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Extracellular Matrix And Angiogenesis In The Uterine Myometrium And Small Growing Leiomyomas In Women Of Reproductive Age

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Abstract: This study investigates the role of extracellular matrix (ECM) accumulation and angiogenesis in the growth of small uterine leiomyomas in women of reproductive age. Histological and immunohistochemical analyses were conducted on myometrial and leiomyoma tissues to evaluate ECM content, vascular remodeling, and hormone receptor expression. The results demonstrate that excessive ECM deposition and distorted vascular architecture are early features of leiomyoma development. The nodules show high progesterone receptor expression, altered apoptosis, and active smooth muscle proliferation from vascular zones, suggesting a coordinated interaction between ECM remodeling, angiogenesis, and hormonal regulation in leiomyoma pathogenesis.

Keywords: Leiomyoma; Angiogenesis; Extracellular matrix; Progesterone; Apoptosis;

I. Introduction

Uterine leiomyoma is the most common benign tumor of the genital system in women of reproductive age with unknown etiology. It occurs in more than 70% of cases and is observed in every 4th to 5th woman (1,2,3). Although it typically does not lead to a fatal outcome, it can cause a number of complications: infertility, uterine bleeding, anemia due to heavy and prolonged bleeding, inability to work, dysfunction of pelvic organs, pain syndrome, and others (1,2,3,7).

Leiomyomas exhibit structural features that differentiate them from normal uterine smooth muscle tissue. A prominent distinction is the excessive accumulation of extracellular matrix (ECM) components, including MMPs, collagen, fibronectin, proteoglycans, laminins, activin, growth factors, cytokines, and steroids (5,6,7,8,9). Several studies have indicated that ECM accumulation is regulated by growth factors (12,13), cytokines (14), and steroid hormones (2). Furthermore, ECM acts as a regulator of the stability and activity of these growth factors. Through the degradation of ECM components, MMPs and other proteolytic enzymes release growth factors and trigger the activation of multiple signaling pathways.

It is well established that the extracellular matrix surrounds cells [23,24], providing both structural and biochemical support to adjacent cells [25,26,27]. Despite variability in ECM composition across different tissue types, its core functions remain consistent: cellular adhesion, intercellular communication, and differentiation (28).

Researchers hypothesize that uterine leiomyoma may represent a response to an inflammatory process and myofibroblasts are believed to play a key role in fibrosis development, as they synthesize ECM components to support tissue repair and maintain

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homeostasis during inflammatory states (15,16,17,18). Growth factors such as TGF-βs and activin-A are known to contribute significantly to myofibroblast differentiation during fibrosis (19). The biological role of myofibroblasts is to produce ECM proteins in order to restore tissue integrity following injury. Upon completion of tissue repair and restoration of homeostasis, myofibroblasts typically undergo apoptosis. If this apoptotic process is impaired, the persistence of cells with a myofibroblastic phenotype can result in excessive ECM production and progressive fibrosis.

The significant role of sex hormones, particularly estrogen and progesteron receptors, in the development of uterine fibroids is also well known. The concentration of estrogen and progesterone receptors is higher in leiomyomas than in the surrounding myometrium, though lower than in endometrial tissue. Nonetheless, in terms of hormonal sensitivity, fibroid tissue more closely resembles endometrium than myometrium. Estrogens, growth factors, and immunoreactive insulin are believed to have a synergistic effect on myometrial tissue, contributing significantly to the formation and growth of fibroids (28). Conversely, numerous clinical and laboratory studies suggest that mitotic activity in the myometrium increases under the influence of progestins (26,29).

Progesterone exerts its physiological effects by interacting with its target cells via progesterone receptors (PR-A and PR-B). It plays a dominant role in the growth and development of uterine leiomyomas, as evidenced by increased mitotic activity in fibroid cells during the secretory phase of the menstrual cycle. Additionally, the involvement of other hormones—including androgens and prolactin—in the pathogenesis of uterine leiomyomas has been extensively documented (10,11,22).

According to scientific research, hypoxic conditions may promote the development and growth of leiomyomas.However, some researchers argue that despite the presence of severe hypoxia, leiomyomas do not express hypoxia-related genes, indicating a diminished or absent cellular response to tissue hypoxia (20,21).

Although leiomyomas have been the subject of extensive scientific research and their distinguishing features from the myometrium have been studied using multicomponent research methods, it should be noted that interest in the growth and development processes of leiomyomas remains ongoing.This is because they themselves undergo a process of rejuvenation, are resistant to treatment, and surgery remains the only definitive and consistent method of cure.

Based on the above considerations, the aim of our study is:

To evaluate the role of the extracellular matrix (ECM) and assess angiogenesis in the processes of growth and development in normal uterine smooth muscle and in small, developing leiomyomas in patients of reproductive age.

II . Materials and Methods

Research Methods and Objectives

To identify the characteristics of the extracellular matrix (ECM) and angiogenesis in the myometrium and small growing leiomyoma nodules in women of reproductive age diagnosed with uterine leiomyoma.

1. Histomorphological analysis was performed on hematoxylin and eosin (H&E) and Masson's trichrome-stained tissue sections.
2. Mathematical gradient-based modeling was applied to analyze the linear and radial blood vessels in the myometrium, as well as the remodeled and distorted vasculature in leiomyoma nodules. A specialized computer program was developed for the digital processing and quantification of vascular structures in histological slides.
3. Molecular characterization was carried out using immunohistochemical analysis, employing the following markers: estrogen receptor (ER), progesterone receptor (PR), smooth muscle actin (SMA), CD34, and caspase.

