

P R A C E O R Y G I N A L N E

ginekologia

Expression of VEGF isoforms and their receptors in uterine myomas

Ekspresja izoform VEGF i ich receptorów w mięśniakach macicy kobiet

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Abstract

Objectives: The aim of the study was to determine the expression of VEGF (vascular endothelial growth factor) isoforms and their receptors in uterine myomas.

Material and methods: The study included 40 women with myomas of reproductive age and 40 perimenopausal women (the study group). Myometrial samples (the control group) were taken from 10 women undergoing hysterectomy for ovarian tumors and 10 older women undergoing hysterectomy for uterine prolapse.

Results: A significantly increased expression of VEGF-A has been found in myomas, both small and large, in the younger women, which may be a sign of increased angiogenesis and intensive tumor growth. In perimenopausal women, the increase of VEGF expression was observed only in the endothelium and vascular smooth muscle.

Conclusion: An important conclusion of this study is that angiogenesis is independent of myoma size, which may suggest intensive tumor growth and the related increased angiogenesis. High expression of VEGF-A and VEGF-R1 receptors in large myomas can probably cause malignant transformation and more extensive growth, regardless of patient age.

Key words: **VEGF / myoma / uterus / woman /**

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Streszczenie

Cel pracy: Celem pracy było próba określenia ekspresji izoform VEGF (ang. vascular endothelial growth factor) i ich receptorów w mięśniakach macicy.

Materiał i Metody: Badania przeprowadzono na 40 chorych z mięśniakami w wieku rozrodczym i 40 chorych z mięśniakami w okresie okołomenopauzalnym. Grupę kontrolną stanowiły próby pobrane od 10 kobiet poddanych histerektomii z powodu guzów jajnika i 10 starszych kobiet poddanych histerektomii po wypadnięciu macicy.

Wyniki: Stwierdzono statystycznie wyższą ekspresję VEGF-A w komórkach mięśniaków małych i dużych u kobiet młodych. Może to skazywać na zwiększoną angiogenezę, a co za tym idzie wzmożony wzrost guza. U kobiet w wieku okołomenopauzalnym wzrost ekspresji VEGF stwierdzono jedynie w śródbłonku i mięśniówce naczyń.

Wniosek: Ważnym podsumowaniem tej pracy, jest stwierdzenie, że angiogeneza jest niezależna od wielkości mięśniaka. Można zatem wnioskować, że wzmożony wzrost guza związany jest ze zwiększoną angiogenezą. Wysoka ekspresja VEGF-A i receptora VEGF-R1 w mięśniakach dużych, może być powiązana ze wzrostem żłobliwości komórek i ich intensywnym rozwojem niezależnie od wieku kobiety.

Słowa kluczowe: **VEGF / mięśniak / macica / kobieta /**

Introduction

Uterine myoma is the most frequent mild tumor of female reproductive organs, originating from the myometrium. The notion 'fibroid', based on the microscopic image, is the least precise but most often used in the English language literature, both scientific and popular. The name 'leiomyoma', referring to the levicellular myoma, most accurately indicates the histological origin of the tumor [1-3].

Various reports published in the United States point to myoma as the most frequent indication for hysterectomy, a condition contributing significantly to the growing costs of treatment and remarkably deteriorating patient quality of life [1, 3]. The prevalence of myomas in women >35 years of age is estimated at 20-50% of the Caucasian female population [1, 4], and up to 80% of the Afro-American female population [3, 5, 6].

Numerous risk factors promoting the development of uterine myomas have been recognized, including early menstruation, age, obesity, oral contraception, and others. However, these cannot be evaluated individually as in many cases only their co-occurrence may lead to myometrial pathology.

Formation of new blood vessels is stimulated by the environmental factors, oncogenes, and cytokines [7, 8]. Angiogenesis is precisely regulated by the system of balanced pro- and anti-angiogenic factors. Numerous biologically active substances have been identified to play a role in angiogenesis, among those the vascular endothelial growth factor (VEGF). The family of VEGF proteins includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and the placental growth factor (PIGF) [9, 10].

VEGF receptors are membrane glycoproteins made of three functional elements: the extracellular domain, the transmembrane, and the intracellular one, showing tyrosine kinase activity [11-13]. The family of VEGF membrane receptors includes VEGF-R1, VEGF-R2, VEGF-R3, and sVEGF-R1 [10, 12].

The family of VEGF proteins stimulates proliferation and migration of the endothelial cells and their organization into the tubular structures, as well as determines the permeability of vessels. Among others, this family includes VEGF-A, VEGF-B,

VEGF-C and VEGF-D. The expression of VEGF isoforms has been confirmed in many cells, tissues, and organs.

The aim of the study was to establish whether there are any differences in the quantity and distribution of VEGF isoforms in myomas of different size, taken from females of various ages, as compared to normal tissue. Another objective of our study was to show whether there exist any differences in the quantity and location of VEGF isoforms, as well as their receptors in the obtained samples.

The role of VEGF and its receptors in cancer has been already established. However, their role in uterine myomas remains to be fully elucidated [14-16]. To the best of our knowledge, our study has been one of the first and very few papers which deal with angiogenesis in uterine myomas in women of different ages.

Material and Methods

Patients

The study group included 40 women with myomas of reproductive age (<45 years, FSH<30 mIU/ml; samples collected during the follicular phase of the menstrual cycle) and 40 perimenopausal patients with myomas (45-55 years, FSH>30 mIU/ml). 20 women undergoing hysterectomy (10 for ovarian tumors and 10 older women for uterine prolapse) constituted the control group.

The inclusion criteria were as follows: myoma detected on USG, eligibility for hysterectomy, and informed consent.

The exclusion criteria were as follows: therapy with any drugs, including hormonal drugs, for at least 3 months before enrollment to the study, neoplastic disease, endometrial hypertrophy, metabolic and systemic disturbances, nicotine use. In our study, we used only material from uteruses with one large myoma or one large and a few small myomas.

The study population was further subdivided.

Group 1 was denoted as the 'control group of reproductive age women': myometrium of young women undergoing hysterectomy for reasons other than uterine leiomyomas (n=10).

Group 2 was denoted as 'small myomas of reproductive age women': leiomyomas of <3cm in diameter (n=20).

Group 3 was denoted as 'large myomas of reproductive age women': leiomyomas of >5cm in diameter (n=20).

Group 4 was denoted as the 'control group of perimenopausal women': myometrium of perimenopausal women undergoing hysterectomy for reasons other than uterine leiomyomas (n=10).

Group 5 was denoted as 'small myomas of perimenopausal women': leiomyomas of <3cm in diameter (n=20).

Group 6 was denoted as 'large myomas of perimenopausal women': leiomyomas of >5 cm in diameter (n=20).

Histology

Tissue samples were fixed in buffered formalin, dehydrated, cleared, and embedded in paraffin.

