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# UTERINE FIBROID AND DIFFUSE UTERINE FIBROMATOSIS – TWO DIFFERENT ENTITIES – A PROSPECTIVE STUDY

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## Summary

Uterine fibroid or fibromyoma is a very common benign human tumor and the most frequent tumor in the woman's reproductive system. Uterine fibroids affect millions of women across the world and they are still the most commonly surgically treated. In Romania too, uterine fibroid is the most common reason for hysterectomy, about 60% of cases have this diagnosis pre-operatively established. Uterine fibroid is a benign tumor that occurs very frequently in women of reproductive age. General prevalence of fibroids is between 20 and 40% [1]. Prevalence varies by age and is higher in the final part of the fertile period, approximately 35-40% of women over 40 years old have uterine fibroids. The reported incidence is highly variable in different studies, in fact, to determine the exact prevalence of fibroids, a correct clinical research should apply ultrasound scanning in a randomly sampled population [2]. On the other hand, women's current trend of delaying pregnancy beyond the age of 30 years but also increasingly frequent disturbances of fertility have led to the need for uterine leiomyomas analysis in relation to fertility. We have assumed a great responsibility approaching this subject of research taking into account the high incidence on the one hand and the fact that the uterine fibroids are considered generally something very "common" in the gynecologic pathology that can no longer bring up nothing spectacular scientifically, on the other hand. We approached a prospective study which will analyze the histological, ultrastructural and immunohistochemical endometrium and myometrium in patients with uterine fibroids well defined compared to patients with diffuse uterine fibromatosis starting from the premise that these two aspects very similar to the uterine pathology are still different at ultrastructural level and in terms of involvement of different profibrotic and angiogenic factors but also in distribution of various hormone receptors.

## Résumé

***Le fibromyome et la fibromatose utérine - deux entités distinctes - étude prospective***

Le fibrome utérin ou le fibromyome est la plus commune tumeur bénigne humaine et il est la tumeur la plus fréquente dans le système reproducteur de la femme. Les fibromes utérins affectent des millions de femmes partout dans le monde et encore ils sont majoritairement traités chirurgicalement. Et en Roumanie aussi, le fibromes utérin est la cause la plus fréquente pour l'hystérectomie, environ 60% des cas ayant ce diagnostic en pré-opératoire. Le fibrome utérin est une tumeur bénigne qui apparait très fréquemment chez les femmes à l'âge de reproduction. La prévalence générale des fibromes est comprise entre 20 et 40% [1]. La prévalence varie selon l'âge et est plus élevée dans la dernière partie de la période fertile, environ 35-40% des femmes de plus de 40 ans ont des fibromes utérins. L'incidence rapportée dans différentes études est très variable, en fait pour déterminer la prévalence exacte des fibromes de l'utérus l'ultrasonographie est nécessaire dans un groupe de population choisie au hasard, statistiquement significative [2]. D'autre part, la tendance actuelle des femmes d'ajourner la grossesse au-delà de 30 ans, mais aussi les troubles de fertilité plus fréquents ont conduit à la nécessité d'analyser le fibrome par rapport à la fertilité. Nous avons pris une plus grande responsabilité pour aborder cette question de la recherche prenant en considération sa haute incidence d'une part, et le fait que les fibromes utérins sont considérés comme quelque chose de „commun" dans la pathologie gynécologique, qui ne peut plus apporter rien de spectaculaire du point de vue scientifique, d'autre part. Nous avons commencé une étude prospective destinée à analyser l'endomètre et le myomètre histologique, ultrastructurale et immunohistochimique chez les patients présentant des fibromes utérins bien définis par

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**Partial results show differences statistically but not so important in terms of epidemiological, clinical and surgical terms. Histological and immunohistochemical data will still be collected and processed to understand the similarities and especially the differences between these two entities.**
**Key words: uterine fibroid, leiomyoma, diffuse uterine fibromatosis, endometrial aspects in uterine fibromatosis, immunohistochemistry, smooth muscle tissue**

