Immunohistochemical study of pathological alterations of peritoneum in patients with terminal renal insufficiency and on peritoneal dialysis

Immunohistolohemjsko ispitivanje patološki izmenjenog peritoneuma kod bolesnika sa terminalnom bubrežnom insuficijencijom na peritoneumskoj dijalizi

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Abstract

Background/Aim. During peritoneal dialysis (PD) an exchange of substances between blood and dialysate takes place through specific histological structures of peritoneum. Peritoneal double-layered serous membrane has, so far, mostly been studied with electron microscopy on experimental animals. The aim of this study was to assess integrity of peritoneal tissue in end-stage renal disease (ESRD) and PD patients using standard light microscopy and immunohistochemical methods. Methods. Peritoneal tissue biopsies were performed on 25 persons: 8 healthy donors during nephrectomy, 9 ESRD patients upon insertion of PD catheter, and 8 PD patients upon removal of the catheter for medical indications. The samples were fixed and prepared routinely for immunocytochemical staining by standardized streptavidin biotin AEC method using a LSAB2
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, Denmark) for collagen IV and analyzed by light microscopy. Results. We observed mesothelial detachment from lamina propria, duplicated basement membrane and much thicker blood vessel walls in ESRD and PD patients, compared to healthy subjects. Differences in histological structure, emphasized with immunostaining, indicated pathological alterations of peritoneal tissue in the renal patients. Conclusions. Immunohistochemistry can be used in studying histological alterations of peritoneal tissue in ESRD and PD patients. This method may indicate possible problems in filtration and secretion processes in this tissue.

Key words: kidney failure, chronic; peritoneal dialysis; peritoneum; pathology; immunohistochemistry; microscopy.

Apstrakt

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HRP kompleta (Dako
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, Danmark) za bojenje kolagena IV i analizirani svetlosnom mikroskopijom. Rezultati. Uočeno je odvajanje mezotela peritoneuma od lamine proprie, duplikacija bazalne membrane mezotela i značajno zadebljanje zidova krvnih sudova kod bolesnika sa TIB i na PD u odnosu na zdrave osobe. Ove promene histološke grade peritoneuma, jasno obeležene immunohistološkim bojenjem, ukazuju na patološke promene tkiva peritoneuma kod bubrežnih bolesnika. Zaključak. Immunohistološke metode mogu se koristiti za ispitivanje promena strukture tkiva peritoneuma kod bolesnika sa TIB i na PD. Ova metoda može pomoći u identifikovanju mogućih uzroka filtracionih i sekrecionih procesa u ovom tkivu koji utiču na efikasnost PD.

Ključne reči: bubreg, hronična insuficijencija; dijaliza, peritoneumska; peritoneum; patologija; immunohistološka; mikroskopija.

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Introduction

Peritoneal membrane is involved in filtration of substances from peritoneal fluid and in the exchange of substances between blood and dialysis fluid within the peritoneal cavity during peritoneal dialysis (PD). The following histological structures of the peritoneum which form the peritoneal membrane are involved in these processes: stagnant fluid layer within peritoneal capillaries, capillary endothelium, capillary basement membrane, interstitium, mesothelium and stagnant fluid film on mesothelial surface within the peritoneal cavity. Peritoneal mesothelium possesses distinctive regenerative ability. Normal myotic activity of rat mesothelial cells is 1% daily, and may rise up to 19% in peritonitis.

It is still difficult to study effects of dialysate on the peritoneal membrane in performing filtration and secretion processes. The tissue was fixed for 24 hours in 10% formaldehyde with 0.1M Sorensen's phosphate buffer pH 7.4, dehydrated in 96% ethanol, then routinely processed for embedding in paraplast. Immunohistochemical staining was performed by using the standard streptavidin biotin 9-amino-ethyl carbosol LSAB® HRP kit (Dako®, Denmark) 12. This method is based on the specific binding of primary antibody to tissue antigens previously incubated with 3% hydrogen in order to inhibit endogenous peroxidase. After 90 minutes incubation with primary antibody for collagen IV, samples were incubated with secondary antibody bound to biotin, and then with streptavidin bound horseradish peroxidase (HRP). This complex: primary – secondary antibody – biotin – streptavidin – enzyme, was then stained with AEC chromogen, resulting in redish-brown product representing positive immunoreaction. Reaction was interrupted by rinsing with distilled water. Samples were then contrasted with Mayer hematoxylin and examined by light microscopy (Opton Photomicroscope III, original magnification 300×) 13.

Morphometric analysis

Outer and lumen diameters of peritoneal blood vessels were determined on vessels transversal sections with 3.1 Soft Imaging System GMbH, Munster, Germany, by direct measuring on the image projected from the microscope on computer screen, using a digital camera (Olympus C3030). Peritoneal blood vessels wall thickness was calculated as difference between outer and lumen diameter. The results were analyzed with ANOVA and Tuckey HSD tests (STATISTICA 5.0, StatSoft, Inc. Tulsa OK); significance was considered at $p < 0.05$.
**Results**

We used immunohistochemical staining to visualize peritoneal structures which were particularly exposed to pathological conditions and therefore suffered alterations: mesothelial basement membrane and blood vessels’ walls. Pathological changes also affect interstitial collagen fibers, especially in the process of peritoneal fibrosis.

Immunostaining for collagen IV in the control group showed single, thin, continuous mesothelial basement membrane. Light microscopy showed numerous, well organized collagen fibers in peritoneal lamina propria, particularly noticeable when stained for collagen IV (Figure 1).

Blood vessel walls in the end-stage renal disease patients showed positive immunostaining for collagen IV (Figure 2) but, with magnification 300 ×, neither qualitative nor quantitative alterations in collagen IV distribution were observed, compared to the healthy subjects.