III . Data Collection and Monitoring

In this study, we examined cases of uterine body leiomyoma in women of reproductive age who had undergone surgery. Our focus was on the characteristics and distribution of extracellular matrix (ECM) accumulation within the uterine myometrium and small, growing leiomyoma nodules. We observed that both the quantity and quality of ECM differ significantly between leiomyomas and the surrounding myometrial tissue.

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The objective of this study was not only to evaluate the formation and accumulation of ECM in the myometrium and leiomyomas but also to assess its role in the early stages of leiomyoma development, the formation of remodeled vasculature, the peculiarities of angiogenesis, cellular proliferation, and the overall progression of leiomyoma growth. Accordingly, we analyzed ECM characteristics and angiogenesis in newly forming, small growing nodules (ranging from 2–4 mm and 4–6 mm in diameter), starting from the earliest pre-leiomyoma processes through to further stages of development.

Both literature data and our findings confirm that leiomyomas are characterized by excessive ECM accumulation, a feature that differentiates them from the uterine myometrium.

We propose that this excessive ECM buildup, under conditions of impaired apoptosis, represents a programmed, stepwise process aimed at forming distorted vascular networks. Furthermore, we suggest that active proliferation of leiomyocytes initiates from the vascular walls.

As supported by literature, vascular walls are rich in progenitor and stem cells, and such cells have been identified within leiomyocytes. Within the ECM microenvironment, leiomyocytes proliferate in a nodular form. According to our observations, ECM accumulation begins in tumor nodules as small as 2 mm in diameter, and increases progressively in volume. This process appears to be driven by the presence of fibroblast growth factors (FGFs) stored within the ECM (53).

The ECM plays a key role in shaping the tumor and sharply demarcating it from the surrounding muscular tissue. It is likely one of the factors that restricts the uncontrolled spread of the tumor mass within the uterine musculature and may reduce the risk of malignant transformation. Importantly, these small growing nodules are encapsulated and clearly separated from the adjacent myometrial tissue.

Histological analysis shows that the uterine myometrium is composed of linear and radial blood vessels, rich in large and medium-sized arteries, arterioles, and capillaries. It contains only minimal ECM, primarily localized around the vascular adventitia and between smooth muscle bundles.

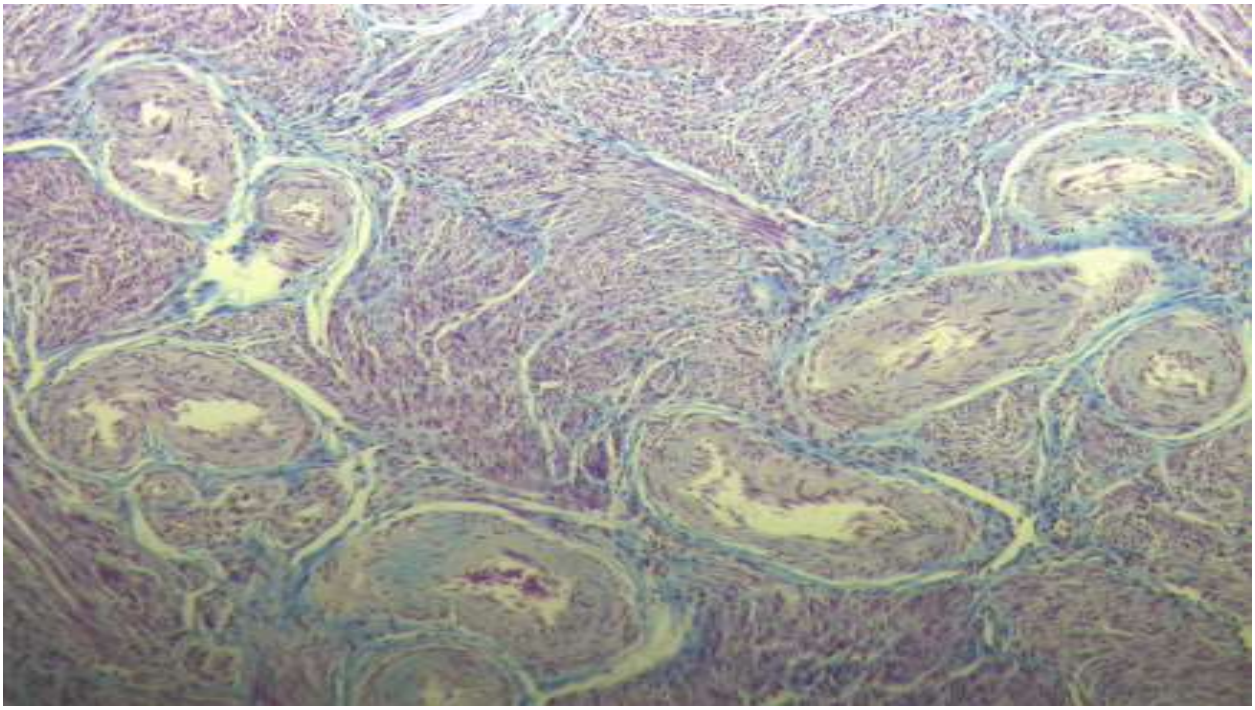

Figure 1. Myometrium.Masson's Trichrome staining. Leika 1000 LED microscope, photo captured with MC170HD camera, magnification ×0.25