**rapport aux patients ayant de la fibrose utérine diffuse, partant du principe que ces deux aspects sont très semblables mais différents dans la pathologie utérine au niveau ultrastructural et en termes de participation des différents facteurs profibrotiques et des facteurs angiogéniques et en termes de répartition variable des divers récepteurs hormonaux. Les résultats partiels montrent des différences statistiquement mais pas si importantes en termes épidémiologiques, cliniques et chirurgicaux. Les résultats histologiques et immunohistochimiques continueront d'être recueillis et interprétés afin de comprendre les similitudes et les différences entre ces deux entités surtout.**
**Mots-clés: fibrome utérin, léiomyome, fibrose utérine diffuse, l'apparition de l'endomètre dans la fibrose utérine diffuse, l'immunohistochimie, tissu musculaire lisse**

## INTRODUCTION

### *Pathogenesis*

The determinant factor of uterine fibromatosis is not known exactly yet, but there are many theories: Conheim's hereditary congenital theory, Klebs-Pillot's vascular theory, Wirchoff's infectious theory. The hormonal theory still proves quite real, uterine fibroids are detected exceptionally before menarche and generally regressing to menopause - Heger, Scitz and Faure incriminating estrogen role in the genesis of these tumors. At present taking into account at least three hormonal factors that may influence the proliferation of uterine leiomyomas: estrogen, progesterone and STH. The leiomyoma presents cyclic proliferation periods, and ceases to grow, generally at menopause, when the levels of sex hormones decrease. Several studies [3,4] reported a rapid increase of fibroid incidence after the age of 40. This could be the result of time-related hormonal changes or an enhanced symptomatology from already existing fibroids. Leiomyomas are tumors in which angiogenesis is very important for their growth. Several studies have demonstrated the role of pro-fibrotic and angiogenic factors in the uterine fibromatosis etiology (cytokines and growth factors): FGF, VEGF; TGF-B; EGF; TGF-A; IGF 1; PDGF; IL1; IL6. Research for the pathogenesis of fibroids and abnormal extracellular matrix (ECM) led to the analysis of a growth factor with profibrotic activity, transforming growth factor $\beta$ (TGF-$\beta$). The $\beta$3 subunit of TGF-$\beta$3 and its signal mediators are overexpressed in leiomyomas compared to normal myometrium [5]. Historically, uterine leiomyomas have not been considered a genetic disease. However much recent clinical evidence indicates that at least some myomata have a genetic mechanism. The HMGA2 gene is expressed in uterine leiomyoma and in other human tissues with a proliferative phenotype, such as fetal tissues, lung, and kidney, but not in the normal myometrium [6]. Recent studies described that 70% of fibroids contained a series of mutations in a transcriptional regulator complex subunit 12 (MED12) [7]. Perot et al. reported that MED12 is frequently mutated in typical leiomyomas (66.6%) [8]. Another study reported that HOXA10 and HOXA11 mRNA expression were significantly decreased in uteri with submucosal myomas compared to those in controls with normal uterine cavity and to uteri with intramural myomas [9]. Therefore, the etiology of uterine fibroids is very complex and still poorly understood, countless studies on the subject are in progress in all academic medical centers around the world.

## MATERIAL AND METHOD

We will carry out a prospective study, begun in January 2015, with a minimum duration of two years that will track the pathological, immunohistochemical, ultrastructural and molecular biology myometrial and endometrial aspects in patients with uterine fibroids well defined - compared with patients with diffuse uterine fibromatosis. We set a statistically significant lot, approximately 200 patients diagnosed with uterine fibromatosis. The cases are recruited and diagnosed at the university gynecological clinics in the "Emergency Hospital Saint Pantelimon", respectively "Dr. I. Cantacuzino Hospital" from Bucharest.

