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Tomasz Bereza[1], Janusz Skrzat[1], Wojciech Szczepański[2], Jerzy Mituś[1],
Krzysztof Tomaszewski[1], Paweł Depukat[1]

VASCULAR STRUCTURE OF OUTER MYOMETRIAL UTERINE LEIOMYOMATA — A PRELIMINARY SEM AND IMMUNOHISTOCHEMICAL STUDY

Abstract: Aim: The main goal of this study was assessment of vascular structure of uterine leiomyomata localized between outer myometrium and endometrium.
Materials and Methods: The study was carried out on thirty two human uteri collected upon autopsy. Vessels were injected with synthetic resin, next corroded and coated with gold, finally observed using scanning electron microscope. Next ten uteri were injected with acrylic emulsion and studies using immunohistochemical staining for von Willebrandt's factor.
Results: Vascular structure of outer myometrial leiomyomata was quite similar to those observed in the middle of muscular layer of uterus, characterized by relatively dense 'vascular capsule', consisted of flattened vein, arterioles and capillaries.
Conclusions: Structure of outer myometrial uterine leiomyomata was similar to those observed during growth within myometrium.

Key words: leiomyomata, myometrium, blood supply, SEM, microcorrosion.

INTRODUCTION

There is a whole list of different factors which may have an influence on uterine leiomyomata risk:

- age at menarche and menopausal status
- reproductive factors
- smoking (which paradoxally seem to decrease the risk of uterine leiomyomata)
- oral contraceptives
- intrauterine devices
- education/social class
- body weight
- infertility
- hypertension
- diabetes
- breast disease [1–5]

Uterine fibroids being the most common benign female tumors (of age 30–55) of the internal genital organs, develop within few locations which are: subserous, intramural, submucous, intracervical and finally intralaminar, when their growth proceeds towards the space between the layers of the broad ligament. These neoplasms are the first indication for surgery among females in the world, maybe apart from the cesarean section [6–10]. However epidemiological studies are rather scanty [11]. The structure of the intramural uterine leiomyomata has been presented in numerous injection studies [12–21] but so far the vascular structure of the leiomyomata growing within the borderline between the serosa and myometrium has not been a subject of an analysis.

Much of the uterine fibroids are asymptomatic, and their detection is a result of coincidental discovery (i.e. gynecological examination, usg, tec.). Other symptoms include: irregularities in uterine bleeding (i.e. menorrhagia), pelvic pain, dysmenorrheal, pressure symptoms, infertility and many others [3, 22].

MATERIAL AND METHODS

The material for this study consisted of 42 uteri obtained during the necropsies of the females aged between 34–48. The angioarchitecture of the tumours was studied using microcorrosion technique, scanning electron microscopy (SEM) [23] and immunohistochemistry, reaction for von Willebrandt's factor.

Thirty two uteri were obtained upon autopsy of women aged 25–56 years, deceased due to causes not related to disorders of the reproductive system. The material was collected 6–24 h after death. Each uterus together with ovaries and cervical portion of the vagina was removed in such a way that relatively long fragments of uterine and ovarian vessels (arteries and veins) were retained.

Immediately after removal, twenty-two uteri were perfused via the afferent arteries with prewarmed (37°C), heparinized saline (12.5 IU/ml heparin; Polfa, Poland, containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine; Polfa, Poland), until the fluid outflowing via the veins was completely transparent (~5 min). Next perfusion was continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma, Germany) in 0.1 mol/l cacodylate buffer, pH 7.4 supplemented with 0.2% lidocaine. Finally, the vascular system was injected with 60–80 ml of Mercox CL-2R resin (Vilene Comp. Ltd. Japan) containing 0.0625 mg/ml methyl acrylate polymerization initiator (Vilene Comp. Ltd., Japan) and the uteri were left in a warm water bath (56 °C) for several hours to allow polymerization and tempering of the resin.

When the polymerization was completed, the uterine tissues were macerated for 5–6 days by repeated baths in 10% potassium hydroxide at 37°C followed by washing with warm (50–55°C) running tap water. The obtained vascular casts were washed for the next 4–5 days in multiple changes of distilled water under mild vacuum conditions, cleaned in 5% trichloroacetic acid for 1–2 days, washed

again in distilled water for 2–3 days and freeze-dried in a lyophilizer (Liovag G2; Aqua Fina, Germany).

The freeze-dried casts were examined macroscopically, gently dissected [24] to expose the vasculature of myomata and stored in an exiccator containing phosphorus pentoxide until the microscopic examination. They were then mounted onto copper plates using colloidal silver and 'conductive bridges' and coated with gold. The casts were examined using a JEOL SEM 35-CF scanning electron microscope at 20–25 kV (Jeol, France).

