DOCTORAL THESIS

ABSTRACT

UTERINE LEIOMYOMA: HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND CLINICAL STATISTICAL STUDY

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*Keywords: uterine leiomyoma, risk factors, histopathological variants, immunohistochemical markers*
INTRODUCTION

Uterine leiomyomas (fibroids, myomas) are benign smooth muscle tumors, found in one in three women over the age of 30 years. Uterine tumors are the most common and the leading cause of hysterectomy in women. Regarding the time of diagnosis they are more common in the fourth and fifth decade of life [1].

The increased incidence of uterine leiomyomas and the absence of onset signs and symptoms, in most cases, are important issues in the attempts of early detection disease.

Our aim is to help to elucidate some aspects of the pathogenesis of the tumor, knowing that in the past decade, there have been some important advances in understanding the molecular mechanisms underlying the tumor genesis, while still other mechanisms are insufficiently explained, despite the many theories advanced.

In this thesis, we propose as the major problem of research, a histological, immunohistochemical and clinical statistical study of uterine leiomyomas and also the effects that they may exercise on the female reproductive tract, with casuistry obtained from the Morphopathology Laboratory and Department of Obstetrics and Gynecology of the Emergency Hospital No 1 Craiova in the period 2003-2012.

The motivation for choosing this theme is determined by the fact that uterine leiomyoma still impresses with their high frequency, limited possibilities of conservative treatment and surgery performed often abusive and unnecessary leading to "mutilation" of many women.

STATE OF KNOWLEDGE

CHAPTER I
MACROSCOPIC AND MICROSCOPIC ANATOMY OF THE UTERUS

The development of the uterus takes place along with other female internal genitalia (fallopian tubes and vagina) from Müller's ducts (paramesonephric ducts). Müller’s ducts present three sections: a cranial section, dilated (ostium), an intermediate section which opens through several holes in the cloacal cavity and the caudal portion, in which the two Müller ducts approaches but remain separated by the septum that will reabsorb in the 3rd month of intrauterine life, when the uterovaginal canal will form. Thus, from the cranial segment and the initial portion of the intermediate segment are formed
the fallopian tubes, the intermediate segment will form the fundus, the body and cervix of the uterus, and from the lower segment of the uterovaginal canal is formed the vagina.

Anatomically, the uterus presents three sections: the upper section, the most dilated, the uterus, a narrower middle region, the isthmus and a lower portion of cylindrical shape, cervix, which is divided by the insertion of the vagina into two areas: supravaginal and subvaginal (vaginal) that protrudes into the vagina. At this segments level there are three cavities: the uterine body cavity, the isthmus canal and cervical canal or the canal of the uterine cervix [2].

Uterine wall presents three tunics: tunica mucosa (endometrium), muscular tunic (myometrium), external tunic (perimeter) and the peritoneal serous. The cervix is the lower portion of the uterus of cylindrical shape. Its wall presents three tunics: mucosa, muscular-elastic (smooth muscle fibers and connective tissue rich in elastic fibers) and adventitia.

The cervix has two areas: exocervix and endocervix. The exocervix is the visible segment in the vaginal cavity, being covered by nonkeratinized stratified squamous epithelium and aglandular chorion and the endocervix or endocervical canal which continues the uterine isthmus consisting of a simple cylindrical epithelium with numerous mucous cells and glandular chorion [3].

CHAPTER II
ETIOPATHOGENESIS OF UTERINE LEIOMYOMA

Uterine leiomyoma are the most common benign tumors of the female genital tract. Their incidence increases with age, and are found in 20% to 50% of women aged over 30 years [4].

Given the development of uterine leiomyoma it is convenient to divide the factors that may be related to tumorigenesis in four categories: predisposing risk factors, initiators, promoters, and effectors. Risk factors are characteristics associated with a disease generally identified by epidemiological studies. Knowledge of these predisposing factors may provide clues to the etiology of these tumors, as well as preventive measures [5].

However, the main promoter of uterine leiomyoma growth, accepted with one consent, is represented by estrogen [6].
CHAPTER III
METHODS OF DIAGNOSIS AND TREATMENT FOR UTERINE LEIOMYOMAS

It is estimated that 20%-50% of women with uterine leiomyoma present with symptoms such as menorrhagia, dysmenorrhea, pelvic pressure, urinary frequency, pain, infertility, or palpable abdominal or pelvic mass [4].

The clinical presentation is variable depending on the size, location, and number of tumors. The four major symptoms of uterine leiomyomas that are appropriate indications for surgery are bleeding, pressure on adjacent organs, pain and infertility.