1. The criteria for including the patiens in the study are:
   - Age: the period of maximum fertility without ruling out the extremes, from age 18 to 60.
   - Patient legally major who has given consent for surgical intervention and for participation into the study.
   - Diagnosis confirmed by ultrasound imaging in most cases or CT / MRI in selected cases.
   - Clear indications of surgery: excessive menstrual bleeding, dysmenorrhoea, metrorrhagias, anemic syndrome, chronic pelvic pain, urinary or sexual dynamic disorders, fertility disorders.

2. Exclusion criteria: The patient refuses the surgery, clinical condition is not a surgical emergency, diagnosis of malignant or pre-malignant gynecological affections, serious associated pathology that contraindicates surgical intervention (heart disease, liver disease, renal, neurological, oncological desease).

For the uniformity of data processing, the ultrasounds are perfomed in similar conditions, endo-vaginal, using the same type of ultrasound machine and 2D of 7.5 Mhz probe. Color Doppler and Power Doppler were used to

study uterine vasculature. Common laboratory investigations were performed: complete blood count, fibrinogen, INR, Quick test, Howell's time, glucose, blood urea, serum creatinine, uric acid, liver tests (SGOT, SGPT), urine analysis. Starting from the clinical observation of the patients, the ultrasonographic appearance, the one intraoperative but also by the morphopathological macroscopic appearance we hypothesized that uterine fibroids with net delimitation and diffuse uterine fibromatosis are two similar aspects of the same disease, but not identical. Uterine fibroids (single or multiple) is a tumor in various forms, sizes and locations at the uterine level but well delimited, sometimes having a peripheral pseudocapsule and ultrasound identifiable vascularization (Fig. 1).

The uterus with diffuse fibromatosis is generally normal or slightly increased in dimensions with an inhomogeneous structure, no ultrasound identifiable formations and a lower vascularization (Fig. 2).

Based on these clinical and ultrasound criteria, the selected lot of pacients was divided from the start, preoperative, in two cohorts:

A. Pacients with uterine fibroids well defined. Uterine fibroid was imaging confirmed (by analysing sizes, number, position, form and vascularisation or for the performance of a exactly differential diagnosis) by transvaginal ultrasound, and

B. Pacients with diffuse uterine fibromatosis, the criteria of admission in this group (in the absence of clearly ultrasound identifiable formations) being: patients with typical symptomatology and suggestive ultrasound aspect, then confirmed on postoperative pathological macroscopic appearance (Fig. 3). Depending on the intra and postoperative appearance of the uterus we reinstated patients in the appropriate cohort when ultrasound diagnosis was not consistent with reality.

The general objectives of our study are:

1. The observation of histological characteristics of the myometrium / endometrium at patients with single or multiple uterine fibroid - well defined, respectively diffuse uterine fibromatosis.
2. The immunohistochemical study of the hormonal receptors in the endometrium and myometrium (ER and PGR) - in relation with the topography / location of the uterine fibroids and quantification of the factors involved in angiogenesis and fibrosis (EGF, TGF-b; FGF; VEGF) as well as the identification of markers for the smooth muscle tissue (H CAL, alfa SMA).
3. The implementation of modern diagnostic methods (immunohistochemistry and molecular biology) to obtain a more accurate diagnosis.
4. Corroborating morfopathological data and immunohistochemistry with the clinical evolution of the patients before and after the surgery and early detection of the oncological diseases.
5. Establishing a therapeutic strategy within the changes of menstrual flow and fertility in patients with coexisting uterine fibromatosis pathology.

