Leiomyoma cellulare in postoperative material: clinical cases

Introduction: Leiomyoma in one of the most common benign endometrial cancers. Location of the myoma in the cervix and the area of the broad ligament of the uterus is rare. Leiomyoma cellulare (LC) occurs in about 5.0% of leiomyoma cases.

Aim of the research: To determine the occurrence of LC among 294 cases of myomas as well as myomas and uterine endometriosis, found in postoperative examinations.

Material and methods: Patients were qualified for the surgery based on a gynaecological examination, an ultrasonographic examination and a histological assessment of scrapings from the uterine cavity. The final diagnosis was given at the Centre of Pathomorphology of the Radom Specialist Hospital in Radom, based on a microscopic examination of postoperative material. Clinical analysis was performed in 16 cases, where LC was diagnosed during postoperative examination.

Results: Among the women operated on due to myomas (n = 179; 60.9%) as well as myomas and reproductive organ endometriosis (n = 115; 39.1%), LC tissue was diagnosed during histopathological examination in 9 (5.0%) and 7 (6.1%) cases, respectively. The average age of the LC patients was 45.6, whereas in patients with myomas as well as myomas and reproductive organ endometriosis it was 49.1. The average age of LC patients was significantly lower in relation to patients operated on due to reproductive organ myomas. In 87.5% of the cases, LC was found in myomas situated in the body of the uterus; and in the remaining 12.5% (2 cases), in the cervix and the area of the parametrium.

Conclusions: Leiomyoma cellulare in reproductive organs concerned mainly the body of the uterus (87.4%), and sporadically the cervix (6.3%) and the area of the broad ligament of the uterus (6.3%).

Streszczenie

Wstęp: Mięśniak gładkokomórkowy (leiomyoma) jest najczęstszym niezłośliwym nowotworem trzonu macicy. Do rzadkości należy umiejscowienie mięśniaków w szyjce macicy i okolicy wiązadła szerokiego macicy. Wśród mięśniaków gładkokomórkowych mięśniak bogatokomórkowy (leiomyoma cellulare – LC) stanowi około 5,0%.

Cel pracy: Ocena częstości występowania LC wśród 294 kobiet z mięśniakami oraz mięśniakami i endometriozą macicy, stwierdzonych w badaniach pooperacyjnych.

Materiał i metody: Do zabiegu chirurgicznego kwalifikowano pacjentki na podstawie wyników badania ginekologicznego i ultrasonograficznego oraz oceny histopatologicznej wyskrobin z jamy macicy. Ostateczne rozpoznanie ustalono w Zakładzie Patomorfologii Radomskiego Szpitala Specjalistycznego, opierając się na wyniku badania mikroskopowego materiału pooperacyjnego. Analizę kliniczną przeprowadzono w 16 przypadkach, w których w badaniach pooperacyjnych rozpozano LC.

Wyniki: Wśród kobiet operowanych z powodu mięśniaków (n = 179; 60.9%) oraz mięśniaków i endometriozy narządów płciowych (n = 115; 39.1%) utkanie bogatokomórkowe mięśniaka gładkokomórkowo-mięśniowego rozpoznano w badaniach histopatologicznych odpowiednio w 9 (5,0%) i 7 (6,1%) przypadkach; łącznie w 16 (5,4%) przypadkach. Średni wiek pacjentek z LC wyniósł 45.6 roku, natomiast pacjentek z mięśniakami oraz mięśniakami i endometriozą narządów płciowych 49.1 roku. Średni wiek kobiet z LC był znacznie niższy w porównaniu z pacjentkami operowalnymi z powodu mięśniaków narządów płciowych. W 87.5% przypadków LC stwierdzono w mięśniakach usytuowanych w trzonie macicy, a w pozostałych 12.5% (2 przypadki) – w szyjce macicy i okolicy przymacicznej.