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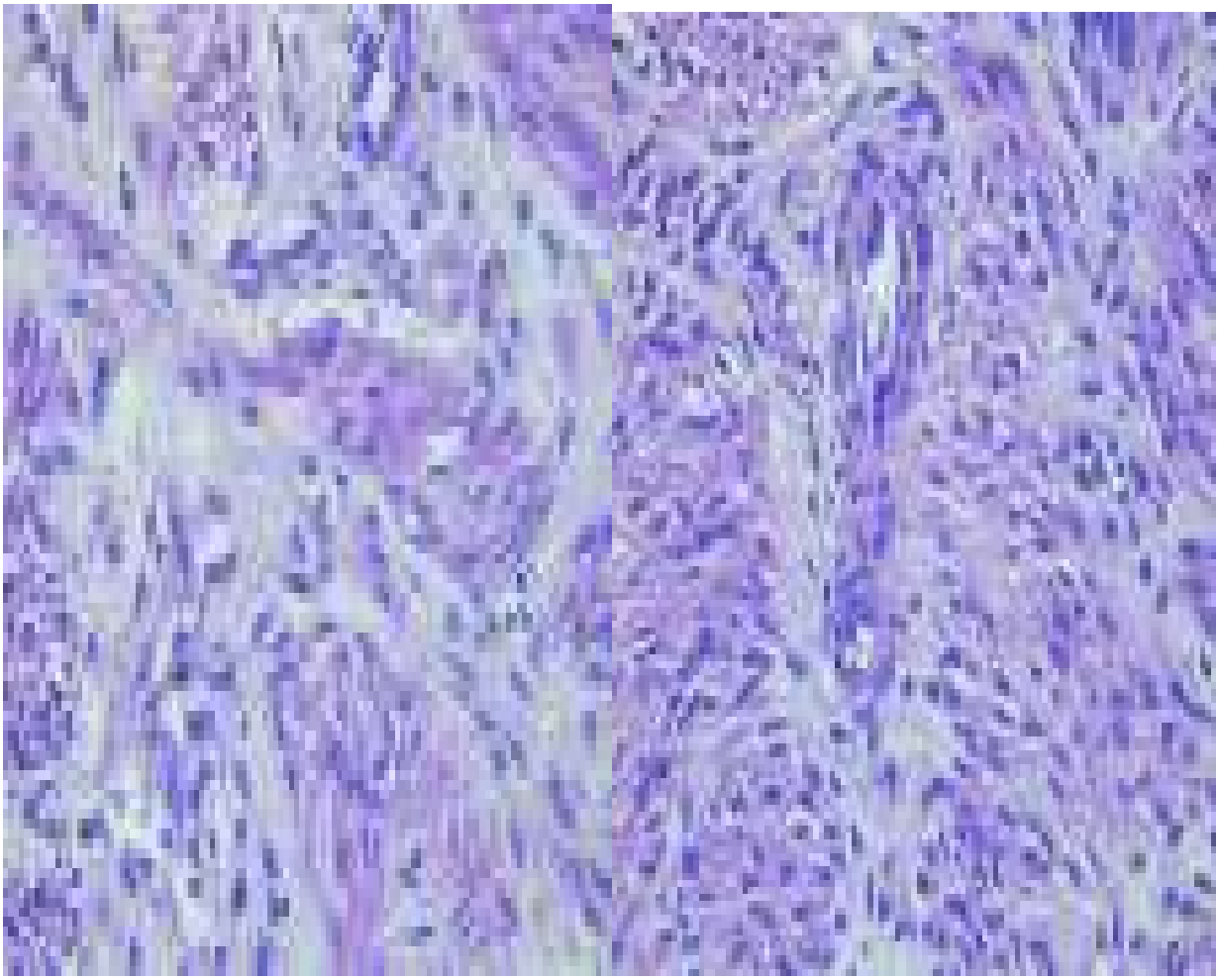
In leiomyomas ranging from 2 mm to 4 mm, histological examination of hematoxylin and eosin (H&E) and Masson's trichrome-stained sections reveals the presence of remodeled and distorted blood vessels, including capillaries and arterioles. Excessive ECM accumulation is observed (Figures 2 and 3), while the number of leiomyocytes is focally reduced relative to the volume of ECM. In leiomyoma nodules ranging from 4 mm to 6 mm, there is a decrease in the number of occluded and distorted vessels and a notable increase in capillary density. These nodules also demonstrate high proliferative activity of leiomyocytes along with reduced ECM content.

Within the leiomyoma nodule, the leiomyocyte-to-ECM ratio appears to be progressive and balanced. At a certain stage, ECM accumulation is observed within the vascular collector, accompanied by activation of angiogenesis, which subsequently provides a favorable microenvironment for leiomyocyte proliferation and continued tumor growth and development.