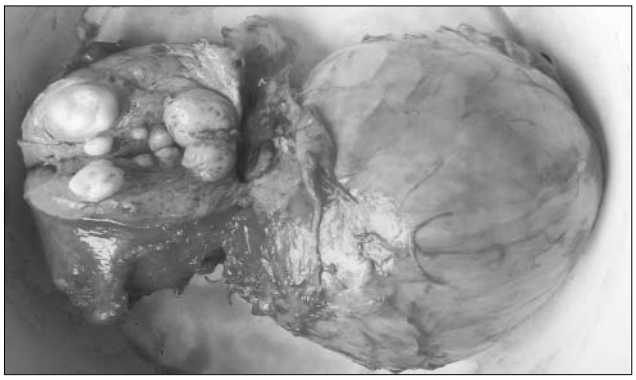


*Figure 1 – Multiple uterine fibroids*

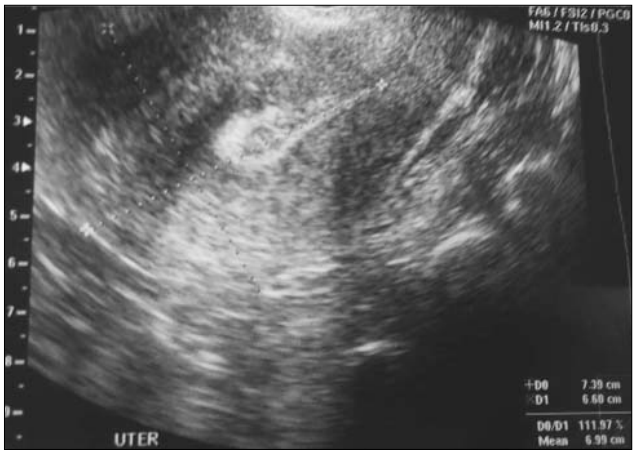



*Figure 2 - Diffuse uterine fibromatosis - sonographic view*

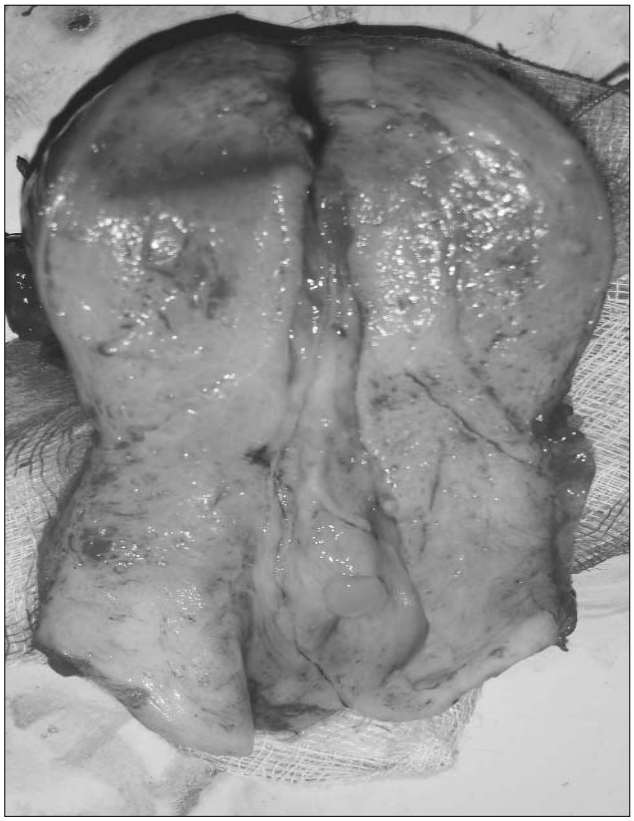


*Figure 3 - Sectioned uterus with diffuse fibromatosis*

Throughout the study we will track and note:

1. Personal data of patients: for every case we made a medical record sheet (personal file) which includes: identity data (age, address, marital status), clinical data from the current clinical observation files (the reasons for hospitalization, physiological and pathological personal history outlining the possible hormonal disruptions), relevant symptoms (abdominal pain, metrorrhagias, dyspareunia, etc.), family history and objective clinical signs.
2. Laboratory exams: ultrasound, CT / MRI, previous biopsies, as well as the accompanying data sheets of the biopsy material and pathological records - detailed macroscopic aspects and histopathological diagnosis.
3. Intra and postoperative data: the duration of the surgery, duration of hospitalisation, intra and postoperative bleeding.
4. Histological sections are obtained after myomectomy and hysterectomy.
5. The certainty final diagnosis is established histologically and data will be corroborated later with the immunohistochemistry results.

