MITOTIC ACTIVITY OF SMOOTH MUSCLE CELLS OF THE MYOMA: DOES HORMONAL STIMULATION HAVE AN EFFECT ON THE NUMBER OF MITOSES?

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Abstract – Myomas develop as a result of increased mitotic (proliferating) activity of smooth muscle cells. In this study we examined the pathohistological samples of 176 myomas and their endometria that were obtained after hysterectomy from patients in the proliferative (follicular) and secretory (luteal) phase of the menstrual cycle. We examined the mitotic activity of the myoma cells in both phases and established that the average number of mitoses in the proliferative phase was significantly larger compared to the secretory phase, and that in the proliferative phase of the cycle there exists a statistically significant convergent association of the number of mitoses in the endometrium and in myomas. The number of endometrial mitoses is significantly larger than in myomas in both phases of the cycle.

Key words: Mitotic activity, myoma, menstrual cycle

INTRODUCTION

Uterine myoma is a benign tumor which originates from the smooth muscle cells or blood vessels of the uterus and is one of the most frequent pathological conditions during the reproductive period of women (Hoffman et al., 1984). The initial neoplastic transformation of smooth muscle cells includes a somatic mutation. Although the initiator of the somatic mutation is still unclear, the mitogenic effects of estrogen and progesterone may increase the propagation of somatic mutations. Proliferation of myoma is the result of clonal expansion and probably includes the complex interaction of estrogen, estrogen receptors, progesterone and its receptors and local growth factors such as epidermal growth factor (EGF), insulin-like growth factor (IGF), platelet-derived growth factor (PDGF) (Hoffman et al., 1984; Nicolle et al., 1999).

The type and progression of the myoma is determined by the structure of the uterus and its level of vascularization. It was determined that estradiol increases the number of glandular mitoses in the endometrium. The binding of thymidine in the endometrium during the follicular phase of the cycle is evidently increased and it reaches its maximum with the largest frequency of mitoses on the 10th day of the cycle. After ovulation, mitotic activity is almost non-existent until the 19th day of the cycle (Ferenzy et al., 1979). It has not been proven that progesterone increases the number of mitoses in the endometrium (Segaloff et al., 1949; Tiltman, 1985).

Numerous authors have determined the number of mitotic figures of myoma in their studies. This number ranges from 2 to 15 mitotic figures on ten microscopic high powered fields (2-15 MF /10 HPF) (Kawaguchi et al., 1989; O’Connor...
An increased number of mitoses affects the growth of the myoma and leads to hyperplasia of the endometrium which causes hypermenorrhea (Prayson and Hart, 1992; Zaouldek and Norris, 1981). Myoma can cause abnormal hemorrhaging in different ways, but the specific mechanism is not obvious in every case.

The number of mitoses represents one of the most significant prognostic factors in the pathology of tumors. A large number of mitotic figures may represent a problem in differential diagnostics regarding the possibility of sarcoma if atypical cells are present. Therefore, the objective of this study was to determine the mitotic activity of myoma and endometrial cells from excised uteri during the proliferative (follicular) and secretory (luteal) phases of the menstrual cycle, and to determine whether there is a connection between the number of mitoses in the myoma and endometrium with regard to the phase of the menstrual cycle.

MATERIALS AND METHODS

For the purpose of this research, we used 176 samples of myoma (from which 88 samples were in the follicular and 88 in the luteal phase of the menstrual cycle) obtained during surgery following abdominal hysterectomy according to Aldridge, performed at the Department of Gynecology and Obstetrics of the Clinical Center “Kragujevac”. The specimens were dehydrated in graded ethanol (70-100%), cleared in xylol and embedded in paraffin. Sections 5 μm thick were cut on Leica SM 2000R and Leica Reinhart Austria microtomes and stained with Hematoxylin-Eosin (HE) and Periodic Acid-Schiff staining (PAS), according to standard protocol (Jones, 2002), as we previously reported (Vukovic et al., 2004; Vukovic et al., 2006). The number of mitoses on the analyzed serial sections of the myomas and endometria in both phases of the menstrual cycle was determined in ten fields with large microscopic magnification (x 400).

For statistical analysis of data, the program package SPSS (Statistical Package for the Social Sciences, version 13.0 for Windows) was used.

RESULTS

All analyzed samples of myomas showed the presence of extensive stroma composed of dense connective tissue – collagen fibers and the fibroblasts between them (Fig. 1). The parenchyme is composed of smooth muscle cells possessing a synthetic phenotype (within the cell nucleus we can notice euchromatin) (Fig. 2). A large number of cells have mitotic figures which indicates that they are in different stages of proliferation/mitoses (Fig. 3). In all of the analyzed samples we observed endometrial hyperplasia with a pronounced proliferating activity.

The comparison of the results of the number of mitoses in the myoma during the follicular and luteal phase of the cycle shows that the arithmetic averages of the number of mitoses in the myoma during the follicular phase of the cycle are significantly higher compared to the arithmetic averages of the number of mitoses in the myoma.