The vascular beds of next ten uteri were perfused with saline and next injected with the solution of acrylic emulsion (Liquitex R, Binney and Smith, USA) [25]. The emulsion was successfully used in some previous studies [26, 27]. The specimens were collected mainly from large fibroids >3 cm in diameter. All tissue specimens were fixed in 10% formalin and embedded in paraffin wax. Tissue blocks were sectioned (4 μm) and section mounted on aminopropyltriethoxysilane (APES)-coated slides. The endothelial cell marker was factor VIII-related antigen (FVIII). Next tissue blocks were deparaffinised, hydrogen peroxidase (3%) in methanol was applied to tissue sections as an endogenous peroxidase block for 10 min. Protein blocking steps included the application of 10% normal rabbit serum prior to the application of the primary antibody (anti Human von Willebrandt factor, Dako, USA) in 20% fetal calf serum. After incubation with primary antibody, a biotynyled secondary antibody was applied followed by horseradish peroxidase-streptavidin conjugate, and visualization with the chromogen 3-amino 9-ethylchlorcarbazole (AEC, Zymed, USA) which identifies tissue antigens with a red-brown stain. Serial sections were immunostained using the endothelial cell marker. Negative controls used substitution of the primary antibody with the non-immune serum for the polyclonal factor VIII antibody. The study was approved by Ethical Committee of Jagiellonian University (KBET/121/8/2007).

RESULTS AND DISCUSSION

Sampson [17, 18] was probably the first who proposed the role of vascular disorders in the development of presented above symptoms associated with uterine fibroids. Blood supply of uterine leiomyomata was studied by Holmgren, Faulkner, Farrer-Brown et al., Walocha et al. [12–16, 19–21].

Uterine leiomyomata or fibroids are the most common solid, benign, smooth muscle cell tumours in women, affecting up to 70% over 30 years of age. They represent an important and significant public health problem because of associated symptoms (pelvic pain and pressure, uterine bleeding) and side-effects (infertility, loss of pregnancy). It is proved that 25% of women in the third to fourth decades of life seek care for leiomyoma-related symptoms. However the cause of leiomyomata still remains obscure. It is known that there are a number of risk and protective factors for and against fibroid growth related to oestradiol

and that there are increased numbers of sex steroid receptors in fibroids compared with myometrium. Human uterine leiomyomata express higher levels of peroxisome proliferator-activated receptor γ, retinoid X receptor α, and all-trans retinoic acid than myometrium [28]. Other factors that may be associated with fibroids etiology include peptide growth factors such as the insulin-like growth factor (IGF) family and epidermal growth factor (EGF), transforming growth factor beta 3 (TGFβ-3) as well as possible chromosomal and subsequent gene changes within these tumours [29-37]. The corrosion casting technique combined with scanning electron microscopy (SEM) is the best currently available method for morphological examination of both normal and pathological vascular networks [20, 38, 39]. SEM offers high resolution and quasi-three-dimensional image [23]. FVIII forms part of the von Willebrand factor complex, plays a part in the coagulation cascade and its expression is reduced in smaller, less mature blood vessels. The vascular staining for this factor is no different to the other vascular markers, i.e. CD 31, CD34. The present study has been undertaken to compare the results of studies of vascular network of human uterine fibroids located within outer myometrial layer using classical injection, using acrylic emulsion, SEM and immunohistochemistry.

The characteristic feature of larger leiomyomata was so called "vascular capsule" [40–46] (Fig. 1). In most of specimens we could observe an avascular fissure (Fig. 2) located between leiomyoma and myometrium. Veins of the "capsule" were present in the form of irregular flattenings. We observed also the presence of intra-

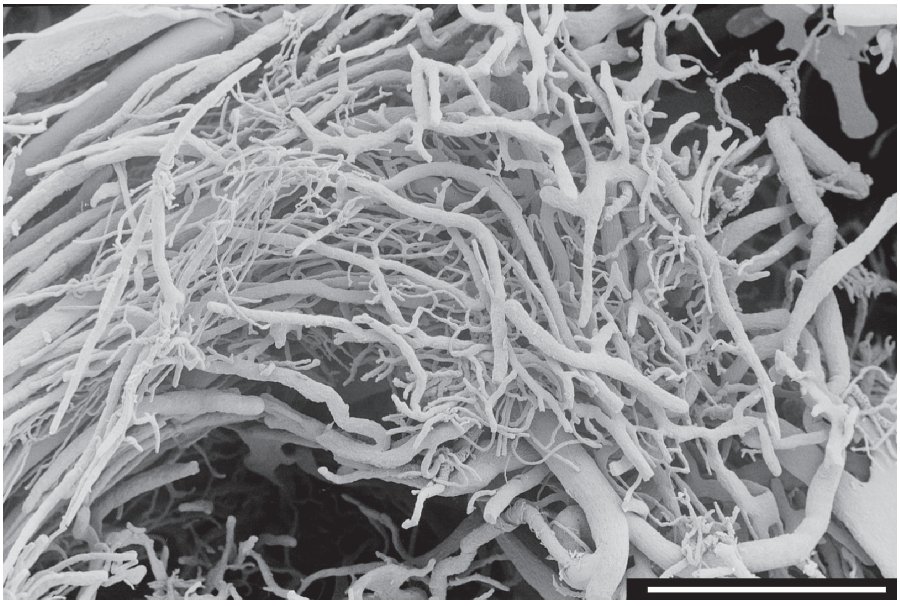

Fig. 1. Corrosion cast. SEM. Dense "vascular capsule of middle-sized leiomoma (~11–12 mm) growing at the border region between subserous and intramural region. Bar = 1000 μm.

parenchymal septa which commonly gave off the branches. Immunohistochemical staining proved the presence of small not injected vessels which penetrated the substance of tumour (Fig. 3).

