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BLOOD VESSELS OF THE INTRATUMORAL SEPTA IN UTERINE LEIOMYOMATA

Abstract: The angioarchitecture of fibroid intratumoral septa was studied using 32 uteri obtained during necropsies of the females aged between 35–57. The whole vascular bed of 16 uteri was injected with synthetic resin Mercox CL-2R and then the uteri were corroded in potassium hydroxide. Next 16 uteri were injected with acrylic emulsion, Liquitex R. Their vascular bed was studied using immunohistochemistry for von Willebrandt's factor.
Immunohistochemistry allowed to visualize the vessels within the intratumoral septa, while SEM allowed to differentiate the vessels, which were mainly the venules and the veins. Apart from the veins the intratumoral septa were consisted of small arteries and capillaries.

Key words: uterine fibroids, corrosion casting, immunohistochemistry, septa, pseudocapsule.

INTRODUCTION

Uterine leiomyomata are usually multiple tumours of the female internal genital organs which seem to be the clones of the single smooth muscular cells [1]. They use to develop their own, specific vasculature [2, 3]. The whole process of growth of new vessels within uterine leiomyomata is controlled by many factors, although it may also happen that tumor up- or downregulates creation of angiogenic factors, causing dysbalance in activity of factors which are pro- or antiangiogenic [4–12]. Females with fibroids may present with irreducible chronic pelvic pain, menorrhagia, dysmenorrhea, infertility, recurrent miscarriage, preterm delivery or preterm rupture of the placental membranes. Different studies showed that peripheral region of fibroid, the so called capsule or pseudocapsule or vascular capsule is a place of significantly increased activity of different members of family of vascular growth factor [13–17]. Walocha *et al.* [2, 18] distinguished two types of vascularization of intramural uterine fibroid: a type where peripheral vessels form relatively dense vascular capsule, while the center of the lesion seems to be poorly vascularized and type two, where foci of intensive regression of tumor are separated by strong vascular septa. In this last location multiple tumours are

characterized by the presence of reach vascular, connective tissue intratumoral septa. Their vascular structure was the main interest of this study.

MATERIAL AND METHODS

Studies were carried out on 32 human uteri obtained during necropsy 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, sixteen uteri were perfused via the afferent arteries with prewarmed (37°C), heparinized saline (12.5 IU/ml heparin; Polfa, Poland, containing 3% dextrane (70kDa) 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). Next the casts were embedded in a mixture of poliethylenoglycols [19].

The casts were gently dissected 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.

The vascular beds of next sixteen uteri were perfused with saline and next injected with the solution of acrylic emulsion (Liquitex R, Binney and Smith, USA) [20]. 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.

RESULTS

High frequency rate of uterine leiomomata cause these tumours to be the most common benign neoplasms of female internal genital tracts. Fibroids can develop in different location: we can distinguish fibroids of the corpus and the cervix; they can grow subendometrially, intramurally and in the subserous location. In each location however multiple tumours are characterized by the presence of intratumoral septa. The smallest leiomyomata observed in SEM were usually avascular — potentially strong center of hypoxia localized in the middle of the lesion is able to stimulate angiogenic factors which produce the vascular capsule in the periphery of the tumor. Middle sized tumors are characterized by well-developed vascular capsule and could be occasionally traversed by small vessels (arteries and veins) [21, 22]. Large, bigger than 1 cm leiomyomata contain rather poorly organized network of blood vessels. Farrer-Brown suggested that direction of blood vessels follows chaotic course of smooth muscle fibers [23–25].

Typical HE study of slides showed the presence of large vessels (both arteries and veins) within the intratumoral septa (Fig. 1, Fig. 2). Immunohistochemical reaction for von Willebrand factor showed small not injected vessels (Fig. 3). SEM allowed us to perform qualitative evaluation of the blood vessels, which were mainly the venules and the veins (Fig. 4). As one can see in the figure the veins were mainly compressed, probably by growing tumor masses and resembled finger-like flattening. Apart from the veins the intratumoral septa were consisting of the few small arteries and more numerous capillaries.

Fig. 1. Uterus of 39 year old female. Corrosion cast. SEM. Veins, small arterioles and capillaries of the tissue septa within a large fibroid (F). Artery — A; V — vein; V* — venule; C — capillary. Bar = 1000 μm.