Figure 1. Parenchymal and stromal elements of myoma. Parenchyme is composed of smooth muscle cells possessing a synthetic phenotype (within the cell nucleus we can observe euchromatic regions). Stroma is composed of regular dense connective tissue – collagen fibers and the fibroblasts between them (histochemical staining of PAS, x 256)
during the luteal phase of the cycle. (Table 1, Graphic 1).

Comparison of the mitotic index of the endometrium of both phases suggests that this index in the follicular phase is significantly higher compared to the mitotic index of the endometrium in the luteal phase of the cycle. The difference was tested with the Wilcoxon-Mann-Whitney test (rank-sum test) and the significance was on the level of p<0.05 (p = 0.009).

Also, in the follicular phase of the cycle, the number of mitoses in the endometrium is significantly higher compared to the number of mitoses in the myoma. The difference was tested using the Wilcoxon matched pairs rank test and significance was found at the level of p<0.05 (p = 0.007).

The average number of endometrial mitoses in the luteal phase of the cycle is significantly higher compared to this number in the myoma during the same phase of the cycle (Table 2).

The results of the examination of the correlation between the number of mitoses in the endometrium and myoma of the same uterus in both the luteal and follicular phases of the cycle have shown that there is a statistically significant convergent association of the number of mitoses in the endometrium and myoma and p<0.05 (r = 0.680, p = 0.015). In the luteal phase, there is no statistically significant convergent association p>0.05 (r = 0.265, p = 0.406).

DISCUSSION

The results of this study have shown that in the first phase of the menstrual cycle there is increased mitotic activity of the endometrium and myoma as well as a high degree of correlation between the number of mitoses in the myoma and its adjacent endometrium. It was also established that there is a connection between the number of mitoses in the myoma and the phase of the menstrual cycle. The high proliferative activity of the cells observed in our samples is in accordance with the available literature (Matsuo et al., 2001).

### Table 1. The difference in arithmetic averages in the number of mitoses in the follicular and luteal phases

<table>
<thead>
<tr>
<th>Number of mitoses</th>
<th>Max</th>
<th>Min</th>
<th>X ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myomas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>11</td>
<td>3</td>
<td>7.58 ± 2.68</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>8</td>
<td>2</td>
<td>3.92 ± 1.62</td>
</tr>
</tbody>
</table>

$t = 4.057 : p = 0.000$
According to the available literature, the growth and development of myoma depends mainly upon the mitotic activity of the cells. The results of this study have shown that the mitotic activity of the myomas is determined by the phase of the menstrual cycle, which is in agreement with the results of other researches where this difference is related not only to the phase but also to age (Prayson and Hart, 1992).

This observation can be explained by the fact that estrogen is the main initiator of growth of the myoma. There are significant biochemical proofs which support the important role estrogen has in stimulation of myomal growth. Long-term application of Gonadotropin Releasing Hormone (GnRH) antagonists is associated with hyperestrogenemia and a decrease in myomal volume (Tiltman, 1985). According to the results of other authors, the concentrations of estradiol in the plasma do not differ significantly in patients with myoma compared to the control group (without myoma). It should be noted that the concentration of estradiol in the local circulation of the uterus is higher in women who are diagnosed with myoma (Savitskii, 1991). Evidence has been presented that the concentration of estradiol is significantly higher in the myoma compared to the myometrium, especially during the follicular phase of the menstrual cycle (Otubu et al., 1982). On the other hand, there are also data which show a significantly lower conversion of estradiol into estrone in the myoma compared to the myometrium. These differences in the conversion levels may be the result of the relative accumulation of estrogen in the myoma. The enzyme 17-hydroxy-dehydrogenase accelerates the conversion of estrogen into estradiol. The concentration of this enzyme is lower in the myoma which leads to higher accumulation of estradiol in the tumor tissue (Yamamoto et al., 1984).

The biological effect of the estrogen on the target tissues is achieved through estrogen receptors. Considerable evidence obtained from immunohistochemical research of myomal tissue has revealed significantly higher concentrations of estrogen receptors in the myoma as compared to the local myometrium (Otubu et al., 1982). The surface of the endometrial cavity of a normal uterus in the reproductive period is approximately 15 cm² and the surface of the endometrial cavity of a uterus when myoma is present can be over 200 cm². Hyperplasia of the endometrium, as a reflection of the local hyperestrogenic condition additionally increases the surface of the endometrium. There is a correlation between the amount of menstrual blood flow and the surface of the endometrium (Deglidish and Loewenthal, 1970).

The results of other studies have shown that there is a significant increase in the concentration of estrogen receptors in myomas compared to the myometrium of the same uterus. It is a paradox that during application of GnRH, the increase of estrogen receptors is even greater and followed by a decrease of estrogen in circulation. This increase in the number of estrogen receptors probably derives from the decrease in estrogen concentration so that all receptors would be available to estrogen which is
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otherwise reduced (down regulation) (Rein et al., 1990).