Immunohistochemical studies

Paraffin sections (5 µm) were dewaxed and rehydrated. The sections were treated with 10 mM citrate buffer, pH 6 (30 min. at 95°C), or Tris-EDTA pH 9 (45 min. at 95°C) in water bath for antigen retrieval, then treated with 1.5% (v/v) H₂O₂ in methanol for 20 min. for quenching of endogenous peroxidase activity. Nonspecific binding was reduced by incubation in 1% BSA for 60 min. Next, the slides were incubated with rabbit anti-VEGF-A (Santa Cruz Biotech. Inc., USA), goat anti-VEGF-C (Abcam, Cambridge, USA), rabbit anti-VEGF-D (Abcam, Cambridge, USA), rabbit anti-VEGF-R1 (Santa Cruz Biotech. Inc., USA), rabbit anti-VEGF-R2 (Abcam, Cambridge, USA), rabbit anti-VEGF-R3 (Abcam, Cambridge, USA) polyclonal antibodies or mouse anti-VEGF-B (Santa Cruz Biotech. Inc., USA) monoclonal antibodies for 22h at 4°C. After washing, the sections were incubated with biotinylated goat anti-rabbit, horse anti-mouse, and rabbit anti-goat immunoglobulins (Vector Laboratories Inc., Burlingame, USA), respectively and next with avidin-biotinylated peroxidase complex (Vector).

The bound antibodies were visualized with DAB and H₂O₂ according to supplier's instructions (Vector). Finally, the tissues were stained with Gill's hematoxylin, dehydrated, and coverslipped. Negative controls were performed by substituting the primary antibodies with rabbit IgG, mouse IgG and goat IgG, respectively.

Optical density analysis

In each positively stained cell, the intensity of staining was measured as the optical density of the reaction product, with the image analyze program NIS-AR. For each analyzed area, average optical density was calculated. Three sections for every studied protein and every patient were analyzed. Ten fields were examined in each section. Finally, the arithmetic mean and standard deviation were calculated.

Statistical analysis

Normal distribution of the data was confirmed by the Shapiro-Wilk test. Measured values were analyzed with the one-way ANOVA. Turkey test was used for post-hoc analysis. The differences were considered as statistically significant at the significance level of p<0.05. Results are presented as mean±standard deviation. The statistical analysis was performed with Statistica 10 (StatSoft, USA).

Results

VEGF-A

Membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells. Moreover, among the controls, the staining reaction has been observed in gland cells and myometrial myocytes.

In the control group comprising reproductive age women, 70% of the cells were stained, while in perimenopausal controls only 50% of the cells were stained. In case of both myoma groups, in younger women the immunohistochemical reaction was observed in 100% of the cells, whereas among the perimenopausal women the percentage of the stained cells was 80% for small and large myomas. It was only the control group of perimenopausal women which showed the vascular muscular coat reaction higher than that in the myometrium. In the remaining cases, the immunohistochemical reaction was comparable to that of vascular myocytes.

In both, reproductive age and perimenopausal women, the expression of the VEGF-A factor was statistically significantly higher than in controls, regardless of myoma size. In the group of small myomas in reproductive age females, the level of the expression of the VEGF-A isoform was 135% of the control values, while the level observed in the older females was 120% of the control values. The highest growth in the expression of this factor was observed in large myomas in females from both age groups. In younger women, the level reached 140% of the control values and in the perimenopausal group – 125% of the control values. None of the age groups showed any differences between large and small myomas (Figure 1, Figure 2).

VEGF-A expression in perimenopausal controls was the same as in reproductive age controls. The same evaluation of the study groups showed the expression of this isoform in small myomas, lower in the older women, scoring 85% of the values for reproductive age women. In the group with large myomas, the level of VEGF-A expression in perimenopausal women reached 90% of the values recorded in younger women.

VEGF-B

The immunohistochemical reaction was observed only in the endothelial membrane of blood vessels. The reproductive age women showed statistically significant growth of VEGF-B expression in the group of small myomas, reaching about 140% of the control level. The expression of VEGF-B in large myomas was comparable to controls, while the group of small myomas showed statistically significant decrease, down to the level of 75% of the control values. In perimenopausal females, a decreased expression of VEGF-B was observed, evident particularly in the group of large myomas, which showed two-fold reduction, as compared to controls. The results were statistically significant when compared to both, controls and the group of small myomas, in both cases reaching practically a two-fold decrease in the expression of the discussed isoform (Figure 3, Figure 4).

The expression of VEGF-B in perimenopausal controls was clearly higher than in the reproductive age controls, reaching 160% of the values revealed in younger women. Similar evaluation of the study groups showed the same expression of this isoform in small myomas in both age groups. In the group of large myomas, the level of VEGF-B expression in perimenopausal women reached about 75% of the values revealed in younger females.

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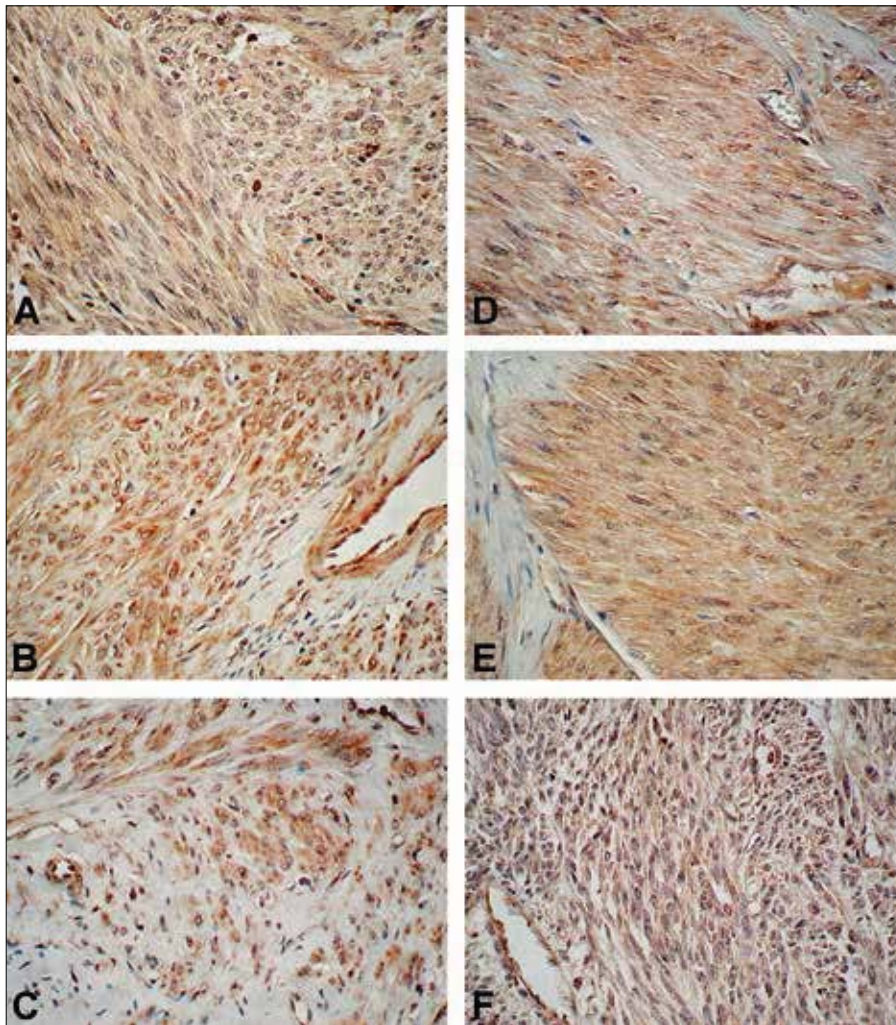


Figure 1. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with rabbit anti-VEGF-A polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

VEGF-C

The membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells. Moreover, the staining reaction has been observed in gland cells and myometrial myocytes.

