Vascular architecture of human uterine cervix visualized by corrosion casting and scanning electron microscopy

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Submitted on July 29, 2011; resubmitted on December 5, 2011; accepted on December 12, 2011

BACKGROUND: In contrast to the uterine corpus, the vascular architecture of the human cervix has been the subject of only a few studies, mostly dealing with the ectocervical mucosal vessels. This study presents the vascular system of the cervical wall surrounding the endocervical canal visualized by the best currently available technique, corrosion casting combined with scanning electron microscopy.

METHODS: Uteri collected at autopsy (n = 20) were perfused via afferent vessels with fixative followed by Mercox resin and corroded after polymerization of the resin. The obtained vascular casts of the cervix visualizing all vessels including capillaries were examined in the scanning electron microscope.

RESULTS: The vascular system of the cervix was nearly completely replicated in only two (10%) of the samples. In the wall of the cervix, four distinct vascular zones surrounding the endocervical canal were observed: (i) the outer zone containing larger vessels, arteries and veins of 0.3–1 mm diameter; (ii) the zone containing arterioles and venules; (iii) the zone of endocervical mucosal capillaries showing a very high density, parallel arrangement and relatively few interconnections and (iv) the innermost, subepithelial zone containing small veins running along the endocervical canal.

CONCLUSIONS: Despite the loss of the delicate ectocervical mucosal vessels from the cast during the corrosion step, we have successfully visualized the majority of the cervical vasculature. The vascular pattern of the human cervix, especially that of the endocervical mucosa, may facilitate the adaptation of the cervical vasculature to the extensive remodeling of the cervix during parturition.

Key words: blood vessels / corrosion casting / uterus / cervix

Introduction

The vascular system of the uterus has a unique remodeling capability associated with the menstrual cycle, implantation and pregnancy (Rogers, 1996; Osol and Mandala, 2009). This characteristic has stimulated the research in this field from the 19th century to the present, with the use of various methods of blood vessel visualization (Manconi et al., 2010). The vascular and microvascular architecture of the human uterus was also extensively studied (Dalgaard, 1946; Farrer-Brown et al., 1970a,b,c; Bulletti et al., 1985; Walocha et al., 2003; Hamid et al., 2006) but the vast majority of the relevant publications dealt with the vasculature of uterine corpus. In contrast, there are very few studies on the vascular architecture of the human uterine cervix (Zinser and Rosenbauer, 1960; Gillet et al., 1973; Sekiba et al., 1979) and they mostly describe the vessels of the ectocervical mucosa. Hence, the aim of the present study was to visualize the entire vascular architecture of the human cervix using the best currently available technique, corrosion casting combined with scanning electron microscopy (SEM).

Materials and Methods

Twenty uteri were obtained at autopsy of women of reproductive ages (20–45 years), deceased from causes not related to disorders of the reproductive system. The material was collected no later than 24 h after death. The study was approved by the Bioethics Committee of the Jagiellonian University (approval KBET/121/8/2007). 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, the uteri were perfused via the afferent arteries with prewarmed (37°C), heparinized saline (Heparin, Polfa, Poland, 12 IU/ml) containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine, Polfa), until the fluid outflowing via the veins was completely transparent (5–10 min). Next, perfusion was continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma) in 0.1 M 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 methacrylate with the polymerization initiator (Vilene Comp. Ltd.) and the uteri were left in a warm water bath (56°C) for 12 h to allow polymerization and tempering of the resin.

When the polymerization was completed, the uterine tissues were macerated for 5–6 days by repeated soaking in 10% potassium hydroxide at 37°C followed by washing with warm (50–55°C) running tap water. The obtained vascular casts were washed in several changes of distilled water, 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).

