Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 4 (53) No. 2 - 2011

METABOLIC RELATIONSHIPS OF THE MYOCYTES AND THE CONNECTIVE TISSUE IN UTERINE FIBROMATOSIS

L.L. NEICA[1] I. PREDOI[2]

Abstract: *Much researched, uterine fibromatosis is a topic of interest, displaying incomplete elucidated aspects, related to the etiology, pathology, histophysiology and histogenesis of this uterine pathosis, classified under uterine benign tumors of the female reproductive system. As a result of specialized research, this paper discusses the findings of metabolic features for leiomyofibroma to be different from those constants in the uterine fibroma itself.*

Key words: *myocyt, leiomyofibroma, fibroleiomyoma.*

1. Introduction

Uterine fibroma includes its morphological variants fibroleiomyoma or leiomyofibroma, depending on the ratios of connective and muscle component, it represents a health problem with increased incidence of this disease in the gynecologic practice.

Numerous books and chapters focusing on specialized treatments have been written about this condition. In the last decade, there has been considerable progress in understanding the tumor's morphology, yet there remain many imprecise aspects, specifically those concerning the etiology and so this topic necessitates further research attention.

This investigation follows the aforementioned need for further research, in the hope to find, through classic and modern methods, characteristic, microscopic data that can add new information for understanding certain mechanisms involved in the emergence and evolution of uterine fibroids, increase and strengthen the knowledge of uterine histophysiology and improve the practical applications of such knowledge to the practice of specialized medicine.

2. Materials and Methods

The papers will be handed on quality printing machine paper, of colour flat white, in the standard form described herein, printed by a quality printing machine on a single facet of the page, and also in electronic format, identical with the printed copies.

Research took place in Brasov at the Emergency Clinical Hospital, Department of Pathology. Atomo - pathological samples were collected from 194 female patients ages 21 to 72 who were diagnosed and treated surgically at the Obstetrics and Gynecology Hospital in Brasov in 2010.

[1] Centre "Advanced Research on Mechatronics", *Transilvania* University of Braşov.
[2] Organization of appurtenance for the second author.

Collected fragments were sectioned and studied through histochemical methods in order to highlight the following enzymatic activities: NADH2-cytochrome-C reductase (diaphorase), lactate (LDH), pH9,4 ATPase.

Fragments were also prepared and investigated with electron microscopy.

3. Results

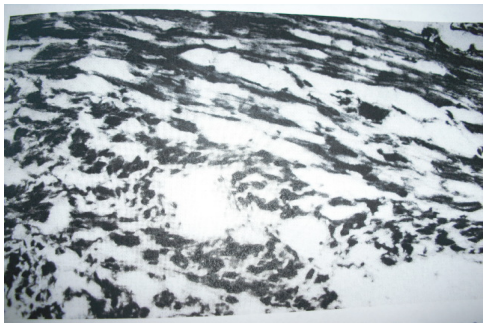

Fig. 1. *Uterine Leiomyofibroma. NADH2-cytochrome-C-reductase. Intense reaction and very intense in the muscle fibers of the myometrium, occurring in bundles arranged longitudinally sectioned and transversal.* ×150

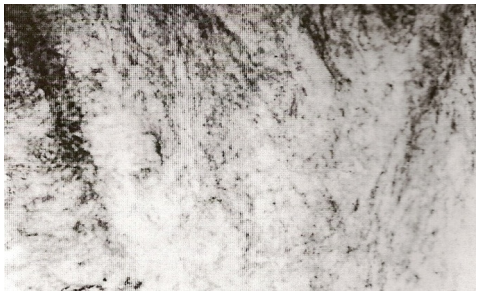

Fig. 2. *Fibroleiomyoma cancer. NADH2-cytochrome-C-reductase. Smooth muscle fibers isolated and grouped with diaphorazic reaction medium, intense and very intense, separated by a rich connective tissue with abundant matrices in which rare reactive connective cells and thin collagen fibers are found, most segmented* . X90

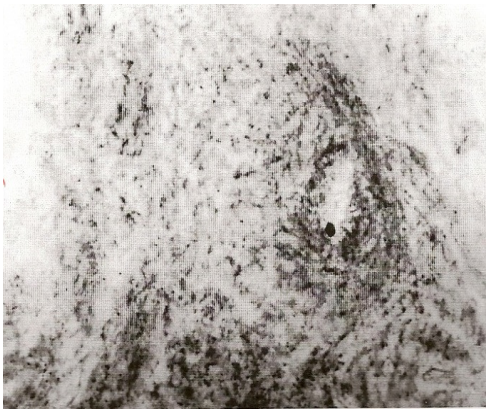

Fig. 3. *Uterine Fibroleiomyoma. NADH2-cytochrome-C-reductase. Zones with different intensities of enzymatic reaction in the smooth muscle fibers and in connective cells. The artery sectioned transversal shows intense diaphorazic activity in the endothelium, medium myocytes and in connective cells from adventitia.*x90.