In reproductive age women, the immunohistochemical reaction was shown by 50% of myocytes in the control group and proved to be much stronger than in the myometrium. All cells of small myomas showed the staining reaction and it was stronger in comparison to vascular myocytes. In large myomas, 70% of the cells were stained at the level comparable to the vascular myocytes. In perimenopausal women, the staining reaction was observed in 70% of the myometric cells in the control group and it was weaker in comparison to vascular muscular coat. The immunohistochemical reaction, comparable to vascular myocytes, was observed in all cells of small myomas and in 80% of the cells of large myomas.

The expression of VEGF-C in small myomas was higher than in controls, in both age groups. In reproductive age women, the expression of this isoform was twice as high as in controls, reaching 140% growth in older women. In large myomas in the reproductive age females, VEGF-C expression showed

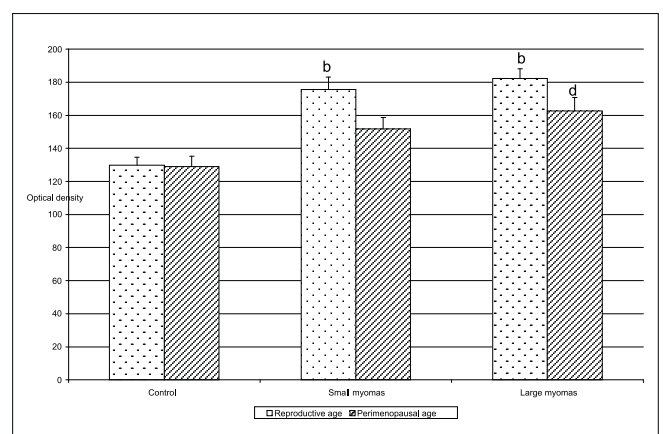


Figure 2. Optical density of the reaction product for VEGF-A, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between: b - the control group and any type of myomas for reproductive age women, d - the control group and any type of myomas for perimenopausal women.

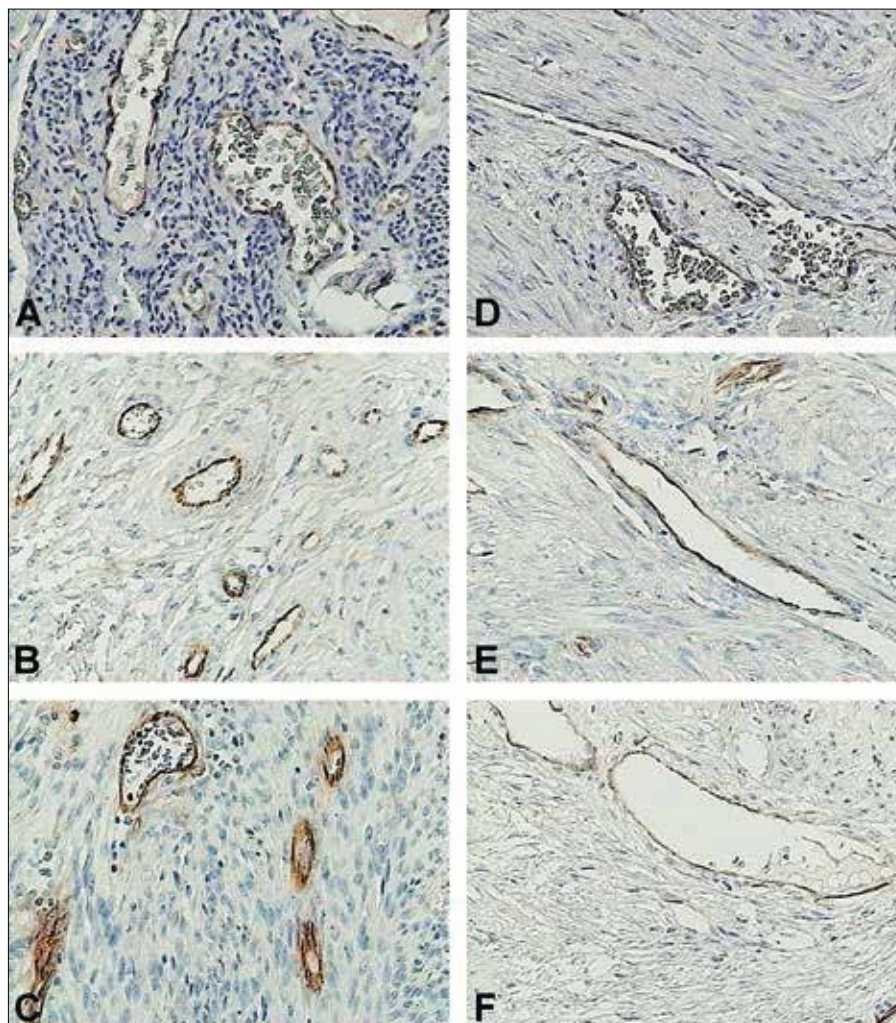


Figure 3. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with mouse anti-VEGF-B monoclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

statistically significant growth, reaching 170% of the control level, while in small myomas it was significantly lower, reaching 85% of the level recorded for that group. In perimenopausal women, VEGF-C expression in the group with large myomas was comparable to controls. However, the value was significantly lower as compared to small myomas, sharing <70% of the expression level observed in that group (Figure 5, Figure 6).

VEGF-C expression in perimenopausal controls was higher than in reproductive age controls, reaching 145% of the values revealed in younger women. Similar evaluation of the study groups showed that the expression of this isoform observed in small myomas was the same in both age groups. In the group with large myomas, VEGF-C expression in perimenopausal women was lower and reached about 80% of the values revealed in younger women.

VEGF-D

Similarly to VEGF-C, also in this case the membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells, as well as in gland cells and myometrial myocytes.

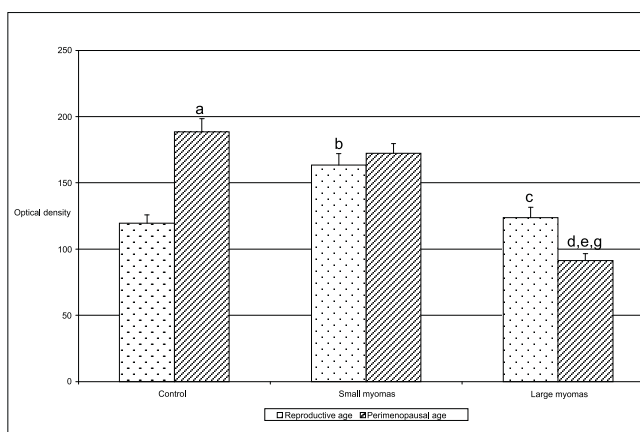


Figure 4. Optical density of the reaction product for VEGF-A, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between:
 a - control groups,
 b - the control group and any type of myomas for reproductive age women,
 c - small and large myomas in reproductive age women,
 d - the control group and any type of myomas in perimenopausal women,
 e - small and large myomas in perimenopausal women,
 g - large myomas in both age groups.