Figures 2 and 3.Leiomyoma nodules measuring 2 mm to 4 mm. Leika 1000 LED microscope, photo captured with MC170HD camera, magnification ×0.65

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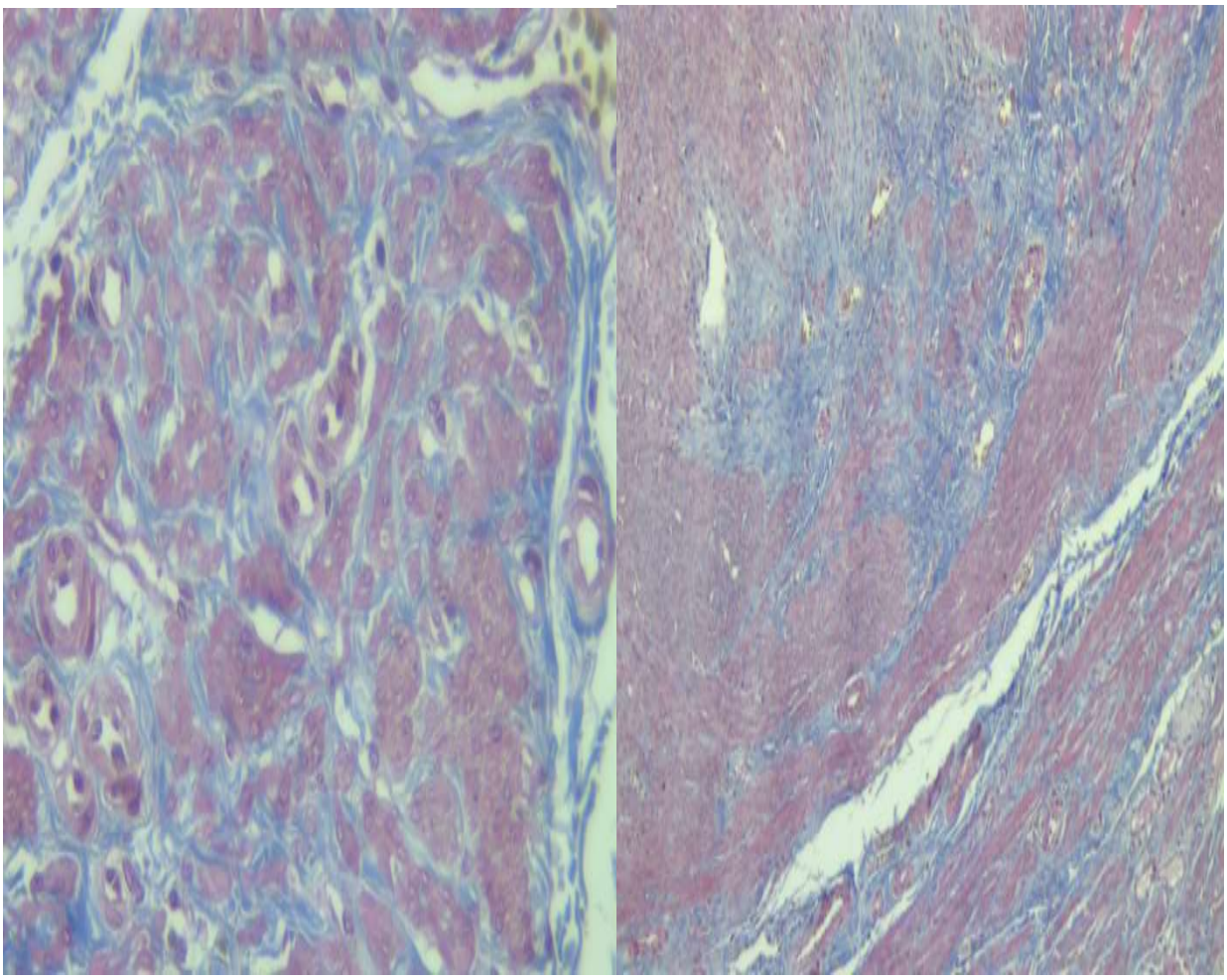

Figures 4 and 5. Leiomyoma nodules measuring 4 mm to 6 mm. Leika 1000 LED microscope, photos captured with MC170HD camera, magnifications ×0.65 and ×0.25

The issue of the development of leiomyoma from remodeled blood vessels remains a subject of debate. The majority of researchers, based on the identity of leiomyocytes, believe that the tumor results from the proliferation of a single cell. However, considering that the muscular and adventitial layers of blood vessels within a leiomyoma nodule are rich in stem and progenitor cells, and that progenitor cell characteristics have been identified in leiomyocyte cells, this suggests that the proliferation of leiomyoma cells may begin within the area of the remodeled vascular collector — from multiple points within it (Iobashvili–Phailodze, 2023). This observation is strongly supported by multiple cases we have encountered of solitary, large, encapsulated leiomyomas containing numerous intranodular ''daughter'' nodules of varying size. (see photo 6.7).