Wnioski: Leiomyoma cellulare w narządach płciowych występuje głównie w trzonie macicy (87.4%), natomiast sporadycznie w szyjce macicy (6.3%) i okolicy więzadła szerokiego macicy (6.3%).
Introduction

Leiomyoma uteri is a non-epithelial, mesenchymal, benign monoclonal tumour formed by smooth muscle cells and a framework constructed of fibrous connective tissue [1, 2].

So far, however, it has not been clarified whether this is formed of uterine muscle cells or vascular smooth muscle cells [3].

These tumours develop as genetically abnormal clones of cells originating from one precursor cell where the original mutation took place. Multiple myomas do not belong to the same clone; each develops irrespectively of the others [4]. Their more common occurrence in first-degree relatives suggests a genetic background of the disease [5].

Cytogenetic analyses showed that chromosomal abnormalities are observed in around 40% of leiomyoma uteri cases. Chromosome aberrations are present in submucosal, subserosal and intramural myoma cells in 12%, 29% and 35%, respectively. The heterogeneity of the aberrations indicates a multiplicity of genetic mechanisms connected with the formation of leiomyomas. In women who are carriers of the mutation, there is a heightened risk of uterine sarcomas and they more frequently suffer from tumours in the postmenopausal period [5–7].

The hereditary occurrence of myomata uteri is also stressed. It was noted that there is a heightened risk of myoma occurrence in monozygotic twins compared to dizygotic twins. It was found in a population with a family history of myomas that the average number of myomas was twice as high as in the control group. A significant increase in the vascular endothelial growth factor A (VEGF-A), one of the seven subtypes of VEGF glycoprotein, was observed in myoma tissue after surgeries in the group with a family history of myomas [7, 8]. Inheritance, in an autosomal dominant manner, was found, among others, in cases of skin myoma co-morbidity with myomata uteri. The condition referred to as Reed syndrome or MCUL1 (multiple cutaneous and uterinae leiomyomata) is connected with a mutation of the gene encoding fumarase (a Krebs cycle enzyme, located on chromosome 1) [9–11].

In formation of the myomas, an important role is played by factors from the superfamily of transforming growth factors β (TGF-β), both as an inhibitor and a stimulator of the development of the tumours, as well as the regulator of their fibrosis. Transforming growth factor-β also affects the micro-environment of the myoma as an immunosuppressive agent and an agent stimulating angiogenesis [12, 13]. Active participation, especially that of TGF-β3, was found in the transformation of a normal uterine muscle cell into its tumorous phenotype. Overexpression of TGF-β3 plays an important role in forming the extracellular matrix (ECM) characteristic of myomata uteri [14, 15].

Proteins of the BMP-1/mTLD (bone morphogenic protein 1)/(mammalian Tolloid) subfamily are important regulators in production of the extracellular matrix and synthesis of an anti-angiogenic agent, perlecan, from a component of the basement membrane [1].

Hormone-dependence of myomata uteri differs from the hormone-dependence of the myometrium. This regards the number of oestrogen receptors, the number of progesterone receptors, the expression and activity of aromatase and the intensity of proliferation and apoptosis [2, 16].

Leiomyoma can occur in any part of the uterus. They occur rarely in female reproductive organs other than the uterus, in the broad ligament of the uterus and the ovary [17–20].

In 20–50% of women with leiomyoma uteri, there are disorders associated with their presence (excessive menstrual or non-menstrual bleeding, aches and pains, or impairment of fertility). The frequency and severity of these disorders are connected with the number of myomas, their sizes and locations. Based on histopathological examinations of the removed uteri, the presence of leiomyoma was noted in almost 77% of the women of the reproductive age [21].

Leiomyoma uteri is found in 40% of women who are above 50 years old [22]. Among the patients with myomas, two thirds of them have multiple myomas of different sizes [17].

In pregnant women, myomas occur in 1–2%. At the end of the pregnancy, the so-called red degeneration of a fibroid, caused by a haemorrhagic infarct in its central part, can be the cause of peritoneal symptoms [23]. Nowicka et al. described a case of leukaemoid reaction in a pregnant woman in the twentieth week of her fourth pregnancy, caused by inflammatory changes of a uterine myoma and a urinary tract infection. After cefuroxime and metronidazole therapy, in the patient’s case, the baby was delivered by Caesarean section in the 38th week of the pregnancy [24].