Although these observations point to the fact that the intramyomatose hormonal pattern is hyper-
estrogenic, there is no data that estrogen directly influences the growth of the myoma. The mitogenic
effects of the estrogen are probably mediated through other factors and their receptors. The presence of
several estrogen-regulated genes has been proven in uterine myomas. Expression of estrogen-regulated
genes is higher in the uterine myomas compared to the adjacent endometrium. There is abundant evidence
which points to the conclusion that the estrogenic stimulation of progesterone receptors (Adams et al.,
1993), epidermal growth factor (Yeh et al., 1991) and insulin-like growth factor (Chaves et al., 2004) are
included in myomal growth. Estrogen is also included in the regulation of the synthesis of the extracellular
matrix components of the myoma. It was determined that estrogen directly stimulates the production of type
I and III collagen (Stewart et al., 1994). Based on these observations, some research has pointed out that
estrogen hypersensitivity may have a key role in the pathogenesis of the myoma (Anderson et al., 1993).

Recent studies have shown the increase in the mRNA expression of progesterone receptors as well
as the increase of protein levels of progesterone receptors in myomal tissue, indicating that signalization intermediated with progesterone receptors is associated with myomal growth (Brandon et al., 1993). Other studies have also
determined that the production of mRNA of the epidermal growth factor in the myoma is increased
only during the follicular phase, leading to the conclusion that the progesterone is the main intermediate in the production of the epidermal growth factor which promotes myomal growth (Harrison-Woolruch et al., 1994). In vitro,
progesterone leads to up-regulation of Bcl-2 gene in the myomal cell culture. The increase in Bcl-2 levels
can inhibit the normal process of the apoptosis, increasing the growth potential of the myoma
(Matsuo et al., 2001).

The results of this study show that the average number of myomal mitoses in the follicular phase of
the cycle were 7.58/10 HPF which is significantly higher compared to the luteal phase when they were 3.92/10
HPF. Also, the results of this study have shown that the mitotic index of the endometrium during the follicular
phase of the menstrual cycle was significantly higher compared to the mitotic index of the endometrium in
the luteal phase. By comparing the number of mitoses in the endometrium and the number of mitoses in the
myoma in the same sample, we observed that a statistically significant correlation (p<0.05) in the follic-
ular phase of the menstrual cycle which points to the way that myoma can cause abnormal hemorrhaging.

Research has found significantly increased mitotic activity, based upon the number of mitoses under high microscopic magnification, in myomas obtained from women treated with medroxyprogesterone acetate compared to the untreated control group (Tiltman, 1985). Other
literature data revealed the link between the phases of the menstrual cycle and the number of myomal mitoses (Kawaguchi et al., 1989). The samples of myoma from 181 patients have shown that the number of mitoses was significantly
higher in the luteal phase (12/100 HPF) than in the follicular phase (3,8/100HPF) or during menstruation (8,3/100HPF). The authors concluded that the increase of mitotic activity during the luteal phase indicates that the growth of
the myoma is caused by progesterone (Kawaguchi et al., 1989).

The study of Tiltman (1985) found in 14 women that were operated on for uterine myoma,
1.6 mitoses on 100 fields under high magnification. Other researchers have reported 2 to 15 mitotic
figures in leiomyomas on 10 fields under high magnification (Zaouldek and Norris, 1981) and that the rate of myomal mitoses was 4.2-10.2/10 HPF during the luteal phase of the menstrual cycle (Prayson and Hart, 1992). The number of mitoses in some studies was 5-9/10 HPF and in the others
2-7/10 HPF (O’Connor and Novris, 1998; Perrone and Dehner, 1988).
CONCLUSION

The increase in mitotic activity of the endometrium and myoma is evident in the first phase of the menstrual cycle. There is a high degree of correlation between the number of mitoses in the myoma and its adjacent endometrium. It is possible that this is one of the ways that myoma can cause abnormal hemorrhage. This be significant for further consideration of conservative uterine myoma treatment.

Our results also suggest that there is a link between the number of mitoses in myoma and the endometrium and the phase of the menstrual cycle. We also conclude that the increase in the mitotic activity of both endometrium and myoma during the first phase of the menstrual cycle is caused by estrogen (the dominant hormone of the follicular phase of the menstrual cycle).

REFERENCES


МИТОТСКА АКТИВНОСТ ГЛАТКИХ МИШИЋНИХ ЋЕЛИЈА МИОМА: ДА ЛИ ХОРМОНСКА СТИМУЛАЦИЈА УТИЧЕ НА БРОЈ МИТОЗА?

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Сажетак – Миоми настају услед повећане митотске активности (пролиферације) глатких мишићних ћелија. У овом раду испитивани су патохистолошки узорци 176 миома и припадајућих ендометријума, добијених након хистеректомије у пролиферативној и у секреторној фази менструалног циклуса. Одређивање је митотска активност ћелија миома и ендометријума у обе фазе и утврђено је да је средња вредност броја митоза у пролиферативној фази значајно је већа у односу на секреторну фазу циклуса, као и да у пролиферативној фази циклуса постоји статистички значајна конвергенција асоцијација броја митоза у ендометријуму и у миомима. Број митоза у ендометријуму је статистички значајно већи у односу на број митоза у миоми у обе фазе менструалног циклуса.