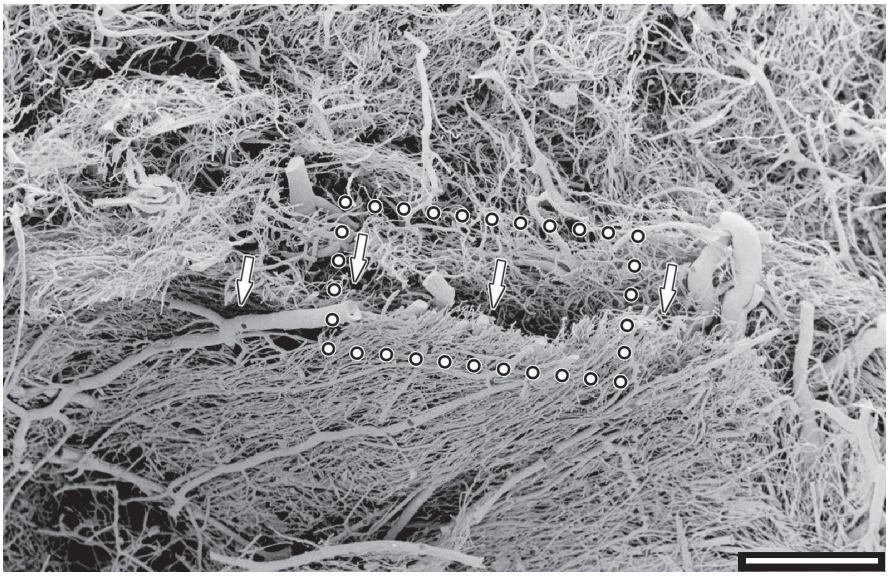

Fig. 2. Corrosion cast. SEM. Uterine corpus — coronal section. Avascular gap (arrows) located between the leiomyoma (downwards) and mometrium (upwards). Bar = 1000 μm.

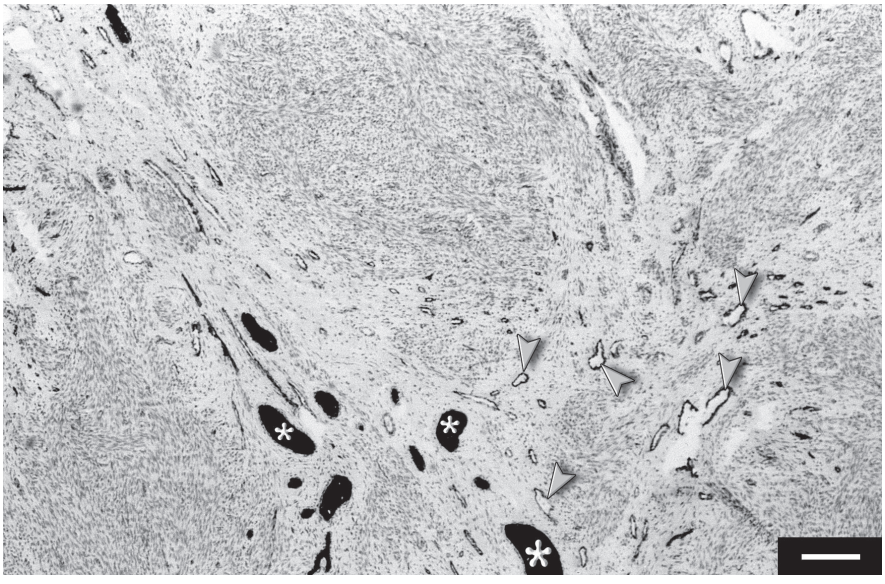

Fig. 3. Light microscope sspecimen. Outer intramural leiomyoma. Arteries injected with acrylic emulsion Liquitex R (Binney and Smith, USA). Immunohistochemical staining for von Willebrand factor marks not injected small vessels (arrowheads). Injected arteries (*). Bar = 100 μm.

The vascular structure of outer myometrial tumours was comparable to those seen in the uterine cervix or intramural of the very early stage of the development. The vessels were mostly arranged in the periphery of the tumour what was observable in SEM. The vascular capsule was loose and less dense than that seen in the typical intramural tumours, so they presented the exophytic model of tumour growth.

Immunostaining proved the fact that single vessels penetrated the substance of the lesion, while using SEM they were mostly invisible.

CONFLICT OF INTERESTS

None declared.

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[1] Chair of Anatomy
Jagiellonian University Medical College
Head: prof. dr hab. Jerzy A. Walocha

[2] Chair of Patomorphology
Jagiellonian University Medical College
Head: dr hab. Dariusz Adamek

Corresponding author:
lek. med. Paweł Depukat
Chair of Anatomy
Jagiellonian University Medical College
ul. Kopernika 12, 31-034 Kraków, Poland
Phone/Fax: +48 12 422 95 11
E-mail address: depukatp@poczta.onet.pl