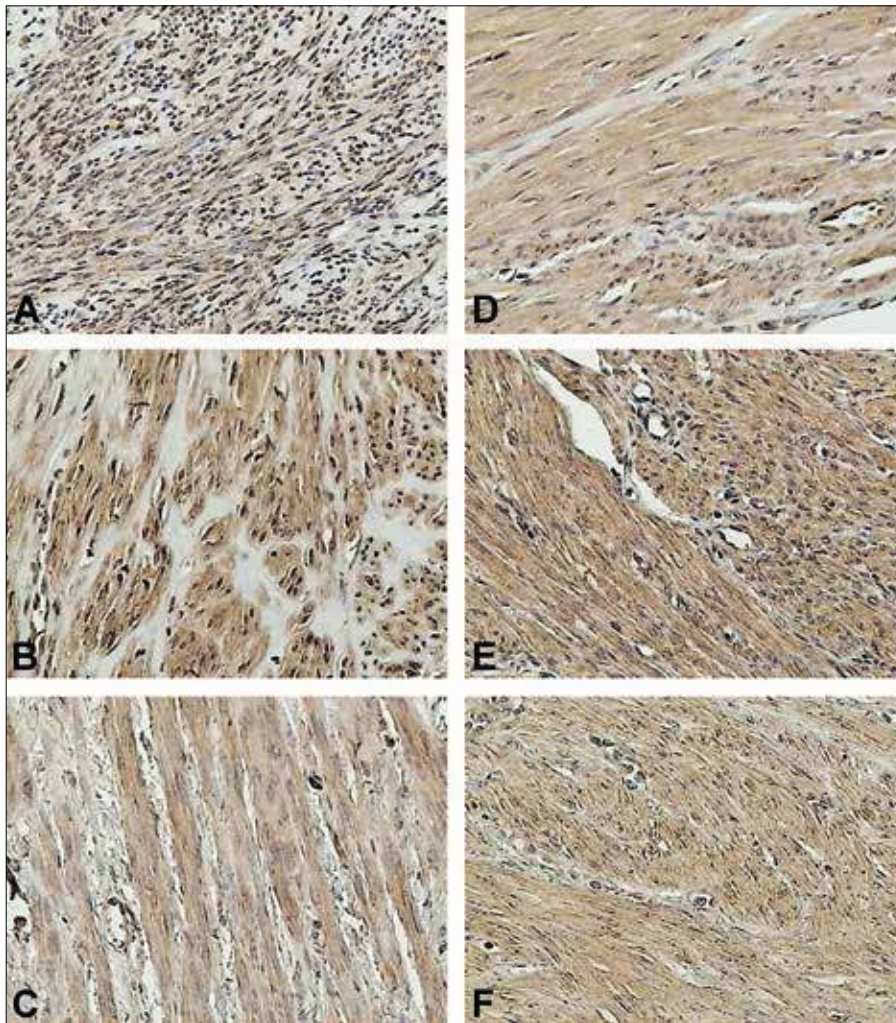
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Figure 5. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with goat anti-VEGF-C polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

The immunohistochemical reaction in the control groups was observed in 50% of the myocytes of the reproductive age women and in 70% of perimenopausal women. In case of large and small myomas in both age groups, the staining reaction was observed in 100% of the cells. The reaction intensity in the myometrium was comparable to myoma cells or myocytes in controls.

Evaluation of changes in VEGF-D showed a statistically significant growth of the expression of this factor only in the reproductive age women group. It was the case in both, small and large myomas, while a slightly higher reaction was observed in small myomas (155% of the control values). The level of VEGF-D expression in small and large myomas in perimenopausal females maintained a constant level and showed no statistically significant differences as compared to controls. Similarly, no differences were observed between the groups with different myoma sizes, both in reproductive age and older women (Figure 7, Figure 8).

VEGF-D expression in perimenopausal controls was clearly higher than that observed in reproductive age controls, reaching 170% of the values revealed in younger women. The same evaluation of the study groups showed a similar expression of this isoform observed in small myomas in both age groups.

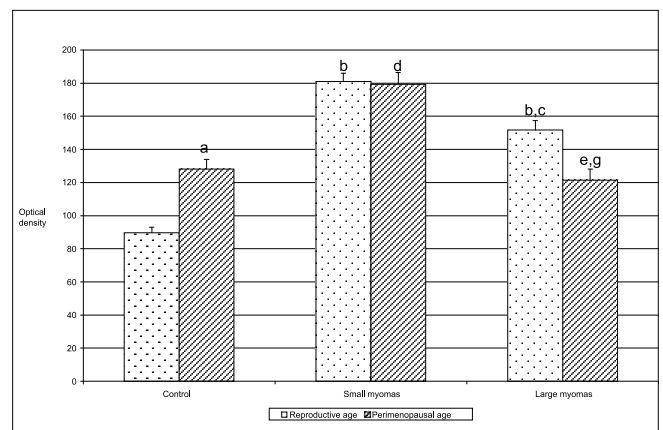


Figure 6. Optical density of the reaction product for VEGF-C, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between:

- a - control groups,
- b - the control group and any type of myomas for reproductive age women,
- c - small and large myomas in reproductive age women,
- d - the control group and any type of myomas in perimenopausal women,
- e - small and large myomas in perimenopausal women,
- g - large myomas in both age groups.

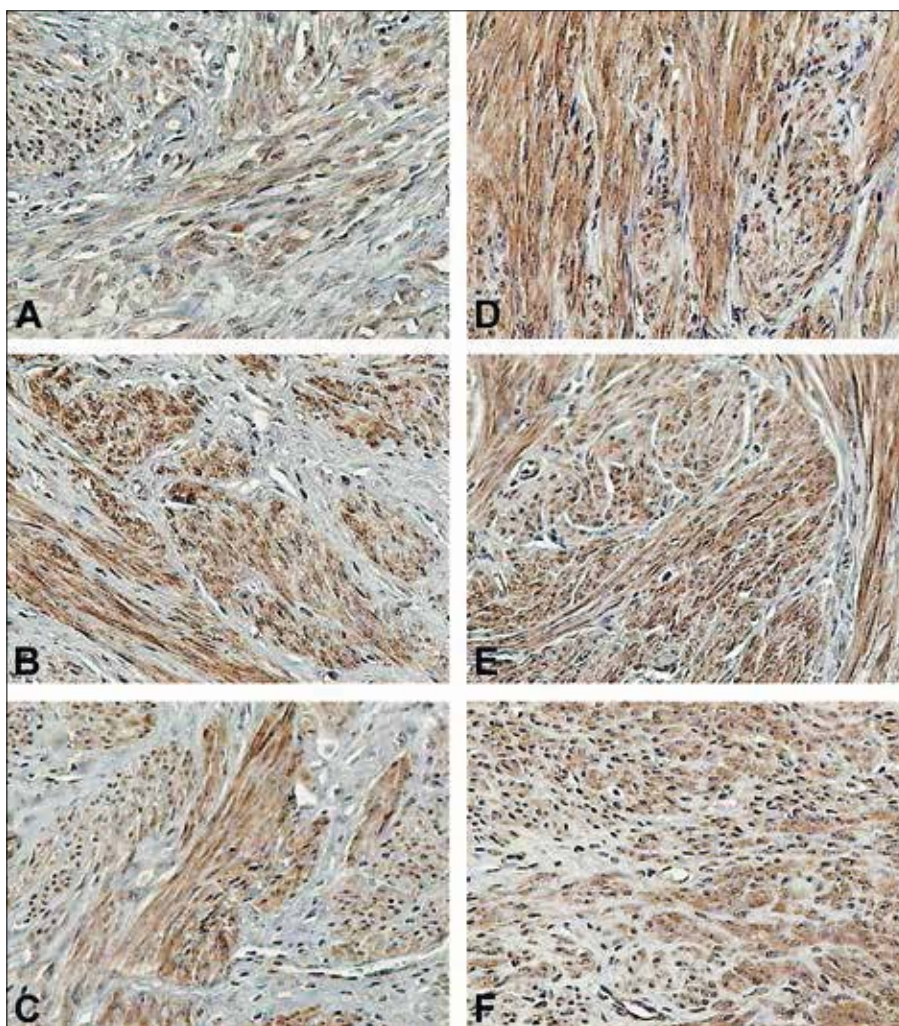


Figure 7. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with rabbit anti-VEGF-D polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

In the group with large myomas, the level of VEGF-D expression in perimenopausal women was higher and reached about 120% of the values revealed in younger females.