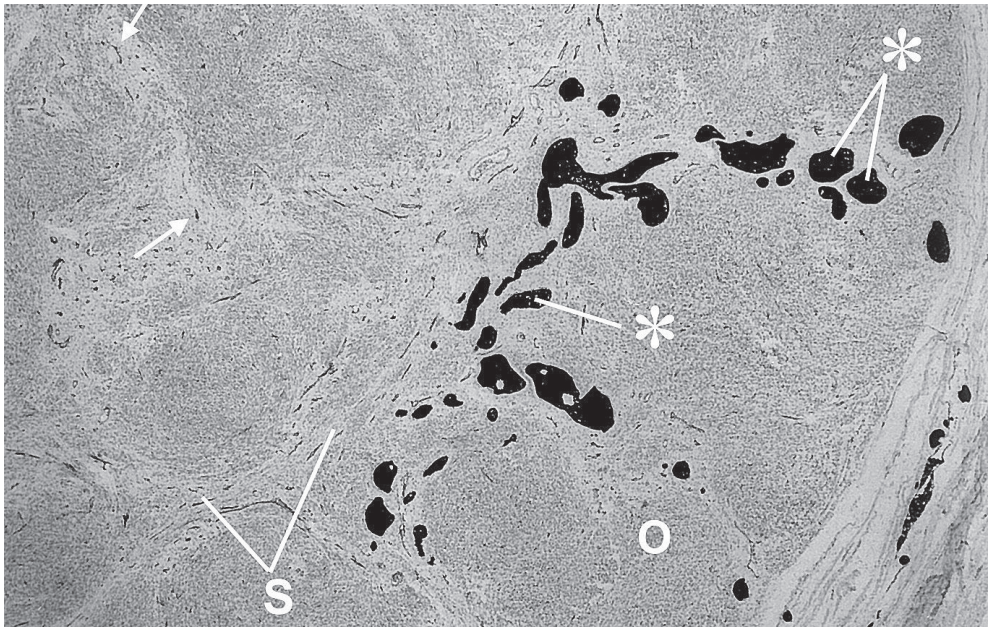

Fig. 2. Uterus of 46 year old female. Arteries injected with acrylic emulsion Liquitex R (Binney and Smith, USA). HE. Magn. 20 ×.

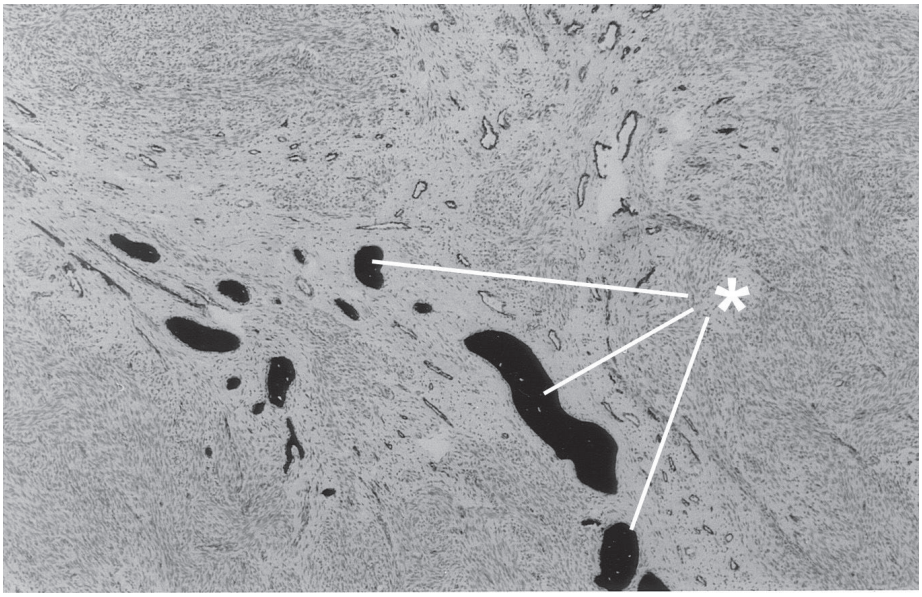

Fig. 3. Uterus of 47 year old female. Veins injected with acrylic emulsion Liquitex R (Binney and Smith, USA). HE. O — outer fibroid region. S — septa; * — injected veins; → — non-injected capillaries. Magn. 20 ×.