Cellular leiomyoma is one of the histological subtypes of leiomyoma which is rarely diagnosed (< 5.0%). This tumour is characterised, in the histological image, by large dense clusters of individual muscle cells, without atypia and with low mitotic activity, up to 5 mitotic figures/10 HPF, with a sparse component of connective tissue elements. It is also referred to as leiomyoma cellular (LC) [3, 18, 25].

Aim of the research

Authors’ own observations concerning the presence of LC in postoperative material from removed myomas and myomata with concurrent endometriosis are presented in the research.

Material and methods

In the seventeen-year-long period (1985–2001) at the Department of Gynaecology and Obstetrics of the
The following were noted in 15 preoperative histopathological examinations of scrapings:

- adenocarcinoma cylindrocellulare partim papillare G II – 1 case,
- hyperplasia endometrii glandularis cum adenodysplasia – 2 cases,
- mucosa corporis uteri in stadio secretionis partim hyperplastica – 2 cases,
- mucosa corporis uteri in stadio secretionis partim hyperplastica – 1 case,
- endometrium in stadio proliferationis – 2 cases,
- endometrium in stadio secretionis – 3 cases,
- endometrium atrophicum – 2 cases,
- endometrium in stadio praem menstruationis – 2 cases.

Clinical analysis was performed in 16 cases, where LC was diagnosed during postoperative examination.

**Results**

Among the women operated on due to myomas (n = 179) as well as myomas and uterine endometriosis (n = 115), LC tissue was diagnosed during histopathological examination in 9 (5.0%) and 7 (6.1%) cases, respectively; 16 (5.4%) cases in total.

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- endometrium in stadio proliferationis – 2 cases,
- endometrium in stadio secretionis – 3 cases,
- endometrium atrophicum – 2 cases,
- endometrium in stadio praem menstruationis – 2 cases.

The standard Student’s t-test with 292 degrees of freedom was used in statistical comparisons of the age of the LC patients in relation to the patients with myomas as well as myomas and uterine endometriosis. A borderline level of significance (the so-called p value) was obtained at around 0.1. The average age of the LC patients, 45.6 ±5.64 (the age range being 31–54), was significantly lower than the age of patients operated on due to myomas as well as myomas and uterine endometriosis, where it was 49.1 ±5.64 (the age range being 27–81).

Detailed data concerning the age of the patients in individual groups of operated patients is presented in Table 1. All of the women in the group of LC patients were multiparas (they gave birth from 2 to 5 times).

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In the group of patients with LC, diagnosed in postoperative histopathological examinations, the most common surgeries performed were intracavitary removal of the uterus, 8 (50%), with or without adnexa; and hysterectomy, 4 (25%) cases. The details are presented in Table 2.

In seven cases (45%) the surgeries were performed in acute moderate controlled haemodilution (AMCH) by drawing two units of the patient’s blood before the operation. The presence of a focus of haemorrhagic necrosis in cellular myomas was found in two (12.5%) cases.

In patient T.T., aged 31, on the fifth day after the third natural labour and two previous Caesarean sections, a vesico-uterine fistula was found. There was a need to perform peripartum removal of the body of the uterus without adnexa and to perform transfusion of 1500 ml of whole blood and 300 ml of RBC mass of a homologous blood type. The presence of intramural LC, partly with haemorrhagic necrosis, was stated in the histopathological examination of the removed body of the uterus. Leiomyomas as well as LC were stated in the body of the uterus (examination no. 317597/88). The presence of partial haemorrhagic necrosis in the LC, in the removed body of the uterus, was also noted in patient S.L., aged 54. In one case, in patient W.H., aged 42, an LC borderline case was stated (leiomyoma cellular malignant/casus limitans/ intramurale corporis uteri). A surgical and gynaecolog-
logical operation (cholecystectomy and hysterectomy with adnexa) was performed in patient K.K., aged 48. Myomas and endometriosis of the body of the uterus, and the presence of a myoma in the area of the left parametrium, determined in a histopathological examination to be leiomyoma angiogenes cellulare oedematosum, were found in the case of patient M.Z. (47), who underwent an emergency operation due to peritonitis (around 1000 ml of foul-smelling pus was found in the peritoneal cavity).