VEGF-R1

The membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells. Moreover, the staining reaction has been observed in gland cells and myometrial myocytes.

In the control group of reproductive age women, 70% of the cells were stained, with only 50% in perimenopausal females. In both myoma groups in younger women, the immunohistochemical reaction was observed in 100% of the cells. In perimenopausal females, the percentage of the stained cells was 80% for both, small and large myomas. It was only the perimenopausal control group which showed a vascular muscular coat reaction stronger than in the myometrium. In the remaining cases, the immunohistochemical reaction was comparable to the vascular myocytes.

The expression level of the R1 receptor was the highest in large myomas of women in both age groups. In reproductive age

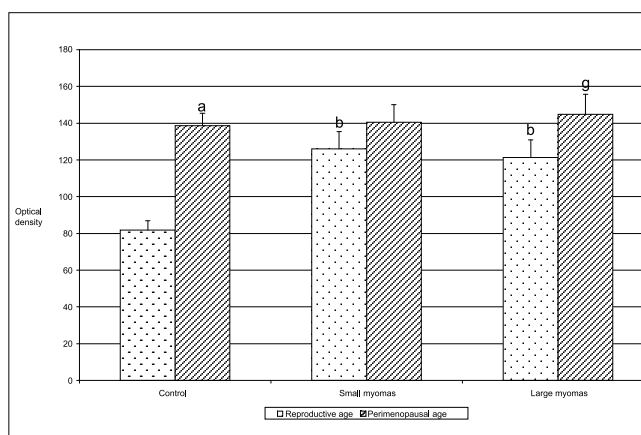


Figure 8. Optical density of the reaction product for VEGF-D, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between: a - control groups, b - the control group and any type of myomas for reproductive age women, g - large myomas in both age groups.

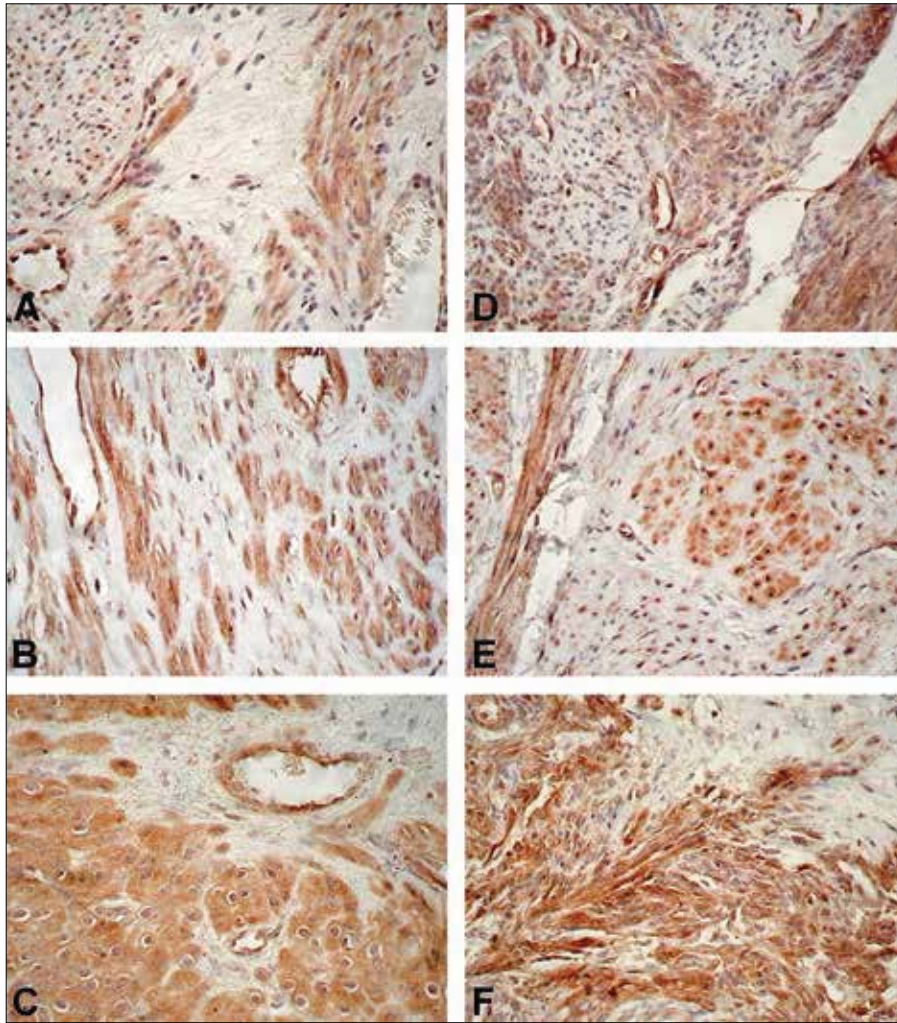
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Figure 9. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with rabbit anti-VEGF-R1 polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

females, the growth was statistically significant as compared to controls, in small (approx. 120%) and large (approx. 150%) myomas. A comparison of the expression in the test groups revealed the values in the group with large myomas to be higher than in the small myomas, reaching 125% of the level recorded for that group. The expression of the R1 receptor in small myomas of the perimenopausal women was comparable to controls, while a slight increase was observed in the group with large myomas. The result was statistically significant as compared to small myomas (Figure 9, Figure 10).

The expression of the receptor in perimenopausal controls was higher than in the reproductive age controls, reaching 125% of the values revealed in younger women. Similar evaluation of the study groups showed that the expression of this receptor, observed in small myomas, was the same in both age groups. In the group with large myomas, VEGF-R1 expression in perimenopausal women was similar (in both age groups), to the values revealed in the small myoma group.

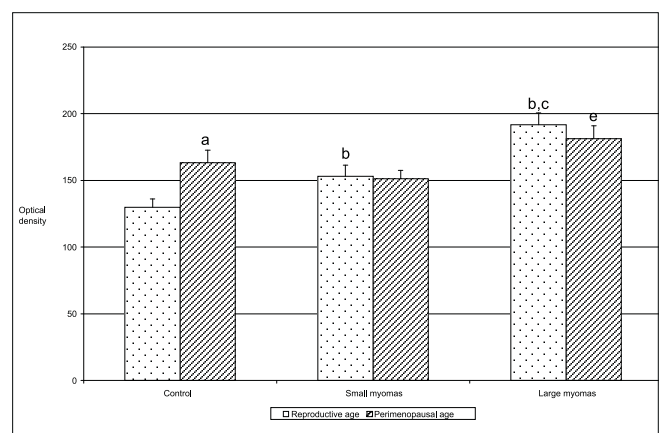


Figure 10. Optical density of the reaction product for VEGF-R1, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between:

- a - control groups,
- b - the control group and any type of myomas for reproductive age women,
- c - small and large myomas in reproductive age women,
- e - small and large myomas in perimenopausal women.