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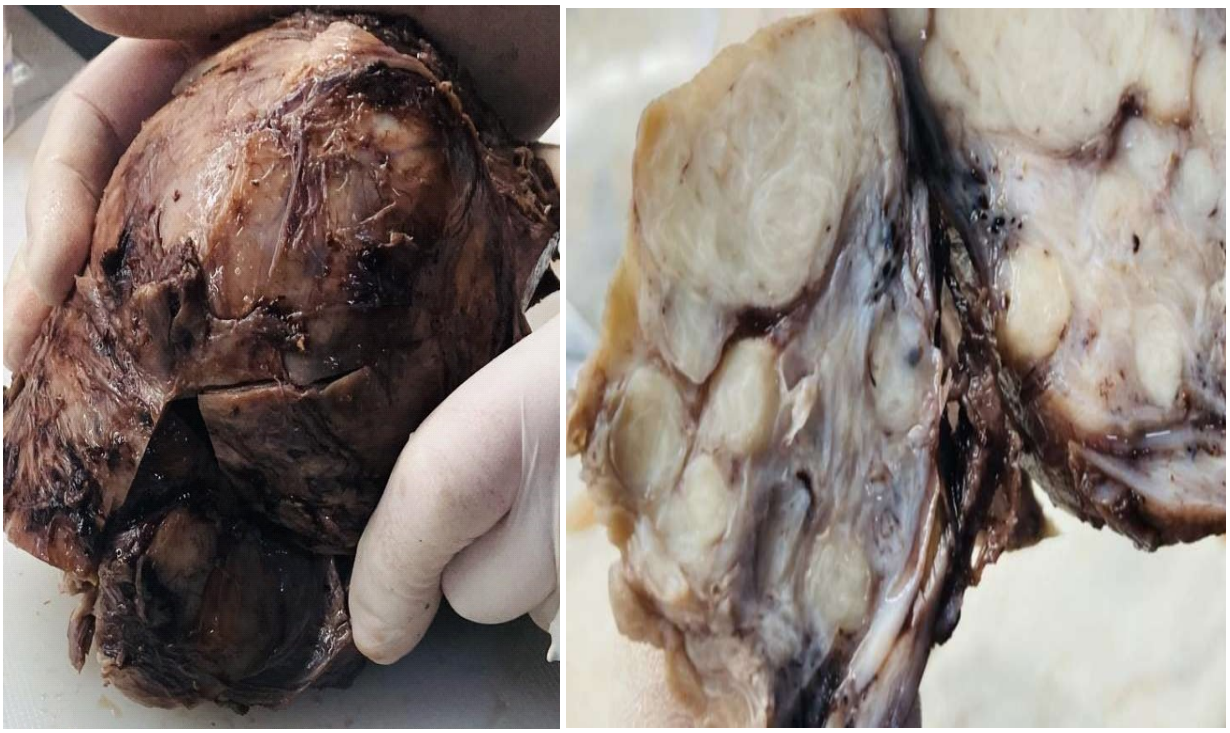
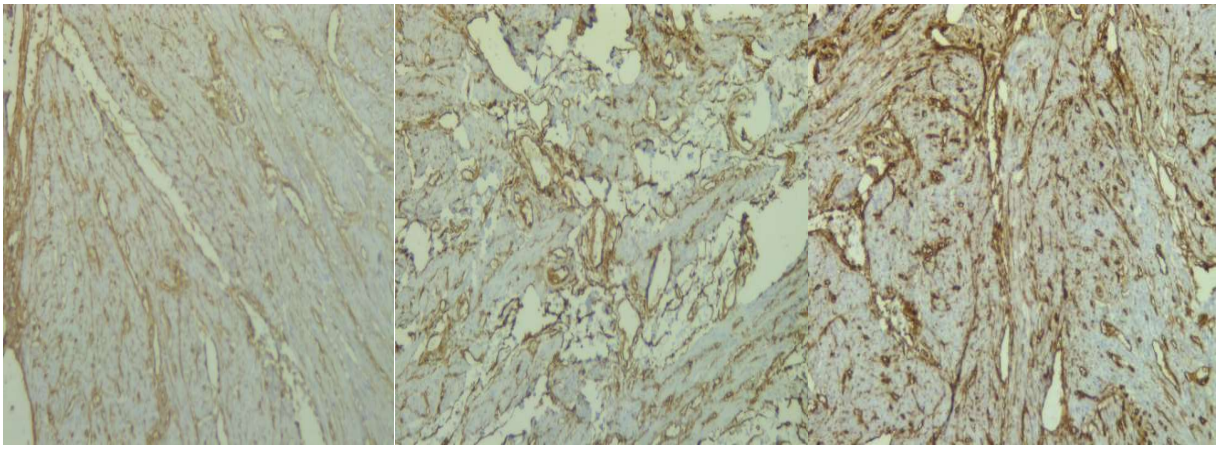


Photo 6.7.A nodule measuring 12 cm. On the cut surface, multiple "daughter" nodules are visible.

Immunohistochemical Study Results

As part of the immunohistochemical analysis, expression of the following markers was detected both in the myometrium and in the small growing leiomyoma nodules measuring 2–4 mm and 4–6 mm in size:

Moderate expression of CD34 (upt o 40 %) was observed in the myometrium, where a linear and radial vascular architecutre was identified. In contrast, the 2-4 mm and 4-6 mm nodules showed a large number of aberrant blood vessels with high CD34 expression-up to 80 %. (Figure 8, 9, 10).



Photos 8, 9, 10.Myometrium; nodules measuring 2–4 mm and 4–6 mm; vascular collector.

CD34 expression observed using Leika 1000 LED, photo capture: MC170HD, magnification ×0.65.