Leiomyoma cellulare in the cervix was found in patient G.T., aged 42, operated on due to endometriosis of the body of the uterus. In the case of patient O.I., aged 50, operated on due to a malignant tumour of the body of the uterus (ca. corporis uteri gr. IIB G2), the presence of LC and endometriosis was found in the body of the removed uterus, apart from the malignant tumour of the endometrium (Histopathological examination: body of the uterus (examination no. 561684/99) – Adenocarcinoma cylindrocellulare par tim papillare endometriotides G. II, partim adeno squamosum cum infiltratione superfitalis myometrii. Leiomyoma cellulare et endometriosis corporis uteri. cervix (examination no. 561685/99) – Infiltratio carcinomatosa canalis cervicalis et colli uteri).

The co-existence of LC with endometriosis of the body of the uterus was stated in 7 (45%) of the 16 cases with LC.

The presence of multiple LC in the examined group was stated in 8 (50%) of the cases; including 3 cases with endometriosis of the body of the uterus.

The weight of the biggest uterus with LC, that of patient F.M., aged 51, was 2050 g, and its dimensions were 19.0 cm × 18.5 cm × 14.0 cm.

Pathological results in the endometrium were found in 7 (45%) cases, in postoperative histopathological examinations.

**Discussion**

Numerous subtypes of leiomyoma, with LC among others, were isolated histopathologically [3]. Cell-rich tissue of leiomyoma was found in histopathological examinations in 16 (5.4%) cases among the women operated on due to myomas as well as myomas and endometriosis (n = 294).

A similar percentage (4.3%) of LC patients among women operated on due to myomas was found by Banaczek et al. [26]. According to various authors, cytogenetic abnormalities are found in about 40–50% of uterine myoma tissues [7, 27].

Chromosome aberrations found among the uterine myomas were classified into six cytogenetic subgroups. Deletions on chromosome 7 and translocations involving chromosomes 7, 12 and 14 are the most frequent mutation changes in the genome of myoma cells [7, 28, 29].

Translocation in chromosomes 12 and 14 occurs in around 20% of chromosomal damage [7]. Zeng et al. found a significant decrease in the expression of suppressor genes, which may be one of the causal links in uterine myoma growth [30].

In the research of Sung et al., the discovery of an increase in the expression of gene p53 in atypical cellular myoma tissues was significant. The product of this gene, protein p53, has a stimulating effect on the process of cell apoptosis [31].

Among the 16 cases of LC, one (6.3%) borderline case of a cellular myoma was found.

Research on the expression of p16 protein, which is an inhibitor of cyclin-dependent kinase 2A which is a tumour suppressor protein coded by the CDKN2A gene, was significant in recent times. An interrelation between the expression of p16 and the percentage of atypical cells in the structure of the myoma has been proven. The significant role of this protein in the development of myoma and Carcinosarcoma is well documented [32, 33].

Interesting observations have been noted concerning mutations in the NBS1 gene which codes the protein nibrin, which takes part in the repair of double strand breaks in DNA. These damages result in chromosomal aberrations and rearrangements of chromosomes lead-
ing to cellular death or to a tumorous transformation. Chromosomal rearrangements in uterine leiomyoma concern chromosomes 1, 2, 6, 7, 12, and 14 [34–36].

Czapczak et al. found that in a group of uterine leiomyoma patients, carriers of the R215W mutation in exon 6 of the NBS1 gene were three more common than in the general population. These authors are of the opinion that it cannot be ruled out that mutations of the NBS1 gene, especially R215W, increase the risk of development of uterine leiomyoma of a certain type [37].