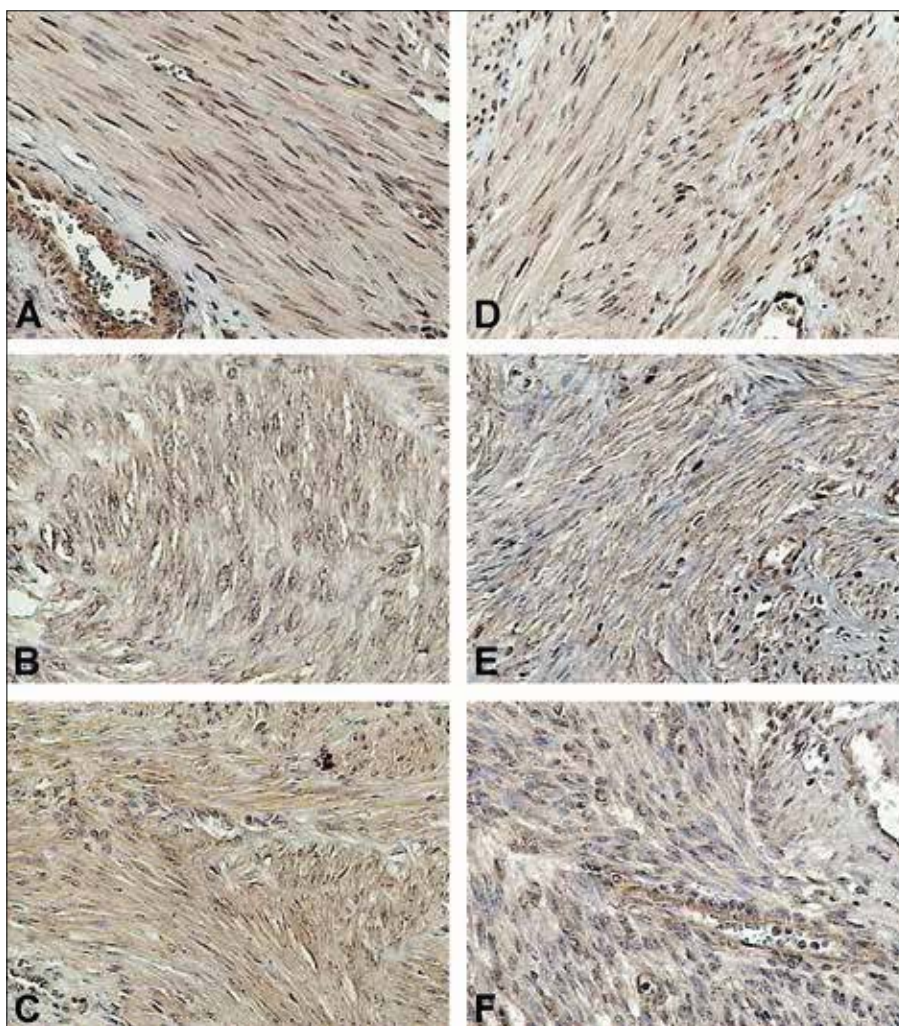


Figure 11. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with rabbit anti-VEGF-R2 polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

VEGF-R2

The membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells. Moreover, in the control groups, the staining reaction has been observed in gland cells and myometrial myocytes.

The immunohistochemical reaction, at a constant level of 30% of the cells, was observed in reproductive age women in both myoma groups and the control myometrium. The same results were observed in perimenopausal controls. In both control groups, the vascular myocyte reaction was stronger than in the muscular coat. In both, small and large myoma groups of perimenopausal women, the staining reaction was observed in 20% of the cells. In small myomas the reaction was stronger, while in large myomas it proved to be comparable to the reaction in vascular myocytes.

The expression level of the R2 receptor in the reproductive age women in both study groups showed no differences, as compared to controls. The perimenopausal group showed a statistically significant decrease in the expression of the R2 receptor in both, large and small myomas, reaching in both cases about 75% of the level observed in controls (Figure 11, Figure 12).

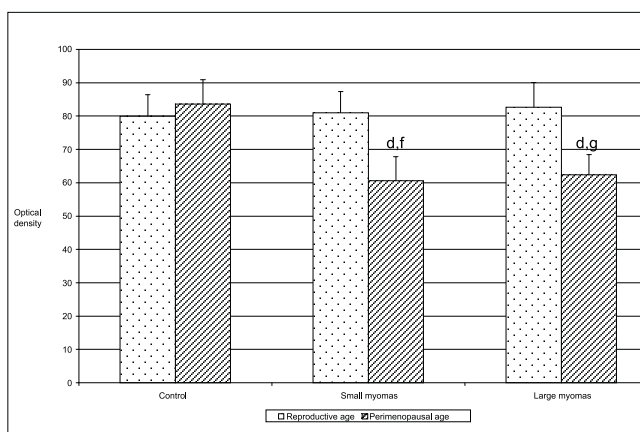


Figure 12. Optical density of the reaction product for VEGF-R2, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between: d - the control group and any type of myomas in perimenopausal age women, f - small myomas in both age groups, g - large myomas in both age groups.

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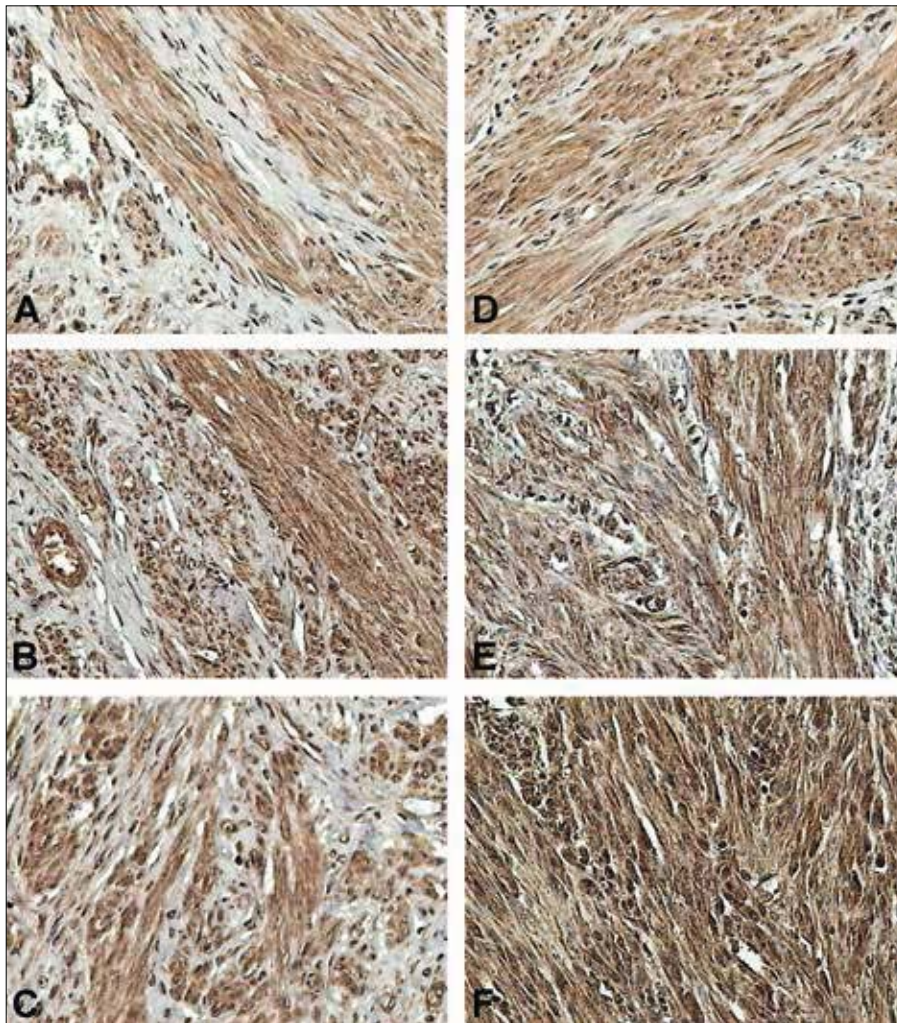


