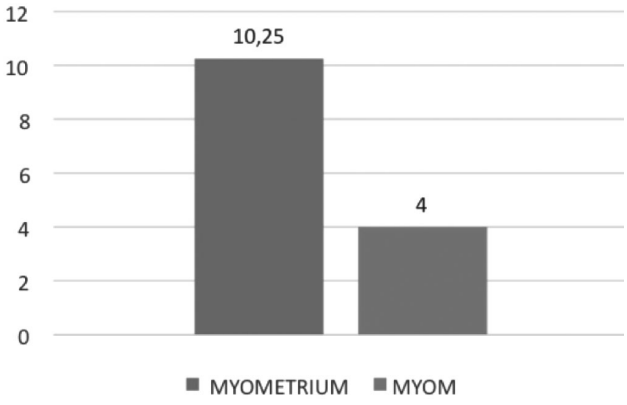

Figure 9. — Number of blood vessels.

Table 1. — *Semiquantitation of immunohistochemical staining results.*

	Myometrium	Myoma
Actin	+	+++++
Desmin	+	++++
S-100	++	+
PGP 9,5	+	++
CD56	+	+++

Table 2. — *Muscle fascicle, collagen of connective tissue and blood vessel counts.*

	Mean±SD	Median (min/max)
Myometrium		
Muscle fascicle	189.10±409.23	175.81 (146.68/239.89)
Collagen	131.75±450.04	119.60 (84.99/211.80)
Blood vessel	10.50±2.41	10.25 (8.00/13.50)
Myoma		
Muscle fascicle	49.91±119.93	51.21 (31.86/67.70)
Collagen	341.90±587.78	332.42 (271.42/448.94)
Blood vessel	4.33±1.63	4.00 (3.00/7.00)

Histological sections of myoma uteri showed a fibroid structure due to increase of collagen fibers. Myocytes in the fibroid structure lost their characteristic structure, with mitotic and cytoplasmic atrophy, vacuolization, and had a pale cytoplasm. In Masson's trichrome stained sections of myomas and muscle fibers with intense dark blue. With H&E stained sections, more collagenous activity was observed and disorganized arrangement of smooth muscle fibers and collagen bundles also were thicker in fibroids than in normal tissue (Figure 3). In the sections that stained with Van Gieson, cytoplasmic vacuolization was common and may have reflected loss of myofilaments or atrophy in myocytes (Figure 4). Figure 5 shows an amorphous (hyaline) matrix between elongated and atrophic myocytes and decreased vascularity due to the increase fibroid areas.

Actin (Figures 6a, b) and desmin (Figures 6c, d) antibody are responsible for contraction in smooth muscle cells and CD56 (Figures 6i, j) antibody indicates an increase in positive carcinomas showing significantly strong immuno-

reactivity in the myometrial layers of myoma uteri with semiquantitative evaluation of immunohistochemical staining. Similarly, semiquantitative evaluation of immunohistochemical staining for S-100 (Figures 6e, f) and PGP 9.5 (Figures 6g, h) showed significantly weak immunoreactivity due to the decrease vascularity in the myometrial layers of myoma uteri (Table 1).

When muscle fascicle diameter ($p = 0.004$) and counted blood vessels ($p = 0.004$) were measured of the myoma uteri, they were significantly decreased compared to normal myometrium. On the other hand, diameter of collagen fibers ($p = 0.004$), of the normal myometrium was significantly decreased than myoma uteri (Table 2) (Figures 8, 9).

Traditional learning methods in anatomy and histology focus on the use of textbooks, 2D illustrations or diagrams. Spatial relations are difficult to appreciate, and this certainly applies to the histomorphological differences between normal uterus and myoma uteri. Visualising these types of structures in 3D with interactive educational material can greatly improve the understanding of spatial relationships and retention of that knowledge. Therefore, light microscopic images were reconstructed into 3D images, which made normal and pathologic findings more apparent (Figures 7a–c).

Discussion

Because smooth muscle is important for coordinated contraction of the myometrium, disorganized arrangement of smooth muscle fibers signals an important departure from the normal contractile structure [4].

The present authors focused their investigation on smooth muscle fascicles, collagen organization, and blood vessel content of normal and uterine fibroid tissues. The fibroids exhibited more collagen than normal uterus; normal uterus had more smooth muscle fibers and was more vascular than fibroids. The present observations are consistent with previous reports [4, 5, 10]. They are also consistent with a structural transformation from smooth muscle cells in normal uterus to fibroblasts in fibroids. Flake *et al.* reported that after structural transformation from smooth muscle cells in normal uterus to fibroblast-like cells in fibroids, collagen deposition within the tumors was variably interfascicular, intrafascicular or a combination of these and that the collagenous stroma was produced by the myocytes [4]. These investigators also reported a loosely parallel orientation of fibers and a disorganized arrangement of the fibroids. They found that Masson trichrome, H&E, and Van Gieson stained fibroids exhibited more collagen than normal tissues.

Flake *et al.* found pale cytoplasm in myomas and myocytes that were transformed to slender and elongated structures in myoma tissues [4]. Atrophy of the cytoplasm and nuclei of myocytes was common. These investigators also reported cytoplasmic vacuolization, which may reflect loss of myofilaments and other changes such as hyalinization related to severe atrophy. [4].

Weiss *et al.* reported a 3D study of the muscle and collagen fiber architecture of the human uterus [10]. Immunohistochemical staining of alpha-smooth muscle actin demonstrated that fibrotic leiomyomas consisted of abundant collagen fibrils arranged in a non-parallel manner, whereas in healthy myometrium collagen bundles adjacent to smooth muscle cells, they were sparse and well-aligned; the present findings were comparable.

Interstitial ischemia results from excessive production of collagen, which causes decreased microvascular density, increased distance between myocytes and capillaries, nutritional deprivation, and myocyte atrophy. The end stage of this process is death of myocytes. Further studies are required to determine whether necrosis may be used to distinguish ischemic necrosis from malignant uterine smooth muscle tumors.

Anatomy teaching is undergoing significant changes owing to time constraints, limited availability of cadavers, and advances in computer-assisted learning. Web3D offers the ability to simulate the spatial relationships among anatomical structures. More research is required, however, to evaluate these resources, before they are introduced routinely into the undergraduate medical curriculum [11].

The present authors investigated histomorphological differences between uterine myoma and normal uterus using histochemical and immunohistochemical methods. In addition, light micrographs were reconstructed into 3D images. In this way, normal and pathologic findings were rendered more understandable. Use of the 3D method presented here can give further insight into the scientific research of the uterus.

References

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Corresponding Author:
T. PEKER, M.D.
Gazi University Faculty of Medicine
Department of Anatomy
Beşevler 06510 Ankara (Turkey)
e-mail: tpeker@gazi.edu.tr