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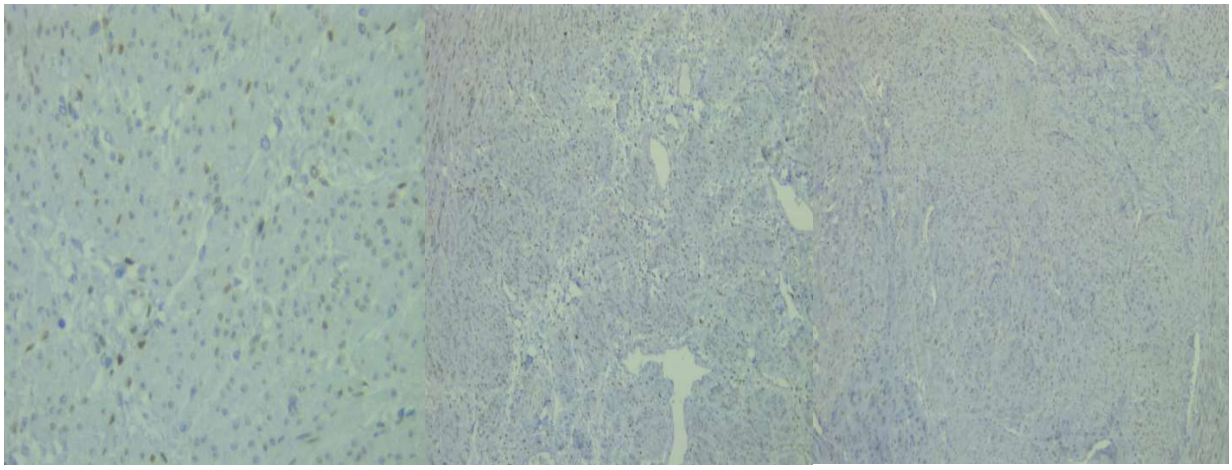
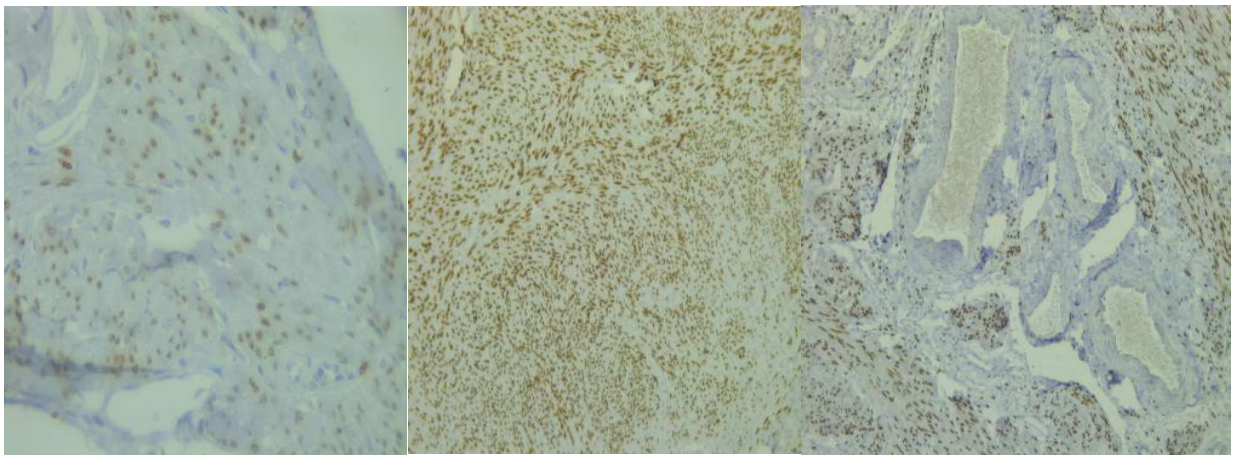
ER (Estrogen Receptor):Minimal expression is observed in the myometrium, while no expression is detected in the nodule itself. Photos 11, 12, 13.



Photos 11, 12, 13 .Myometrium; nodules measuring 2–4 mm and 4–6 mm; Estrogen Receptor (ER) expression. Imaged with Leika 1000 LED microscope; photo captured using MC170HD camera at ×0.65 magnification.

Progesterone expression is significantly higher in the nodules compared to the adjacent myometrium. The strongest positivity—up to 90%—is observed in nodules measuring up to 6 mm. Photos 14, 15, 16.



Photos 14, 15, 16. Smooth muscle tissue; nodules measuring 2–4 mm and 4–6 mm; Progesterone Receptor (PR) expression. Imaged using Leika 1000 LED microscope; photo captured with MC170HD camera at ×0.65 magnification.

Smooth Muscle Actin (SMA) expression is markedly elevated compared to normal levels, with particularly strong expression observed in nodules measuring 5–6 cm. A characteristic feature is active perivascular proliferation originating from the endothelium, which supports the role of blood vessels in the growth and development of the nodules. Photos 17, 18, 19.