Figure 13. Immunohistochemical staining of uterine samples from reproductive (A)–(C) and perimenopausal age women (D)–(F) with rabbit anti-VEGF-R3 polyclonal antibodies. (A), (D) myometrium of control groups; (B), (E) small myomas; (C), (F) large myomas. Magnification 200x.

The expression of the R2 receptor in perimenopausal controls was the same as in the reproductive age controls. Similar evaluation of the study groups showed that the expression of this receptor, observed in small and large myomas, was the same in both age groups (lower in perimenopausal women). In both cases, the decrease reached about 75% of the values revealed in younger women.

VEGF-R3

The membrane and cytoplasmic reactions have been observed in the endothelial cells, the blood vessel muscular coat, and the myoma cells. Moreover, in the control groups, the staining reaction has been observed in gland cells and myometrial myocytes.

In the reproductive controls, the staining reaction was observed in 70% of the myocytes. The immunohistochemical reaction in perimenopausal controls was observed in 80% of the myocytes and was comparable to the blood vessel cells. In the first case, the vascular myocyte reaction was stronger than in the myometrium.

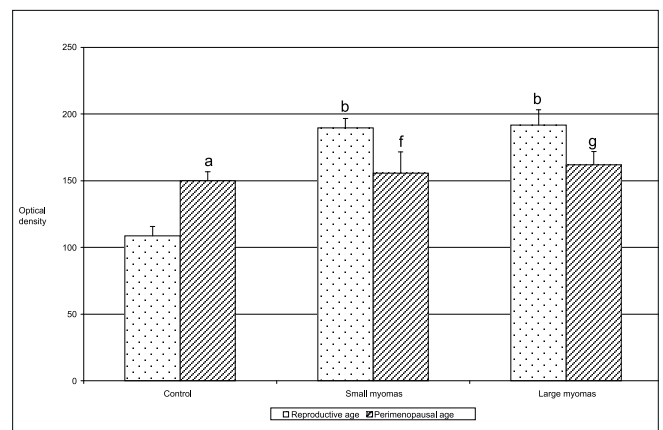


Figure 14. Optical density of the reaction product for VEGF-R3, depending on myoma size and patient age.

The letters indicate statistically significant changes at $p < 0.05$ between:

- a - control groups,
- b - the control group and any type of myomas for reproductive age women,
- f - small myomas in both age groups,
- g - large myomas in both age groups.

In small and large myomas of the reproductive age women, 100% of the cells were stained at the level comparable to that observed in the vascular myocytes. In both myoma groups of perimenopausal women, the reaction was also comparable to vascular myocytes, however it was observed only in 80% of the small myoma cells and only in 60% of the large myoma cells (Figure 13, Figure 14).

The growth in the expression of the R3 receptor was statistically significant in both study groups and reached about 175% of the control level in each of the cases. In perimenopausal women, the statistically significant growth of the expression was observed only in the group with large myomas. The expression of the R3 receptor in the group with small myomas, within the same age range, was comparable to the control values. No differences in the expression between the study groups were shown.

The expression of the R3 receptor in perimenopausal controls was higher than in the reproductive age controls, reaching about 140% of the values revealed for younger females. Similar evaluation of the study groups showed that the expression of this receptor, observed in small and large myomas, was the same in both age groups (lower in perimenopausal women). In both cases, the decrease reached about 80% of the values revealed in younger women.

Discussion

Our study showed a distinct growth of the VEGF-A expression in both, small and large myomas, which is consistent with the results obtained by Hague et al. [17]. It could indicate a high rate of tumor growth as manifested by intensified angiogenesis. Noteworthy, the expression of angiogenesis was independent of myoma size. Vascular density and the endothelial proliferation index remained unchanged throughout the menstrual cycle in the myometrium, the endometrium, and the leiomyoma. This was consistent with some earlier studies on the myometrium and the endometrium [18, 19]. Vascular density correlated with the proliferation index in the endometrium, the myometrium and the leiomyoma. Different results, which may have been the effect of a different methodology applied, were reported by Harison-Wolrych et al. [20]. The use of frozen tissue samples could have resulted in the measurement error. Nevertheless these authors did not exclude the role of VEGF-A in the angiogenesis within myomas.

Leiomyomas are thought to show poor vascularization. However, we proved indirectly that vascular density of leiomyomas is comparable to that observed in normal myometrium. We also demonstrated that vascular density of the myometrium in higher in a uterus with leiomyoma, as compared to controls. This could be due to excessive release of angiogenic stimuli by leiomyomas, stimulating angiogenesis in the surrounding myometrium. It is also likely that females showing extremely high vascular density of the myometrium or an elevated expression of VEGF-A or aFGF and bFGF may be predisposed to develop leiomyoma. This appears interesting when viewed in the light of a known family predispositions to leiomyoma [21, 22].

VEGF-B deficiency has no effect on angiogenesis in most organs [23], as much as VEGF-B deficiency does not influence the pathological angiogenesis in most of the evaluated organs, such as injured skin, hypoxic lungs, or ischemic retina.

Some results support the concept of VEGF-B function restricted to the 'survival' factor rather than the angiogenic one. This explains, and to a certain extent combines, the conflicting opinions about the 'angiogenic' character of VEGF-B. This means that acting as a survival factor, VEGF-B has also some minor role in inducing blood vessel growth [24, 25].

High level of VEGF expression observed in leiomyomas appears to be a possible target of therapeutic intervention, making use of antibodies specific for VEGF to treat myomas. Specific, monoclonal antibodies, capable of inhibiting VEGF, have become available [26,27]. In fact, it was shown that removal of VEGF signaling results in apoptosis of the vascular cells and subsidence of the neoplastic lesions. Surprisingly, the use of blocking, anti-VEGF antibodies revealed little side effects [28, 29].

In light of the above considerations, one must not forget about the receptors, the ligand for which is the vascular factor VEGF. One of those is VEGFR-1. The role of this receptor remains debatable. It is present in membranes of endothelial cells, monocytes, macrophages or neoplastic cells of solid tumors [30]. Extremely interesting observations were made during the experimental studies on tumor biology. For example, it was shown that stimulation of proliferation, migration and increase of the invasive potential of the tumor cells result from activation of VEGF-R1. Our studies pointed to the increased level of this receptor, particularly in large myomas, which may be an indication of enhanced proliferation and higher rate of the mass increase, as compared to small myomas.