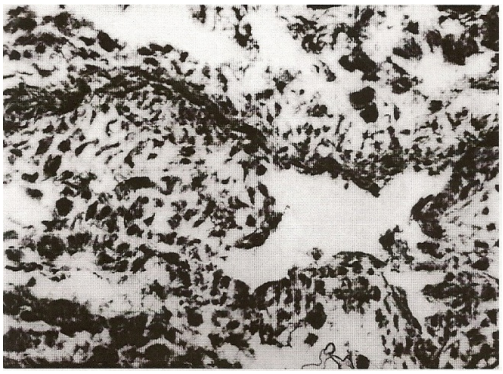

Fig. 4. *Leiomyofibroma. Lactatdehydrogenase. Increased image of the connective-vascular space from the myometrial muscle bundles. Intense reaction is observed, which predominates in the vessels at the endothelial cellular level and reactions of different intensities in the connective cells around the vein*. X 190

Fig. 5. *Uterine Fibroleiomyoma ATP-ase ph 9,4. Very intense enzymatic reaction in vessels in most myocytes and very weak reaction in fibroblasts.*

4. Discussion

Observations made from the optic microscope allow for unique and interesting aspects to be discussed. This refers to the metabolic characteristics of the two forms of the benign tumor researched, respectively of the uterine leiomyofibroma and fibroleiomyoma, of their common factors, but most importantly of their distinctive factors.

The variety of histopathological tissue and cellular modifications suggest that basic morphophysiopathological conclusions to the understanding of the organization, evolution of uterine fibromas and their treatment through pharmaceutical and surgical therapy.

From the start it is important to remember the major difference in relation of smooth muscle fiber to connective tissue, with predominance of myocytes in leiomyoma and connective tissue in the fibroma itself.

Compared with the normal appearance the leiomyofibroma presents like a compact mass of smooth muscle fibers laid out one beside the other, with extremely reduced inter-fibril space in which you can find a weak environment of extracellular matrix (2). It is important to note the appearance of the muscle fibers, which slightly dimensionally modified, shorter but with larger diameters than normal, fact which is well known by anatomic pathologists.

Structurally these smooth muscle fibers, contain an active euchromatic nucleus with nucleolus, and in the sarcoplasm the common organelles, of which the number of mitochondria seem to be higher, present are also myofilament specific organelles and lysosomes. They also contain a higher percentage of glycogen, present in the sarcoplasma, in the form of isolated granules, aggregated and relatively frequent lipid vacuoles (1).

If to these structural aspects, one adds also the intense enzymatic activities for the aerobe and anaerobe oxidative enzymes, for the membrane ATP-ase it can be appreciated that in the leiomyoma, muscular activity is present and active at values higher than in the normal ungestated myometrial fiber.

Through the histochemic test, it was possible to observe the oxidative aerobic mitochondrial activity through the reaction for diaphorase (NADH2-cytochrome-C-reductase) compared to the glycolytic anaerobic cytosilic activity for lactatdehydrogenase.

These enzymes' modifications, showed based on the aforementioned observations, that the glycolytic metabolism is dependent on the predominant tumor component (leiomyoma or fibromyoma), on the location (submucosal, intramural,subserosal) , on the relation of aerobic or anaerobic enzyme or of the membrane and on the relation of the cellular structures inside the myoma (myocytes, connective cells, vessels).

The type of tumor that is most enzymatically active is represented by leiomyofibroma, in which the uterine muscle fibers in predominant numbers, are very intensely reactive for both glycolytic enzymes researched. In these myocytes it

seems that the mitochondrial enzyme activates as much as the cytosolic one does (lactatdehydrogenase) therefore showing that in the muscle cells in a pathologic situation it activates the cytochemistry, the organelles as much as the cytoplasm.

In this way, tumorous muscle hyperplasia triggers through its development enzymatic activities which generate the necessary energy for its development and function (7).