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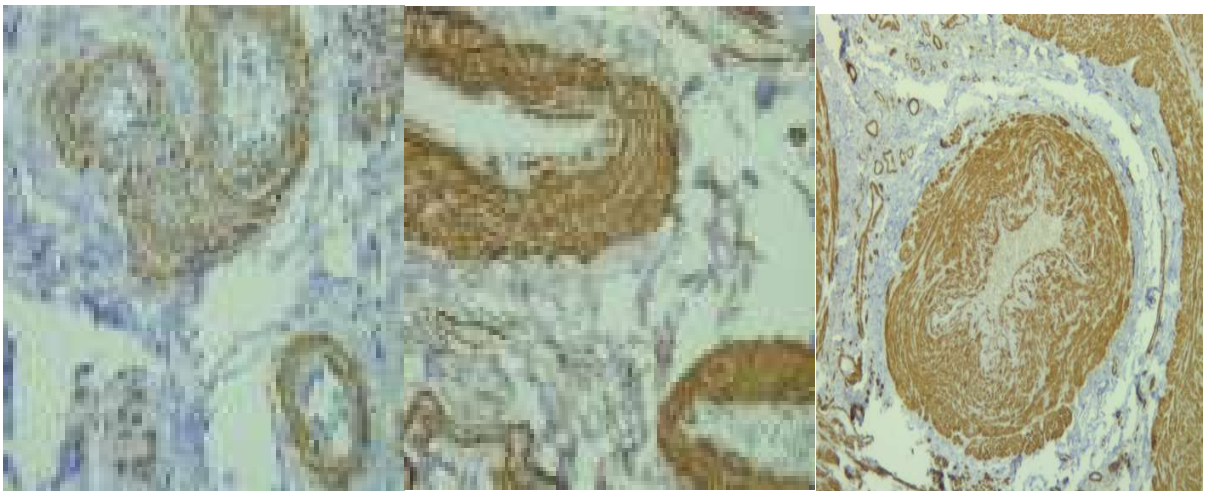
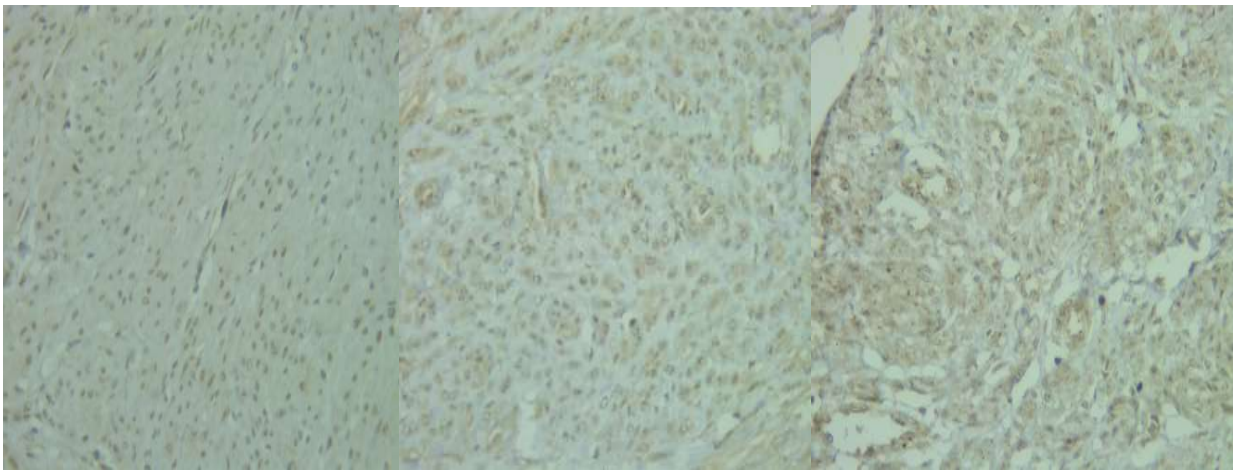


Photos 17, 18, 19 Smooth muscle tissue; nodules measuring 2–4 mm and 4–6 mm; SMA (Smooth Muscle Actin) expression.Imaged using Leika 1000 LED microscope; photo captured with MC170HD camera at ×0.65 magnification.

Normal expression of Caspase-3 is observed; however, in the small proliferating nodules (2–4 mm and 4–6 mm), limited or altered activity is noted, indicating an imbalance in apoptotic regulation. Photo 20.21.22



Photos 20, 21, 22. Smooth muscle tissue; nodules measuring 2–4 mm and 4–6 mm; Caspase-3 expression.Imaged using Leika 1000 LED microscope; photo captured with MC170HD camera at ×0.65 magnification.

To assess the linear and radial vascular architecture in the uterine myometrium, and the remodeled, deformed vasculature within leiomyoma nodules, a dedicated mathematical gradient modeling method was developed. A specialized computer program was created for the digital processing of blood vessels in histological micropreparations. Using this mathematical method, applied to CD34-stained immunohistochemical slides (Photos 23, 24, 25), it was determined that: The uterine body myometrium is characterized by linear and radial blood vessels, representing 0.2% of the vascular area. In contrast, remodeled and deformed blood vessels are predominantly observed in leiomyoma nodules: Most frequently in 2–4 mm nodules (2.7% vascular deformation), Less so in 4–6 mm nodules (1.5%).

These parameters are visualized in Diagram 1.