Parts of the freeze-dried casts corresponding to uterine cervix and a small fragment of uterine body were excised and examined macroscopically. In order to facilitate sectioning of the casts, they were next embedded at 55°C in a mixture of polyethylene glycols (PEG 2000/PEG 600, 20:1), cooled to room temperature to solidify PEG (Walocha et al., 2002) and gently dissected longitudinally in the plane of the endocervical canal to expose the vasculature of cervix wall. The sectioned fragments were washed with stirred distilled water to remove PEG and stored in an exiccator containing phosphorus pentoxide until microscopic examination. The fragments were then mounted onto copper plates using colloidal silver and ‘conductive bridges’ (Lametschwandtner et al., 1990) and coated with gold. The casts were examined in a JEOL SEM 35-CF scanning electron microscope at 20–25 kV. Casts of arteries/arterioles were distinguished from those of veins/venules on the basis of different imprints of endothelial cell nuclei (Miodoński et al., 1976; Fig. 1, inset).

**Results**

Among the 20 corrosion casts prepared, the vascular system of the cervix was nearly completely replicated in only two specimens obtained from multiparous women—with exception of the...
subepithelial ectocervical vessels. The wall of the cervix showed a high density of blood vessels. Roundish or oval avascular areas observed in the mucosal layer represented the corroded cervical glands. In both the vaginal and supravaginal portions, four distinct vascular zones surrounding the endocervical canal could be observed: (1) the outer zone containing larger vessels; (2) the zone containing arterioles and venules; (3) the zone of endocervical mucosal capillaries and (4) the innermost, subepithelial (pericanalar) zone containing capillaries and small veins (Fig. 1).

Relatively large arteries and veins, 0.3–1 mm in diameter, located in the outermost zone of the cervix wall gave off arteriolar and venular branches observed in the successive zone, characterized by a slightly lower density of blood vessels. The arterioles and venules entered the next zone where they supplied/drained an extremely dense mucosal capillary plexus. Near the internal os, the plexus was irregular but towards the vaginal portion of the cervix it assumed a pattern of parallel capillaries running increasingly obliquely to the axis of the endocervical canal (Fig. 2) and showing relatively few interconnections (Fig. 3). In deeper areas of the mucosa, these capillaries first ran for a short distance perpendicular to the endocervical canal and then bent to assume an oblique orientation (Fig. 4). In the vaginal portion of the cervix, the capillaries were nearly parallel to the long axis of the endocervical canal. As compared with the endocervical mucosal capillaries,
the endometrial capillary network of the uterine body was less dense and the capillaries showed an irregular arrangement (Fig. 5).

The mucosal capillaries were drained by small veins, 100–200 μm in diameter, running along the axis of the endocervical canal and located directly beneath its lumen, in the subepithelial region. The veins followed such a course for some distance and then turned towards deeper regions of the wall (Fig. 6) and joined larger veins located in its outermost zone. The veins were surrounded by a single layer of irregular capillaries forming a plexus of variable density (Fig. 7). Collectively, the subepithelial veins were observed along the whole length of the endocervical canal with the exception of its last segment close to the external os (Fig. 1) and their number, depending on canal segment, ranged from one to four (Fig. 8).

The cervical glands were surrounded by a dense capillary plexuses, either irregular (Fig. 9) or, especially in the case of dilated glands (retention cysts), sometimes composed of parallel capillaries following the course of the gland (Fig. 10). Larger vessels, mostly arterioles and venules, were also observed in the direct vicinity of the glands.

Discussion

In our previous study on vascularization of the uterine myomata (Walocha et al., 2003), we obtained acceptable vascular corrosion casts in ~20% of postmortem specimens of the uterine corpus. For some reason, successful replication of the vascular system in postmortem specimens of the cervix is more difficult, because in the present study only 10% of these were nearly completely replicated. Unfortunately, during the corrosion step, the delicate and highly fragile mucosal microvasculature of the ectocervix exposed on the surface of the cast was lost. This weakness of the present study is ameliorated by the fact that the ectocervical mucosal vasculature was described in detail by other authors (Zinser and Rosenbauer, 1960; Gillet et al., 1973; Sekiba et al., 1979).