Lately, attention is drawn to the role of TSC (tuberous sclerosis complex) genes in the development of malignant and benign tumours. Loss of function of these genes, manifesting in a decreased level of the product of the TSC gene, the protein tuberin, was noted in over 50% of the cases of uterine leiomyoma. This suggests a genetic background of this disease [38–40].

Uterine leiomyoma can also occur as part of hereditary cancer diseases such as hereditary leiomyomatosis and renal cell carcinoma (HLRCC) and Alport syndrome characterised by a defect in the COL4A5 and COL4A6 genes [41].

In the group of our patients with myomas and endometriosis of the body of the uterus, in patient W.A., aged 55, cancer of the right kidney was diagnosed in preoperative examinations. Eight months after urological operative treatment (kidney removal), a gynaecological surgery was performed (removal of the uterus with adnexa – 09.10.1992).

Cytogenetic abnormalities in myomas correlate with the size of the tumour and the location. Uterine leiomyoma, among which chromosomal aberrations are found, are usually larger and a larger percentage of them are submucosal [42, 43].

Based on macroscopic as well as detailed assessment of individual cases, it can be stated that the weight of removed myomas and uteri among women with LC was larger in comparison to that of the cases with leiomyoma. Similar results, after detailed analysis of these parameters on numerous material (99 women with diagnosted LC and 198 with leiomyoma uteri), were obtained by Taran et al. [44]. Lobel et al. stated in genetic research that larger uterine myoma more often contains cells with an abnormal karyotype. In turn, Guan et al. found that tumours with LC were smaller in diameter than leiomysarcoma (LMS) tumours [6, 45].

A significant role is played by growth factors, factors regulating the angiogenesis process and inhibitors of apoptosis in the processes connected with inducing proliferation of uterine muscle tissue to an abnormal form [48].

Vascular endothelial growth factor (VEGF) plays an essential role in transformation processes of normal uterine muscle into myomas, inducing and regulating angiogenesis in normal tissue and tumours [49–52]. According to the opinions of certain researchers, it is a potent mitogen for cells of the vascular endothelium which stimulates angiogenesis, and is probably the strongest growth factor which has so far been identified in myomas. VEGF-A influences the release of nitric oxide and prostaclin, triggers a cascade of coagulation and fibrinolysis in spiral arterioles, and by stimulating endothelial cells to synthesise tissue plasminogen activator activates an extravascular coagulation cascade. High concentration of tissue plasminogen activator in the endometrium, abnormal contractility of the uterine muscle and spiral arteries in the basal layer of the endometrium, thrombosis and necrosis of large venous vessels within the endometrium may cause extensive uterine bleeding in women with uterine myomas [50, 53, 54].

The proliferative potential of uterine myomas is also induced by other growth factors, namely, insulin-like growth factor (IGF), platelet-derived growth factor (PDGF), and epithelial growth factor (EGF) [55].

The role of p53 protein, the product of the p53 gene and an anti-apoptotic protein of the Bcl-2/Bax family is important in the process of apoptosis. A significant increase was found of the expression of the Bcl-2 gene in an abnormal myometrium, remaining in correlation with the induction of progesterone receptors [56]. Blocking the progesterone receptor by a selective modulator of this receptor – CDB4124 – reduced the number of cellular proliferation markers and the concentration of the Bcl-2 protein. It is significant in this case that CDB4124 does not block the progesterone receptors in normal tissue of the uterine muscle [57–59].

Ulpristal acetate, introduced lately into therapy, creates new opportunities for pharmacological treatment of uterine myomas.

Ovarian steroid hormones (17\-estradiol (E2) and progesterone (P4)) play an important role in the pathogenesis of uterine myomas. It is assumed that E2 is the primary element which stimulates the growth potential of myomas. Numerous authors are of the opinion that progesterone (P4) may be the primary factor inducing an abnormal growth of the endometrium. Ishikawa et al. found that there is a correlation between the phase of the menstrual cycle and the cellular proliferative potential of the myometrium. The expression of the Ki-67 protein, connected with cellular proliferation, coded by the MKI67 gene which is an indicator of the mitotic index of dividing cells, increased in the luteal phase and during pregnancy [60].