*a- Histological study:*

The experimental pathology protocol consists in laboratory determinations of all the patiens included in the study: evaluation of serum and intratumoral angiogenesis biomarkers (EGFR, VEGF) and quantify of microvascular density. While the enrollment cases in the study we will make the initial assessment of available and good quality histological material tissue in the corresponding paraffin blocks which we will take 5 micron sections. It will create a bank of frozen biological materials (biobank) to be used in the studies by immunohistochemistry. On the other hand, this biobank will allow the opening of research opportunities both nationally and international. Myometrial and endometrial tissue fragments will be fixed in 10% formalin, processed, included in paraffin, sectioned at 3-5 microns. After Van Gieson stain and HE standard stain , along with pathologist, we will follow: the appearance of glandular epithelial cells, assessing the degree of proliferation, hyperplasia and the degree of atypia; Miometrial cell layout; appearance of stromal cells; vasculature appearance – number, shape, ramifications, presence of blood suffusions or neo-vessels, microvascular density (number of vessels / unit of area).

*b- Immunohistochemical study:*

Determination of receptors expression in uterine tissue will be done by immunohistochemistry techniques using primary antibodies. Histological sections will treat heat for 20 minutes in citrate solution for antigen unmasking. Histological sections will be incubated with primary antibody using the most appropriate dilutions and that according to the manufacturer's technical specifications. Using monoclonal antibodies we study the estrogen and progesterone receptors but also other factors involved in angiogenesis and fibrosis respectively - FGF, VEGF, EGF, TGF-b. We have taken into account the markers that distinguish or appreciate qualitatively or quantitatively the two existing types of tissue in the uterus fibroid – smooth muscle tissue and connective/fibrous tissue (Caldesmon – HCAL, alpha SMA are markers for smooth muscle tissue) and possibly a marker for cell proliferation (Ki-67) and one for vascular appearance (CD34).

## RESULTS

### *Statistical interpretation / Correlations*

We will try to answer the question - there are qualitative differences in myometrium / endometrium in uterine fibroids well delimited versus diffuse uterine fibromatosis? The follow parameters are quantitative but also qualitative! We will try to quantify the qualitative parameters to be processed statistically. Data to be processed, besides the records of surveillance files of patients will be obtained from a detailed morpho-pathological research that will analyze of histologically (muscular or fibrous appearance?) and immunohistochemistry (the presence of receptors / markers) point of view. The data will be processed using SPSS and EPI INFO software, statistical significance was considered at a p-value $<0.05$. To compare qualitative variables will be used Fisher's exact test. We envisage the signs test to for this analysis (null hypothesis: in large group of patients diagnosed with uterine fibroids there is no difference between those with well-defined uterine fibroids and those with diffuse fibromatosis). By logistic regression will be determined parameters independently correlated with the presence of certain signs or symptoms.

### *Partial results*

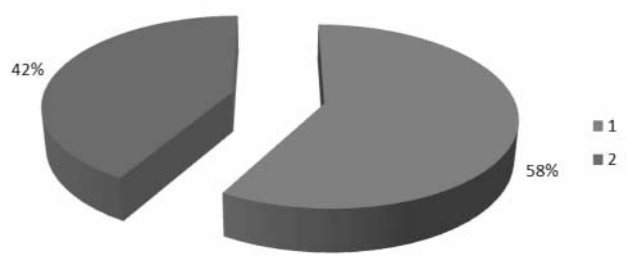
Until now 91 patients were included in the study who met all the previously stated criteria and who have given their written consent. Of these, 53 were diagnosed (based on clinical, ultrasonographic or morphopathological criteria) with uterine fibroids (single or multiple) well defined and 38 patients were included into the group with diffuse uterine fibromatosis (Graph 1).

In the group of patients with uterine fibroids 32 had uterus with multiple nodules and 21 had only one fibroid (Graph 2).