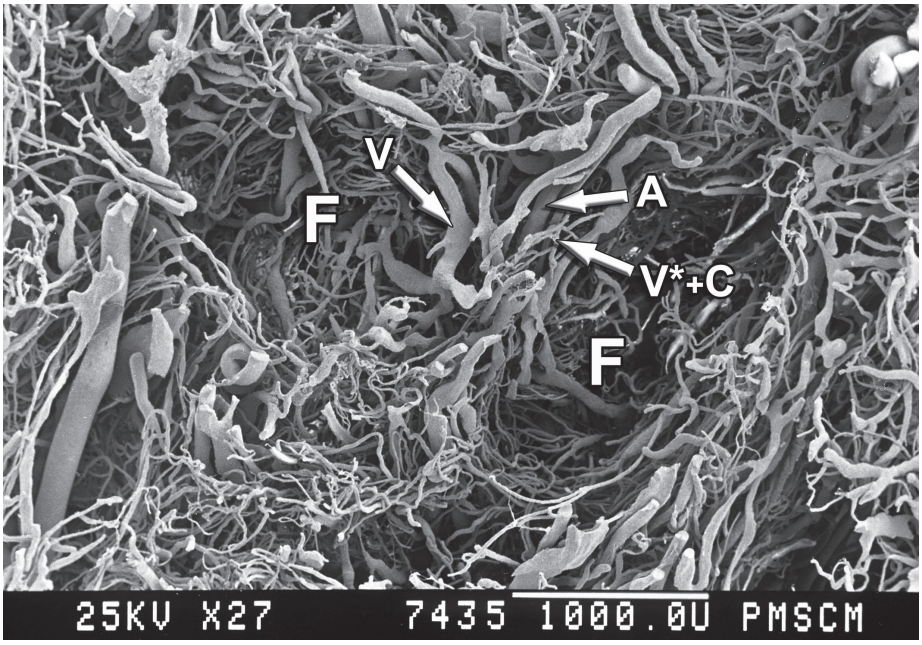

Fig. 4. Uterus of 46 year old female. Veins (*) injected with acrylic emulsion Liquitex R (Binney and Smith, USA). O — outer fibroid region; S — intraleiomomatous septa. Numerous empt small venules and capillaries (arrows). Immunohistochemical reaction for von Willebrand reaction. Magn. 20 x.

DISCUSSION

Several authors, specially these classical [26–29], postulated decreased density of blood vessels in the tumor. It was especially well visible at the border regions between normal and fibroid tissue, where in SEM we could observe avascular gap. It was the most probably resulted from accumulation of connective tissue in the fibroid pseudocapsule [2, 3], which was corroded during specimen maceration. Initial development of small leiomyomatous foci is associated with regression of blood vessels of the host organism. Intramural leiomyomata, specially these of greater size, revealed the presence of connective tissue septa, which separated the foci of increased proliferation. These septa were penetrated by numerous vessels which could be identified in SEM or seen in histological specimens Vessels of septa were consisted mainly of digitate flattened veins and capillaries [2, 3, 18, 30]. Only few arteries were visible. It cannot be excluded that density of the blood vessels within the myometrium and uterine fibroids is similar, because most of the leiomyoma is composed of extracellular matrix. Corrosion casting technique aided by SEM is still a method which allows to observe quasi 3D picture of leiomyoma vasculature — it can be certainly used to evaluate normal and other pathological tissues [31–33]. It is especially interesting tool when it comes to see the "vascular capsule" or intramural septa, however as we proved in our study, SEM lets to see the vascular nature of the inner regions of the lesion. Most of the recent techniques concentrating on treatment of uterine leiomyomas propose mechanical or hormonal intervention [2, 34–38]. Two most common nonsurgical treatments, GnRHa therapy and uterine artery embolization, act both to decrease or even bloc blood flow to the fibroid. In our opinion further investigation on the fibroid treatment regarded to penetration of the medium into the depth of the lesion through the minimal vessels of the septa, should be taken into consideration.

CONFLICT OF INTERESTS

None declared.

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