If this very intense metabolic activity characterizes the intramyometrial myoma, the other two locations have more moderate metabolic activity in the sense that the leiomyomatic tumor, the submucosal and the subserosal trigger slightly lower enzymatic activities which appear rarely intense in some muscle fibers.

This could also be understood through others and through the myometrial abundant vascularization with vessels which are mostly part of the uterine muscle fibers through the absence of adventitia from their structure and this was even more apparent that the arterioles, arteries and veins have tunics that are very endothelial and muscular enzymatically active, as much for the oxidative enzymes, but most importantly for ATP-ase, localized on the cellular membrane. Through this, there are permanent active bidirectional exchanges between the blood and matrix of the surrounding connective tissue or even stronger between the blood and the uterine muscle fibers.

The intracellular topography of the mitochondria in the uterine smooth muscle fibers, indicate the same as did Popescu L.M. (8) in the smooth muscle fibers from the intestine a predominance of the sub sarcolemma, number which maintains itself high but decreasing through myofilaments in the sarcoplasmic axis and the least amount at the nucleus' poles. In all of these mitochondrial locations to role to provide energy in the form of ATP, necessary to all uterine muscle fiber functions, of contraction, of mitosis.

The increased activity of the membrane enzyme, respectively ATP-ase pH9,4 is well linked and present near the cytoplasmic surface of the sarcolemma, of the high number of mitochondria, which through the available ATP provides a well-functioning ionic pump of Ca2+and of Na+/K+, favouring the good and active bilateral exchanges between the myocyte and the extracellular matrix.

In these very lively structural and metabolic conditions at the level of the uterine myoma, it can be assumed that there exists a slightly higher activity of contraction, a higher synthesizing process, more dense inter-fascicular and inter-fibril relations in the form of dense areas supported also by a well-represented cholinergic innervation.

This raises the question of the purpose of these functional stimulations at the level of the uterine leiomyoma. Those of synthesis could better accepted since they are linked to the hyperestrogeny present in these tumors, which leads to uterine muscle hyperplasia, which necessitates new contributions of enzymatic and structural molecules. But the slightly increased contraction- what is the purpose? Normally, the strength and speed of the smooth muscular contraction is smaller than those of other types of muscles, but the slightly modified structural appearance brings it closer to that of the constant in the gestational uterus (the growth in length and girth of the uterine muscle fibers).

Even if a clear functional explanation cannot be suggested, it still remains a sure fact that the myocytes become more active, promoting its functions, one of which is the contractile force (3).

The stimuli which produce and maintain the process are mostly hormonal, estrogen and progesterone being directly involved

through their increased values in fibromas, just as the increased number of receptors for these, also proven by other recent histochemical (5) and immunohistochemical (4), (9) studies.

Connective inter-fibril tissue very poorly represented, but well quantitatively and structurally organized intrafascicularlly separate among them myocytic cells or cellular groups and appears organizationally and metabolically close to normal.

Therefore connective cells, fibroblasts, with positive enzymatic activities, with different degrees of metabolism from cell to cell, confirm their well known functional cycle having normal fibrologenetic function and synthesis of macromolecules from the extracellular matrix component ensuring the presence of collagen, elastin, glycosaminoglycans, proteoglycans, etc.

The other types of connective cells appear just as enzymatically active as do the blood vessels, each with its well defined role in their respective tissue.

Unlike the leiomyofibroma, the pathologic aspect in which the connective tissue is developed, respectively in the fibroleiomyoma, the structural and enzymatic modifications are much more pronounced and greatly diversified, just as is observed in research, the alterations in the cellular organization as well as the matrix organization of the connective tissue appear quite severe, with many varied dystrophies, similar to those described in the pathology (6).

Conclusion

Activity was investigated by histozymatic means NADH2-cytochrome -C-reductase, the enzyme oxidation - reduction aerobic glycolitic activity compared with the activity of lactatdehydrogenaze, aerobic glycolytic enzyme, through fragments of leiomyofibroma and uterine fibroleiomyoma.

Activities of the two enzymes appear characteristic for each one on its own and in comparison, but also in relation to the two forms of benign uterine tumors or to the different structures that goes into them.

Generally both diaphorase and lactatdehydrogenase develop an intense activity in uterine fibroleiomyoma and leiomyofibroma slightly lower in the second type of tumor.