A state of increased mitotic activity and over-expression of progesterone receptors was observed in patients with uterine myomas. A regulatory function of progesterone in relation to certain proteins taking part in cellular proliferation processes (the anti-apoptotic protein Bcl-2 and the proliferating cell nuclear antigen, PCNA) was proven in research on cell lines [61]. A significant increase of the proliferative potential of uter-
ine myoma cells in relation to normal myometrium cells was found using assessment of PCNA [62].

Guan et al. noted a similar average age, 45.3 years old, as in our group of LC patients under study [45]. Banaczk et al. also noted a lower average age of LC patients (44.5 years old) than that of women operated on due to myomas (51.3 years old). However, that difference was not found to be significant [26].

Areas of necrosis in leiomyoma cellular can be stated especially at the end of pregnancy and are referred to as red degeneration of a fibroid caused by haemorrhagic infarction in its central part [3, 23]. Leiomyoma cellular with a focus of necrosis in the body of the uterus was found (in histopathological examination after peripartum removal of the body of the uterus) in patient T.T., aged 31, after the third natural birth with a vesico-uterine fistula complication.

A case of cellular myoma in the broad ligament of the uterus, similar to that noted in our material, was presented as a case study by Chmaj-Wierczowska et al. [18].

No description could be found in professional literature of leiomyoma angiogenes cellulare in the area of the broad ligament of the uterus. There is no description of LC in the cervix either, in available literature.

It can be concluded from a study of literature that diagnosis of LC takes place only after a histopathological microscopic examination of postoperative material.

In differentiating LC from sarcoma stromale and leiomyosarcoma, immunohistochemical examinations are used. The usefulness of marking smooth muscle actin, desmin, h-caldesmon, CD10 and CD44v3 in these examinations is stressed [26, 46, 47]. Taran et al. found that a simultaneous presence of endometriosis was found less frequently in women with cellular leiomyoma uteri who underwent surgery than in the control group of women with typical leiomyoma uteri. These authors are of the opinion that LC patients have a distinct clinical phenotype compared to patients with typical leiomyoma uteri and have certain characteristics common with leiomyosarcoma [44].

Lately, Guan et al. have found a lower level of Ki-67 and expression of PCNA in cancers with LC than in leiomyosarcoma (LMS) [45].

There are no reports in literature on the subject of malignant transformation of LC-type cancers. Mi- chalska et al., based on a study of literature, presented the opinion that LC is not a form of transition into leiomyosarcoma (LMS) [63].

Guan et al., among 78 LC patients, have not found cases of cancerous transformation in 41 of them during long-term observation. However, they advise clinical supervision of LC patients after surgical treatment [45].

The fact that cases have been noted of transformation in the direction of malign cancers of other rare histological subtypes of leiomyoma-type cancers, e.g., lipoleiomyoma, also needs to be taken into consideration in these deliberations [64].

In the USA, around 40% of transabdominal and 17% of vaginal hysterectomies are performed due to uterine myomas [65].

The authors of this study preferred various ways of intrafascial removal of the uterus, including own modifications, in surgical treatment of benign uterine cancers [66]. Some of the authors performed myomectomy in 50% of the cases, in surgical treatment of LC patients [26].

In cases where LC has been diagnosed without accompanying changes of malign nature in the reproductive organs, the patients do not require adjuvant treatment after the performed surgeries [26, 45].

Attempts are also made to use gene therapy in treatment of uterine myomas. Promising results have been obtained in research on animal models with the use of gene therapy to inactivate the oestrogen receptor, which leads to apoptosis of myoma cells and modulation of the proliferation of the myoma’s extracellular matrix [17, 67].

An interdisciplinary cooperation of doctors of various specialisations and scientific workers of various fields of basic science is essential in studies concerning the complicated disease of the reproductive organs, that is leiomyoma cellare.

Conclusions

Leiomyoma cellare in reproductive organs concerned mainly the body of the uterus (87.4%), and sporadically the cervix (6.3%) and the area of the broad ligament of the uterus (6.3%).

References


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