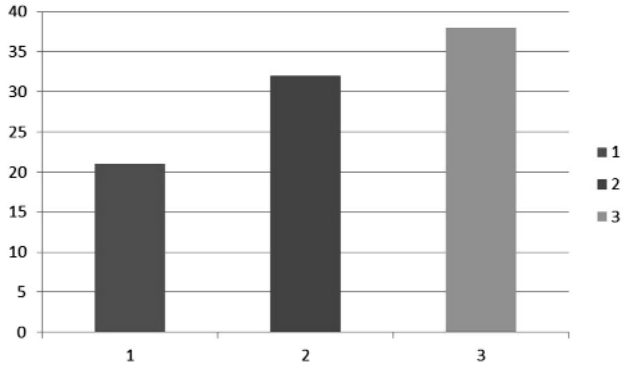
Regarding clinical data were marked significant differences in the two groups (Table 1).

The average duration of hospitalization was almost identical in the two subgroups A and B. Immediate or late complications rate (Table 2) was below 5% in both subgroups. Average duration of operation and intraoperative bleeding was greater in the subgroup A.

The patients are monitored postoperative throughout the entire duration of the study to assess the evolution and to note any late complication. They are undergoing processing statistical data to explain the involvement of certain risk factors (age, family history, body mass index, etc.) in the development of uterine fibroids. Are being




*Graph 1 - 1. Patients with uterine fibroids well defined (A): 53 patients; 2. Patients diagnosed with diffuse uterine fibromatosis (B): 38 patients*




*Graph 2 - 1. Patients diagnosed with single uterine fibroid: 21 patients; 2. Patients with multiple uterine fibroids: 32 patients 3. Patients with diffuse uterine fibromatosis: 38 patients*

finalized preparation of tissular fragments and antibody kits for immunohistochemical study. Final results will be published in the following articles.

## CONCLUSIONS

We believe that our study has an major clinical importance because of the high incidence of uterine fibroids and because of association, in most cases, with endometrial pathology. Although benign, uterine fibromatosis, with her form well defined (fibroids or leiomyomas) sometimes with giant size or in her diffuse form, can cause severe clinical manifestations and permanent interfere with daily life of our patients. Excessive menstrual bleeding, urinary and sexual dynamic disorders, dysmenorrhea, chronic pelvic pain and fertility disorders are very common clinical manifestations that make from this frequent pathology a permanent challenge for gynecologist. Myometrium and endometrium analysis, histological and immunohistochemical, will be the priority aim of our study in an attempt to find more explanations regarding the consequences and implications of uterine fibromatosis in abnormal uterine bleeding or infertility.

The study is ongoing, besides the usual histopathological findings we will continue with a detailed immunohistochemistry research to be able to find new data on the etiology of this disease with great impact in the health of the global female population.

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*Table 1*

| Clinical data | Patients with well defined fibroids ( A: 53 patients) | Patients with diffuse uterine fibromatosis (B: 38 patients) |
|---|---|---|
| Mean age | 43,5 years | 48,4 years |
| Metrorrhagias | 48 (90,5%) | 32 (84,2%) |
| Pelvic pain | 37 (69,81%) | 25 (65,7%) |
| Urinary dynamic disorders | 28 (52,83%) | 8 (21%) |
| Sexual disorders | 32 (60,37%) | 23 (60,5%) |
| Number of sexual partners | 2,3 | 2,5 |
| Significant family history | 28 (52,83%) | 14 (36,8%) |

*Tabel 2 - Complications and monitoring immediately*

| Monitoring 48-72 h/ Complications | Well defined fibroid (A: 53 patients) | Diffuse fibromatosis (B: 38 patients) | p value |
|---|---|---|---|
| Average duration of operation | 74 minutes | 56 minutes | 0,05 |
| Average duration of hospitalization | 2,7 days | 2,8 days | 0,05 |
| Intraoperative bleeding | 110 milliliters | 95 milliliters | 0,05 |
| Supurrative complications | 2 cases | 1 case | 0,05 |
| Pelvic drainage | 135 ml | 160 ml | 0,05 |

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