Diagnostic methods: endovaginal ultrasonography, endometrial biopsy sonohysterografia with saline, hysteroscopy, nuclear magnetic resonance [7,8,9,10]. The differential diagnosis includes: adenomyosis, solid adnexal masses, uterine leiomyosarcoma [11].

Treatment methods: non-surgical treatment, myomectomy to preserve fertility, uterine artery embolization [12,13,14].

PERSONAL CONTRIBUTIONS

CHAPTER IV
CLINICAL AND STATISTICAL STUDY ON RISK FACTORS INVOLVED IN THE ONSET UTERINE LEIOMYOMA IN THE PERIOD 2003-2012

We performed a retrospective study of 1009 patients hospitalized in the II Clinic of Obstetrics and Gynecology of the Emergency County Hospital Craiova in the period 2003-2012 who were diagnosed with uterine fibroid and who underwent surgery.

We analyzed the age groups with the highest degree of risk for this condition, the characteristics of the menstrual cycle (menarche, cycles, menopause), the correlation between uterine fibroids and fertility and associated disorders.

Knowledge of the etiopathogenesis and the results of our study have raised sufficient arguments that there was two factors favoring tumor development, one hormonal and other vascular by providing conditions through which tumor proliferation may be influenced.

For this condition has been described a particular hormonal status, with an increase of its evolution by hormonal loading status (premenopausal), and those induced iatrogenically by administration of estroprogestative, menopause
Chapter V
Histopathological Study of Uterine Leiomyomas

In the histopathological study we investigated the main clinical and morphological features of uterine leiomyomas. Histopathological material came from the cases of the Morphopathology Laboratory of the Emergency Emergency Hospital Craiova and was represented by 56 archived paraffin blocks.

In the morphological study we used the classic histological techniques including paraffin and as staining methods I used:

- Hematoxylin-eosin (HE) for diagnostic evaluation according to the criteria for the classification of tumors of the endometrium set by WHO (2003) [15].
- Masson trichrome with aniline blue highlighting specific collagen fibers (assessing the degree of fibrosis, tumor).
Alcian Blue-Periodic Acid Schiff (PAS AA) to assess the profile of mucins (neutral versus acidic) secreted by tumor cells.

In the the table below are presented histological subtypes of leiomyomas found in our study, the most representative being the cellular ones (Table V.1):

<table>
<thead>
<tr>
<th>LMU subtypes</th>
<th>Number of cases</th>
<th>Percentage%</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMU high celularity</td>
<td>28</td>
<td>50%</td>
</tr>
<tr>
<td>LMU with hyaline degeneration</td>
<td>9</td>
<td>16,07%</td>
</tr>
<tr>
<td>LMU with myxoid degeneration</td>
<td>4</td>
<td>7,14%</td>
</tr>
<tr>
<td>LMU highly vascular</td>
<td>2</td>
<td>3,57%</td>
</tr>
<tr>
<td>LMU with cystic degeneration</td>
<td>2</td>
<td>3,57%</td>
</tr>
<tr>
<td>LMU hemorrhagic infiltration</td>
<td>2</td>
<td>3,57%</td>
</tr>
<tr>
<td>LMU schwannom-like</td>
<td>4</td>
<td>7,14%</td>
</tr>
<tr>
<td>LMU plexiform</td>
<td>5</td>
<td>8,92%</td>
</tr>
</tbody>
</table>

Table V.1. The distribution of the histologic subtypes of uterine leiomyomas

Cellular leiomyomas typically consist of dense cellular fascicles separated by a small amount of collagen. Presents mitotic activity around 5 mitoses / x10 objective. In some cases necrosis and hemorrhage may occur, but mitotic activity and atypical cytology are absent. Given the presence of hypercellularity, it may need a differentiation from endometrial stromal sarcoma in which the fascicle pattern and the presence of blood vessels with thick muscular walls are characteristic of smooth muscle tumors.

Microscopically uterine leiomyomas have a proliferation of fusiform cells arranged in bundles which intersect, separated by variable amounts of fibrous connective tissue. Peripherals are limited by the presence of a pseudocapsule formed by compression of adjacent tissues as shown in Figure V.1.
Fig V.1. Uterine leiomyoma – pseudocapsule, Col. HE x 100.

Fig V.2. Uterine leiomyoma – proliferation of fusiform cells arranged in bundles with different orientations, separated by septa of fibrous stroma, Col. PAS-AA, x100.