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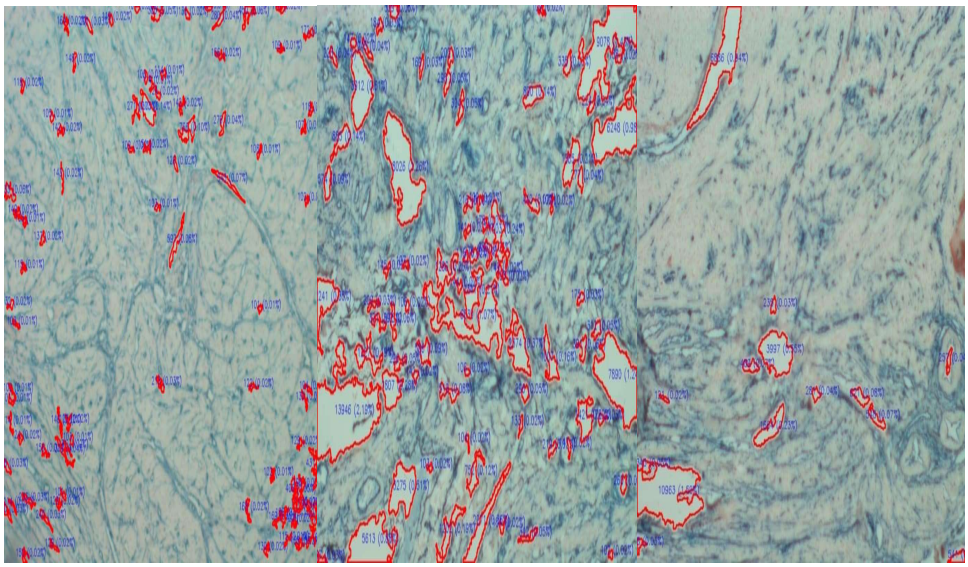
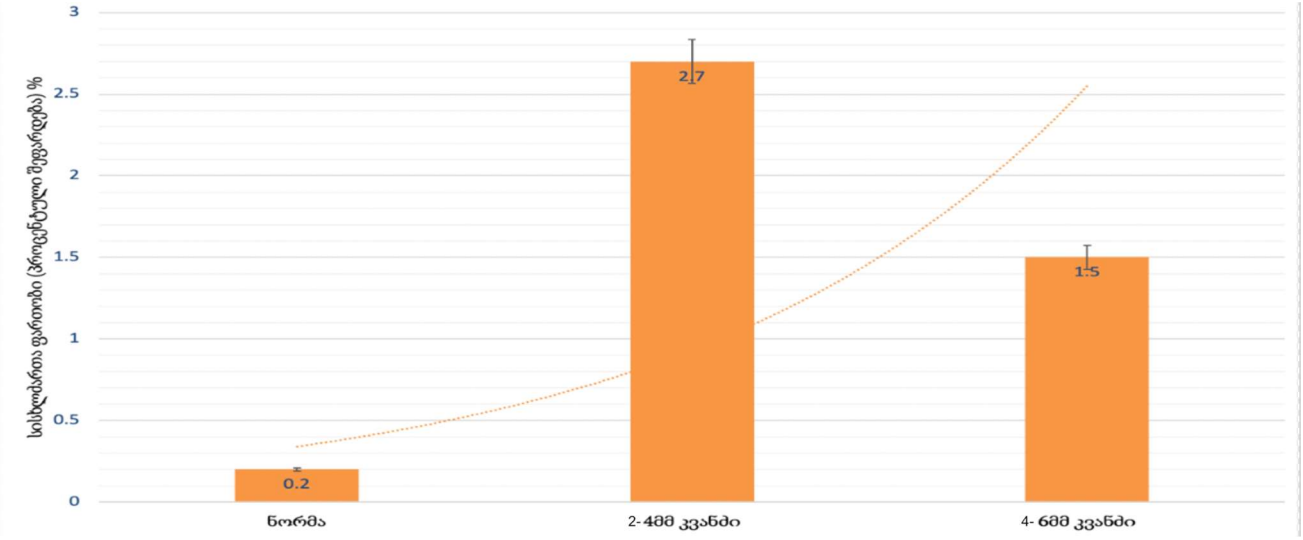


Photos 23, 24, 25. Myometrium and leiomyoma nodules measuring 2–4 mm and 4–6 mm; CD34 expression.Imaged using Leika 1000 LED microscope; photo captured with MC170HD camera at ×0.65 magnification.

Diagram 1.Angiogenesis Characteristics in the Uterine Body Myometrium and Leiomyoma Nodules in women of reproductive age



IV . Discussion and Conclusion

1.In women of reproductive age, during the early stages of leiomyoma development in the uterine body —prior to the formation of remodeled and distorted vascular network — an excessive accumulation of extracellular matrix (ECM) is observed, in contrast to the myometrium. ECM, whose accumulation is normaly regulated by cytokines, growht factors and steroid hormones —and which, in turn serves as a regulator of growth factor stability by limiting their activity —undergoes excessive accumulation and remodeling in pathology. This process, driven by proteolytic enzymes and their inhibitors, result in the release of growth factors.

2.Under condition of excessive extracellular matrix accumulation and dysregulated apoptosis (altered Caspase-3), a distored vascular collector if formed as a result of effect of steroid hormones (with progesterone being dominant) and growth factors. This

vascular structures, characterized by markedly increased CD34 expression compared to the myometrium, is encapsulated, exhibit autonomy, and prossess the ability for independent growth and development.

3. Proliferative activity of leiomyocytes (marked by strong SMA positivity) originates from any point within the entire plane of the remodeled vascular collector. This concept is supported, on one hand, by the presence of progenitor and stem cells within the walls of blood vessels in the collector, and on the other hand, by numerous cases we have observed of solitary, large, encapsulated leiomyomas containing multiple “daughter “ nodules of varying sizes.

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