The aerobic enzyme activity of NADH2-cytochrome-C reductase remains superior to lectatdehydrogenase and with very high intensity in uterine leiomyofibroma smooth muscle fibers and in connective tissue – vascular adjacent.

Diaphorasis reaction occurs equally in all leiomyofibromal structures and very different in intensity in those of the fibroleiomyomal especially muscle fibers dispersed by the connective solid.

The enzymatic appearance "in situ" of the aerobic glycolitic enzyme looks therefore more even and more uniform in leiomyofibroma and very differentiated in fibroleiomyoma. Anaerobic lycolitic enzyme, lactathydrogenase, presents itself approximately equal in intensity and cellular localization in both tumor types.

Thus mitochondrial localization of diaphorasis shows that these cellular organelles are highly active and very intense in all leiomyofibromal myocytes but with unequal functionality in these muscle fibers in the uterine fibroleiomyoma.

By comparison, the anaerobic glycolytic enzyme with its location of activity in the cellular cytosol seems to be more stable, without changing in relation to the two types of tumor.

Among the uterine structures of the uterine leiomyofibroma, of the leiomyofibromas and fibroleiomyomas

respectively, at the biggest disadvantage is the metabolism of the smooth muscle fiber in fibroma, the connective fibroblast cell maintains its functional enzyme cycle linked to the fibrilogenetic process.

Macrophages are present, with a general intense activity, but with many very intense cells in the uterine leiomyofibroma.

Very enzymatically active are the blood vessels of different sizes and types that have a common metabolic denominator at the endothelial level, which always appears highly reactive.

In arterioles and arteries, the middle tumor appears with intense reaction slightly uneven in myocytes, and in fibroblastic adventitia connective cells displays uneven reactions, generally medium or intense.

Blood vessels are intensely reactive to the ATPase membrane, enzyme activity is present in all vascular tunics: the endothelium of capillaries, arterioles, arteries, venules and in myocytes, and in adventitious fibroid vessels of medium and large sizes.

A rich cholinergic innervation intensely positive in the form of isolated or grouped together smooth muscle fibers from fibroleiomyoma and leiomyofibroma lies over them. The richness of the cholinergic threads is also found in adventitia of arteries and veins.

The enzymatic appearance of the fibroleiomyoma appears very different from one area to another depending on pathological changes of the local connective tissue. Therefore areas with negative activities or very weak in the rare cells dispersed by the existing edema, or calcification of the extracellular matrix, giving the impression of an enzyme mosaic.

Thus, two metabolic areas can be distinguished, one narrow pericellular muscular and connective with an enzymatic appearance close to normal and another much more stretched with negative or weak reactions. And in this second area, there occur enzymatic inequalities both within themselves and between them, depending on the intensity of local histopathological alterations.

In fibroleiomyoma there is the need for therapeutic support (hormones, vitamins, mineral salts) as much as possible for structural completeness of the connective tissue in order to maintain a physiological status as close to that of the normal fibroblasts, macrophages, mast cells, all with an important role in the formation and function of the connective matrix.

References

1. Abbas, A. et al. : *Cellular and molecular immunology*. Philadelphia: Ed. Sanders, 1997.
2. Csapo, A.: *Structure and function of muscle*. New York: Ed. Bourne, Academic Press, 1960, pp. 229-262.
3. Cuello, A.: *Immunocytochemstry*. Ed. Wilb, 1993.
4. Felipe, I., Lake, B.: *Histochemistry in Pathology*. London: Ed. Churchill Livingstone, 1990.
5. Florescu, M., Simionescu, C., Nistor, E., Ardeleanu, C.: Histopathologye and immunohistochemical study of endometrial diffuse hyperplasia. In: *Arch. Balcan Medical Union*, 2002, 34,79-84.
6. Moraru, I., Pambuccian, G., Dragomir, C.: *Distrofiile in Anatomia Patologica*. Bucureşti: Ed Medicala*, vol I, 1980, 66-123*.
7. Kudson, A.: Anticogenes and Human cancer. In: *Proc.Nabl.Acad*, SUA, 2007.
8. Popescu, L. M. et col.: Imunologie Medicala, Ed. Bucuresti, 1998.
9. Vitale, G., Linciano, M., Salamanca, S., Ferrari, P.: Anomalous uterine bleeding in the perimenopause: fibromatosis, hyperplastic endomrtriopathy and GnRH- analogs. In: *Minerva Ginecol*, 2008, 46, 317-320.