Fig V.3. Conventional leiomyoma – overview that highlights the fascicle pattern of this tumor, fusiform neoplastic cells arranged in bundles with different orientations, separated by septa of fibrous stroma, Col. Tcr. Masson, x40.

Fig V.4. Uterine leiomyoma – dense cellularity variant, Col. HE, x100.

Fig V.5. Uterine leiomyoma with hyaline degeneration, Col. PAS-AA, x100.

Fig V.6. Conventional leiomyoma – with myxoid degeneration area, between fascicles of fusiform cells are present accumulation of Alcian blue positive material (acid mucopolysaccharides type), Col. PAS-AA, x100.
Microscopic diagnostic parameters in the tumor are: cell type differentiation, atypical cytological mitotic index, presence of areas of necrosis, tumor margins appearance and relationship with adjacent structures.

Atypical cytology and number of mitosis, are important in differentiating atypical or cellular leiomyomas, smooth muscle tumors of uncertain malignant potential and leiomyosarcoma.

For leiomyoma pleads the absence of atypia or their occurrence in mild degrees and the absence of necrosis. When atipia is present in moderate to severe and when the necrosis is absent tumor diagnosis is based on mitotic activity. The presence of less than 10 mitoses / x10 objective, calls for a non-standard leiomyoma, while a number of over 10 mitoses / x10 objective is met in a leiomyosarcoma. If atypia are moderate to severe necrosis of tumor cells is present, the tumor is a leiomyosarcoma regardless of the mitotic index.

CHAPTER VI
IMMUNOHISTOCHEMICAL STUDY OF UTERINE LEIOMYOMAS

We analyzed the medical records of the Morphopathology Laboratory of the Emergency Clinical Hospital No.1 of Craiova and identified 15 patients who were diagnosed with uterine leiomyoma and 5 cases of normal uterine tissue. Uterine leiomyomas tissues were obtained from female patients with symptomatic uterine fibroids at the time of elective hysterectomy and who were not receiving any type of hormone therapy or medication. Normal uterine samples were collected from women during perimenopause to which hysterectomy was performed for medically indicated reasons (excluding endometrial cancer and leiomyoma) and who were not on hormone therapy at the time of operation. The pieces were obtained from interventions practiced in the department of Obstetrics and Gynecology II.

As clinical data we noted each patients age and as pathological parameters we sought histopathological variant of leiomyomas. Histopathological variant was determined using World Health Organization criteria (Tavassoli & Devilee, 2003) [16].

Paraffin blocks from these patients were processed by histological techniques (HE stain) and detailed histopathological investigations were stained with Masson trichrome kit with Alcian blue dye pH 2.5-PAS.

Immunohistochemistry was performed on 4-μm sections from a selected block in each case. Briefly, primary antibodies were used in a dilution of 1:1000 for TGFbeta1 (mouse monoclonal, TB21, AbD Serotec, Albedo,
Romania, Code: MCA797T) for TGFbetaR1 1:300 (rabbit polyclonal, T-19, Santa Cruz Biotechnology, Redox, Romania, Code: SC-402) TGFbeta3 1:500 (rabbit polyclonal, Santa Cruz Biotechnology, Redox, Romania, Code: SC-83) and TGFbetaR3 1:100 (rabbit polyclonal, Abcam, Cheminpres, Romania, Code: ab28366) incubating the slides overnight at 4 °C. Primary antibodies were amplified with biotinylated species-specific and LSAB2 system (Dako, Redox, Romania - Code K0675). Visualization was done with 3,3′-diaminobenzidine (DAB) (Dako, Redox, Romania - Code K3468). For counter stain Mayer hematoxylin was used. Negative staining controls were performed by omitting the primary antibody.

Clinicopathological data:
According to medical records, the average age for investigated leiomyomas was 39, with typical lesions developed relatively late in life (at least 1.5 years later). Most leiomyomas (9 cases, 60% from the cases investigated) were diagnosed histologically as typical cases of proliferating spindle cells arranged in anastomosed fascicles. In the other cases, leiomyomas were classified with morphological variants hyaline (3 cases), epithelioid (2 cases) and atypical (1 case) as we showed in Table VI.1.