Our study found no changes in the expression of the R2 receptor in any of the evaluated groups. It should be remembered that VEGF-R2 is the main receptor through which VEGF-A imposes its effect, i.e. the mitogenic effect, the angiogenic one and promoting permeability of blood vessels. VEGF-R1, on the other hand, shows both, the inhibitory and the stimulating effect toward angiogenesis. It appears, therefore that a possible increase in the myoma's mass is not associated with better vascularization. It is likely that upon the observed, higher level of VEGF-A, the blood vessels in the myoma show distinct growth of permeability, which may improve nutrition of the myoma, due to enhanced diffusion.

Based on our findings as well as the available literature, it seems safe to assume that VEGF-R1 and VEGF-R2 play different roles in the process of physiological and pathological angiogenesis. During early embryogenesis, the receptors perform in the opposite directions in order to achieve balance required for the proper level of angiogenesis. During the adult stage, VEGF-R2 seems to be restricted to the endothelial vascular cells, while VEGF-R1 has a role in both, the vascular cells of the endothelium and the macrophages. VEGF-R1 stimulates the inflammatory process, tumor growth and metastasis, at least partly, in the macrophage-dependent manner. Therefore, the inhibitors which block specifically VEGF-R1 or VEGF-R2 may prove helpful in controlling VEGF-VEGF-R signaling to treat the diseases with maximum effectiveness and minimal side effects. Nevertheless, the relation between the VEGF-R pathway and other signaling pathways should be extensively investigated to ensure full understanding of the molecular base controlling blood circulation in a human body.

Danuta Plewka et al. *Expression of VEGF isoforms and their receptors in uterine myomas.***Ethical statement**

The study was planned according to statement of Human and Animal Rights. Local Ethics Committee approved of the study (Ethical Statement no. KNW/0022/KB/139/09).

Conflict of interest

All authors declare that they have no conflict of interest. All authors read and approved the final manuscript.

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19. Hickey M, Pillai G, Higham JM, [et al.]. Changes in endometrial blood vessels in the endometrium of women with hormone replacement therapy-related irregular bleeding. *Hum Reprod.* 2003, 18, 1100-1106.
20. Harrison-Woolrych ML, Sharkey AM, Charnock-Jones DS, Smith SK. Localization and quantification of vascular endothelial growth factor messenger ribonucleic acid in human myometrium and leiomyomata. *J Clin Endocrinol Metab.* 1995, 80, 1853-1858.
21. Vikhlyeva EM, Khodzhaeva ZS, Fantschenko ND. Familial predisposition to uterine leiomyomas. *Int J Gynaecol Obstet.* 1995, 51, 127-131.
22. Sato F, Mori M, Nishi M, [et al.]. Familial aggregation of uterine myomas in Japanese women. *J Epidemiol.* 2002, 12, 249-253.
23. Li X, Lee C, Tang Z, Zhang F, [et al.]. VEGF B: a survival, or an angiogenic factor? *Cell Adh Migr.* 2009, 3, 322-327.
24. Zhang F, Tang Z, Hou X, [et al.]. VEGF-B is dispensable for blood vessel growth but critical for their survival, and VEGF-B targeting inhibits pathological angiogenesis. *Proc Natl Acad Sci USA.* 2009, 106, 6152-6157.
25. Rissanen TT, Markkanen JE, Gruchala M, [et al.]. VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. *Circ Res.* 2003, 92, 1098-1106.
26. Kim KJ, Li B, Winer J, [et al.]. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature.* 1993, 362, 841-844.
27. Fraser HM, Dickson SE, Lunn SF, [et al.]. Suppression of luteal angiogenesis in the primate after neutralization of vascular endothelial growth factor. *Endocrinology.* 2000, 141, 995-1000.
28. Benjamin LE, Golijanin D, Iltis A, [et al.]. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. *J Clin Invest.* 1999, 103, 159-165.
29. Vajkoczy P, Farhadi M, Gaumann A, [et al.]. Microtumor growth initiates angiogenic sprouting with simultaneous expression of VEGF, VEGF receptor-2, and angiopoietin-2. *J Clin Invest.* 2002, 109, 777-785.
30. Kowanez M, Ferrara N. Vascular endothelial growth factor signaling pathways: therapeutic perspective. *Clin Cancer Res.* 2006, 12, 5018-5022.

References

1. Cheng MH, Wang PH. Uterine myoma: a condition amenable to medical therapy? *Expert Opin Emerg Drugs.* 2008, 13, 119-133.
2. Blake RE. Leiomyomata uteri: hormonal and molecular determinants of growth. *J Nat Med Assoc.* 2007, 99, 1170-1184.
3. Parker WH. Etiology, symptomatology, and diagnosis of uterine myomas. *Fertil Steril.* 2007, 87, 725-736.
4. Bajekal N, Li TC. Fibroids, infertility and pregnancy wastage. *Hum Reprod Update.* 2000, 6, 614-620.
5. Wise LA, Palmer JR, Stewart EA, Rosenberg L. Age-specific incidence rates for self reported uterine leiomyomata in the Black Women's Health Study. *Obstet Gynecol.* 2005, 105, 563-568.
6. Marshall LM, Spiegelman D, Barbieri RL, [et al.]. Variation in the incidence of uterine leiomyoma among premenopausal women by age and race. *Obstet Gynecol.* 1997, 90, 967-973.
7. Fraisl P, Mazzone M, Schmidt T, Carmeliet P. Regulation of angiogenesis by oxygen and metabolism. *Dev Cell.* 2009, 16, 167-179.
8. Nyberg P, Xie L, Kalluri R. Endogenous inhibitors of angiogenesis. *Cancer Res.* 2005, 65, 3967-3979.
9. Ferrara N. Role of vascular endothelial growth factor in the regulation of angiogenesis. *Kidney Int.* 1999, 56, 794-814.
10. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev.* 2004, 25, 581-611.
11. Tammela T, Enholm B, Alitalo K, Paavonen K. The biology of vascular endothelial growth factors. *Cardiovasc Res.* 2005, 65, 550-563.
12. Karkkainen MJ, Petrova TV. Vascular endothelial growth factor receptors in the regulation of angiogenesis and lymphangiogenesis. *Oncogene.* 2000, 19, 5598-5605.
13. Rahimi N. Vascular endothelial growth factor receptors: molecular mechanisms of activation and therapeutic potentials. *Exp Eye Res.* 2006, 83, 1005-1016.
14. Salomon-Perzyńska M, Perzyński A, Rembielak-Stawecka B, [et al.]. VEGF-targeted therapy for the treatment of cervical cancer - literature review. *Ginekol Pol.* 2014, 85 (6), 461-465.
15. Dobrzycka B, Terlikowski SJ, Kwiatkowski M, [et al.]. Prognostic significance of VEGF and its receptors in endometrioid endometrial cancer. *Ginekol Pol.* 2010, 81 (6), 422-425.
16. McMahon G. VEGF Receptor Signaling in Tumor Angiogenesis. *The Oncologist.* 2000, 5 (1), 3-10.
17. Hague S, Zhang L, Oehler MK, [et al.]. Expression of the hypoxically regulated angiogenic factor adrenomedullin correlates with uterine leiomyoma vascular density. *Clin Cancer Res.* 2000, 6, 2808-2814.
18. Hickey M, Lau TM, Russell P, [et al.]. Microvascular density in conditions of endometrial atrophy. *Hum Reprod.* 1996, 11, 2009-2013.