In the patients on peritoneal dialysis even with light microscopy we observed numerous sites of mesothelial detachment from peritoneal lamina propria, especially well noticeable when stained for collagen IV in mesothelial basement membrane (Figure 3a), which is frequently duplicated in the PD patients (Figure 3b). Blood vessel walls showed positive immunostaining for collagen IV (Figure 3c).

On morphometric analysis with magnification 300 ×, it was clearly visible that peritoneal blood vessels in the PD patients had significantly thicker walls than blood vessels of the healthy subjects (48.43 μm vs 28.98 μm; p < 0.01). Furthermore, blood vessel walls were significantly thicker in the PD patients than in the ESRD patients (48.43 μm vs 35.90 μm; p < 0.05), as shown in Figure 4.
When analysing distribution of blood vessels with regard to wall thickness, we observed the vessels with extremely thick walls in the PD patients group, shifting the curve to the right. Both the healthy subjects and the ESRD patients had normal distribution of blood vessels with regard to wall thickness (Figure 5).

![Image](image_url)

**Fig. 5 – Peritoneal blood vessels distribution regarding wall thickness**

ESRD = end-stage renal disease; PD = peritoneal dialysis

**Discussion**

In the present study of the human peritoneum, special attention was payed to immunohistochemical characteristics of elements of submesothelial connective tissue. We demonstrated pathological alterations in peritoneal submesothelial interstitial tissue and in mesothelial and endothelial basement membranes, using positive immunoreaction for collagen IV in these tissue elements in the renal disease patients.

Previous immunohistochemical studies of the peritoneum showed fascicular pattern of collagen IV, fibronectin and laminin fibers and diffuse fibers of collagen I in the submesothelial interstitial tissue. The peritoneal mesothelium and endothelium of peritoneal blood vessels in healthy persons have single basement membranes. They represent a barrier for blood proteins and support for mesothelial and endothelial cells. Positive immunoreaction for collagen IV was found in mesothelial and endothelial basement membrane.

Uremic alterations of serous membranes in the ESRD patients affect the peritoneum as well. Peritoneal tissue is highly delicate and extremely sensitive. Changes of internal environment, inflammation or minor injuries cause cell degeneration and desquamation. Uremic serositis itself causes detachment of mesothelial cells from their basement membrane and cell death. Mesothelial regeneration is condictio sine qua non for maintaining normal physiological functioning of the peritoneum in ESRD, and especially when PD is performed. Denuded regions are colonized with new mesothelial cells of yet undetermined origin. Several possible sources have been proposed: precursors from the bone marrow, mature undamaged mesothelial cells from adjacent and/or opposite peritoneal areas, precursors from lamina propria or free mesothelial cells from peritoneal fluid.

Detailed follow-up of mesothelial regeneration process, cells’ origin and determination of their maturity is possible with immunohistochemical staining of mesothelial cytoskeleton (actin microphylaments, microtubules and intermembrane desmin, vimentin and cyto keratin filament) and determining the amount of cyto keratine and vimentin. Especially important is the presence of cyto keratin 8 and 18, which are specific markers for mesothelial cells. Elements of the mesothelial cytoskeleton may also serve as markers for cell maturity. Young mesothelial cells in culture have equal amount of low molecular mass cyto keratin and vimentin, but during maturation the amount of vimentin rises. Mature mesothelial cells have high molecular mass cyto keratin and vimentin fibers.

The peritoneum suffers various structural alterations during PD. Destruction of mesothelium presents as: decrease in number or complete loss of microvilli, decrease in number of pinocytotic vesicles, widening and degeneration of rough endoplasmatic reticulum, mitochondrial picnosis, apical cytoplasmatic protrusions and paracrystalline inclusions in these cells are observed with electron microscopy.

In physiological conditions, basal surfaces of mesothelial cells are in contact with basal lamina which they produce. Electron microphotographs clearly demonstrate lamina densa and light layer of lamina lucida above it, separating it from mesothelial cells. Immediately beneath lamina densa are collagen fibers organized in fascicular pattern. Collagen is synthesized and secreted by fibroblasts from peritoneal connective tissue to form lamina fibroreticularis. Collagen fibers can be clearly detected with light microscopy with specific immunostaining. In the present study, immunostaining for collagen IV in the PD patients allowed visualisation of detachment of mesothelium from its basement membrane (and consecutive denudement of submesothelial layer) even with basic light microscopy.

A valuable finding in the PD patients is duplication (multiplication) of the mesothelial basement membrane. This was previously observed with electron microscopy. We already found this diabetiformic alteration of the basement membrane using electron microscopy, but in this study we showed that it can also be seen with light microscopy when immunohistochemical staining for collagen IV in the mesothelial basement membrane is applied. This may enable a routine follow-up of peritoneal membrane status during PD.

**Conclusion**

Basic microscopy techniques, such as light microscopy, and relatively low-budget preparation methods (immunohistochemistry) may contribute significantly in studying peritoneal membrane in the ESRD and PD patients. Data obtained using standard antibodies for certain peritoneal structures (collagen IV in mesothelial and endothelial basement membranes) which suffer alterations in ESRD or during PD, are valuable in assessing adequacy of the peritoneal membrane in filtration and secretion processes it performs. Various degrees of damage to the peritoneal membrane represent clinical indication for PD cessation and transfer to hemodialysis.

**References**

On the other hand, timely detection of moderate peritoneal tissue alterations allows undertaking adequate measures (such as changing dialysate or temporary cessation of PD) in order to prevent a complete deterioration of peritoneal membrane, irreversible damage and usability for PD.

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REFERENCES


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