Table VI.1. Histopathological subtypes of the analyzed parts

<table>
<thead>
<tr>
<th>histological subtypes</th>
<th>typical leiomyomas</th>
<th>Hyaline leiomyomas</th>
<th>Epithelioid leiomyomas</th>
<th>Atypical leiomyomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immunoreactivity TGFbeta1 and TGFbetaR1:
In the normal uterine samples TGFbeta1 was expressed specifically in the cytoplasm of glandular epithelial cells and, to a lesser extent, in the cytoplasm of endometrial stromal cells, in myometrium smooth muscle cells and endothelial cells and smooth muscle of the uterine vessels. In addition, the inflammatory cells present in the endometrium and myometrium were also positive for TGFbeta1. A similar immunoreactivity was observed in TGFbetaR1 but with a much lower intensity.

For the samples from leiomyomas, immunoreactivity to TGFbeta1 was higher than in normal myometrium and autologous myometrium samples (Fig VI.1.AC). A similar trend was observed in TGFbetaR1 (Fig VI.1.DF). In
conclusion, the intensity of immuno staining of TGFbetaR1 was lower than that of the corresponding growth factor TGFbeta1.

![Image](image1.png)

Figure VI.1. TGFbeta1 immunoreactivity in A. Samples normal myometrium, X100, B. Samples of typical leiomyoma, X100, C. Samples of atypical leiomyoma X200. Immunoreactivity TGFbetaR1 in: D. Samples of normal myometrium, X100, B. Samples of typical leiomyomas, X100, C. Samples of epithelioid leiomyomas, X10.

**Immunoreactivity TGFbeta3 and TGFbetaR3:**

A weak cytoplasmic reaction, in particular in glandular epithelial cells was observed in normal uterine specimens, to a lesser extent in smooth muscle cells of the myometrium and in the smooth muscle cells and endothelial cells of myometrial arterioles. A more intense reaction was observed for TGFbetaR3 with the pattern of cytoplasmic and membranous expression.

TGFbeta3 immunoreactivity in tumor samples was higher than in normal
myometrium samples and autologous myometrium (Fig VI.2.AC). However, leiomyomas cell reactivity was lower than in endothelial cells of vessels, in the tumoral and normal myometrium. In addition, a weak reactivity was also observed in cells associated with inflammation. A similar trend was observed for tumoral TGFbetaR3 immunoreactivity (Fig VI.2.DF). From a qualitative immuno staining intensity TGFbetaR3 was higher than that of the corresponding growth factor TGFbeta3. Also there were no significant differences on immunoreactivity for TGFbetaR3 and TGFbeta3 leiomyomas samples collected from patients who associate various malignant or benign gynecological conditions.

Figure VI.2. TGFbeta3 immunoreactivity of: A. Samples of normal myometrium, X100, B. Samples of typical leiomyomas, X100, C. Samples of epithelioid leiomyomas, X100. Immunoreactivity TGFbetaR3 in: D. Samples of normal myometrium, X100, B. Samples of typical leiomyomas, X100, C. Samples of epithelioid leiomyomas.
GENERAL CONCLUSIONS

Uterine fibroids exhibit minimal malignant tendency, but can complicate the development of a pregnancy, may mask menopause treatment or confuse the diagnosis of more serious gynecological pathologies.

Current knowledge of etiopathogenesis and the results of our study have resulted in sufficient arguments favoring the existence of two factors for tumor development, a vascular and a hormonal factor, offering conditions through which tumor proliferation may be influenced.

Although a particular hormonal status has been described for this condition, there was an increase of its evolution with hormonal fluctuating status (premenopausal), same for those induced by administration of estroprogestative, iatrogenically menopause representing the most effective treatment and the only one leading to tumor involution. Trying administration of GnRH agonists, which induce an artificial menopause, manages to reduce tumor volume, but with the cessation of treatment, the tumor returns to its original size.

Microscopic diagnostic parameters in the tumor are: cell type differentiation, cytological atypia, mitotic index, presence of areas of necrosis, tumor margins appearance and its relationship with adjacent structures.

The most common varieties of leiomyomas are represented by - cellular leiomyomas. The differential diagnosis of uterine leiomyomas include:
- Muscle tumors with uncertain malignant potential
- Leiomyosarcoma
- Endometrial stromal sarcoma

Atypical cytology and number of mitosis, are important in differentiating atypical or cellular leiomyomas, smooth muscle tumors with uncertain malignant potential and leiomyosarcoma.

Our study demonstrates that leiomyomas have increased expression of TGFbeta1, 3 and their receptors TGFbetaR1, 3 compared to autologous myometrium.

Highest reactivity in leiomyomas was recorded for TGFbeta1 and TGFbetaR3 with significant correlation between their IRS scores both in typical and atypical uterine tumors. This reactivity may have prognostic and therapeutic impact on patients with uterine leiomyomas.
SELECTIVE BIBLIOGRAPHY