Indeed, the studies dealing with angioarchitecture of the human uterine cervix published so far have been focused mostly on the ectocervical mucosal vessels, because of the significance of this region in the development of cervical cancer, the most common and severe pathology of the cervix. Moreover, vascular abnormalities of this region can also be observed by routine colposcopic examination (Joshi et al., 2008; O’Connor, 2008). Two detailed papers published in the 1960s and 1970s employed a gelatin and ink injection technique followed by light microscopy of cleared specimens (Zinser and Rosenbauer, 1960; Gillet et al., 1973). The corrosion casting method combined with SEM is the best currently available technique for morphological examination of vascular networks (Lametschwandtner et al., 1990), because it offers high resolution and a quasi-three-dimensional image of the vessels without interference.
from the non-vascular tissue. There has been only one study of the human cervical vessels using this technique (Sekiba et al., 1979) but it visualized exclusively ectocervical mucosal vasculature under normal and pathological conditions. The present study demonstrates for the first time almost the entire vascular system of the human cervical wall, especially the vasculature of the endocervical mucosa which was only fragmentarily described by Gillet et al. (1973).

The cervical blood vessels are arranged in four distinct zones around the endocervical canal. The existence of such zonation was also demonstrated by magnetic resonance imaging (DeSouza et al., 1994) which revealed the outer zone of larger blood vessels and another high signal area corresponding to the zone of numerous mucosal capillaries. These zones were separated by a low signal stromal zone, in our material corresponding to the zone containing arterioles and venules, which indeed shows lower general density of blood vessels. The zones observed in the cervix are a continuation of the uterine body wall layers: the zone of larger blood vessels corresponds to the vascular layer of the myometrium, while the other three zones belong to the mucosal layer. In the zone of arterioles and venules, relatively straight or wavy arterioles supplying the mucosal capillaries are an equivalent of spiral (coiled) arterioles which are characteristic of the endometrium.
In our study, two findings are quite surprising: the extremely high density of endocervical mucosal capillaries and the presence of small veins located in the direct vicinity of the endocervical canal. Even though the injection of the resin might cause some dilatation of thin-walled capillaries and a slight artifactual increase in the diameter of their casts, the density of mucosal vessels revealed in our specimens is enormous. The density of the endometrial capillary network in the uterine body is lower (albeit still considerable) and it gradually decreases in the transition zone between the isthmus and the cervix. Such high density and regular arrangement of the endocervical capillary network might be related to the extensive remodeling of the cervix during parturition (Word et al., 2007), to which the vascular system must adapt. Since the mucosal capillaries are largely parallel, the dilatation of the cervix could result in the increase in intercapillary distance and such “spreading” of capillaries might be possible because they show relatively few interconnections. Owing to their initial high density, the capillaries would still retain sufficient blood supply to the stretched mucosa. Moreover, a rich blood supply to the endocervical region could facilitate development of such cervical pathologies as placenta previa or lobular endocervical glandular hyperplasia/minimal deviation adenocarcinoma.

Small subepithelial veins running along the endocervical canal have not been described in previous papers dealing with the cervical vasculature. We observed them in two cast specimens and it seems that such veins are a frequent feature of the human cervix. Such a vascular pattern suggests bidirectional draining of endocervical mucosal capillary plexus: centrifugal, by the venules/veins located in the middle and peripheral zones of the cervical wall and centripetal, by the subepithelial veins. This unusual double draining system could be related to the extreme density of mucosal capillaries, as it provides efficient evacuation of blood from the capillary plexus. The endocervical subepithelial veins can also contribute to formation of cervical varices, a rare complication of pregnancy, which may cause bleeding or thrombosis (Hurton et al., 1998; Sammour et al., 2011).

Authors’ roles

J.A.W. was involved in study design, data collection and analysis and manuscript preparation. T.B. and W.K.-P. performed experiments, J.A.L. participated in data analysis and manuscript preparation, A.J.M. was involved in study design and data analysis. All authors approved the final version of the manuscript.

Funding

The study was supported by the statutory funds from the Jagiellonian University Medical College.

Conflict of interest

None declared.

References


