 doi:10.20944/preprints202009.0623.v1

Article

New findings in the study of the pathogenesis of urethral pain syndrome

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Abstract: Urethral pain syndrome (UPS) is still a pathology in which the diagnosis is formulated as a "diagnosis of exclusion". The exact pathogenetic mechanisms are not yet fully understood and clear recommendations for the prevention and treatment of UPS are absent. The goal of the study was to assess the condition of the tissues in the female urethra in UPS, by using transvaginal ultrasound (TVUS) and cross-polarization optical tomography (CP OCT). TVUS showed an expansion in the diameter of the internal lumen of the urethra, especially in the proximal region compared with the norm. Compression elastography revealed areas with increased stiffness (presence of fibrosis) in urethral and surrounding tissues. When studied with CP OCT it was shown that with UPS, the structure of the tissues in most cases was changed: trophic alterations in the epithelium (hypertrophy or atrophy) and fibrosis of underlying connective tissue were observed. The proximal fragment of the urethra with UPS underwent changes identical to those of the bladder neck. This paper showed that the introduction of new technology — CP OCT — in conjunction with TVUS will allow verification of structural changes in tissues of the lower urinary tract at the level of their architectonics and will help doctors understand better the basics of the UPS pathogenesis.

Keywords: cross-polarization optical coherence tomography (CP OCT); ultrasound; urethral pain syndrome; epithelial atrophy; epithelial hyperplasia; inflammation; fibrosis; image evaluation

1. Introduction

The most common reason for women to seek medical attention is dysuria, and it is believed that in 40% of cases urethritis and / or urethral syndrome are involved [1]. According to the US National Institutes of Health, one third of women with chronic pelvic pain (CPP) have urethral pain syndrome (UPS) [2,3]. The European Association of Urology defines UPS as the occurrence of chronic or recurrent episodic pain lasting for more than 6 months, and felt in the urethra, in the absence of proven infection or other obvious local pathology. It is often associated with negative cognitive, behavioral, sexual or emotional consequences, as well as with symptoms suggestive of lower urinary tract, sexual, intestinal, or gynecological dysfunction.

The problem of pain in the urethra with unchanged urinalysis, the absence of any other clinical manifestations, and the absence of somatically explainable causes, is complex and ultimately remain unresolved, since the exact pathogenetic mechanisms are not yet fully understood [4-6]. Neither are there any clear recommendations for the prevention and treatment of UPS, as a result of which the only effective form of medical care, today, is symptomatic therapy — involving the continuing

intake of strong pain medications, antidepressants and anticonvulsants. In the methodological recommendations on CPP, published under the auspices of the Moscow Department of Health (dated 14 July 2016), it is noted that there is no specific accepted treatment for UPS. The approach should be interdisciplinary and the treatment multimodal, with the general principles of chronic pain syndrome management being applied.

The close embryological relationship between the urethra and the bladder makes it likely that there are causes in common with the development of painful bladder syndrome [7]. According to the classification of the International Association for the Study of Pain (IASP, 2019) the mechanism of CPP and possible causes of its occurrence may include vascular lesions, persistent inflammatory processes, or violation of the innervation of organs due to mechanical compression in the pelvic region, but often the reason is not clear [8].

The connective tissue matrix of organs plays a key role in the occurrence and persistence of pain, as shown by the works of a number of authors [9,10]. It is believed that connective tissue, as well as performing its supporting, protective and trophic functions, acts as a network-wide mechanosensitive signaling system — as a global unifying network [9,11].

Thus, it can be surmised that the above reasons for the development of CPP could well be due to factors that affect the state of the connective tissue matrix of the lower urinary tract. However, there are currently no methods for adequate, appropriate study of the structure of urethral tissues. According to the standards for examination of patients with CPP when using the UPOINT classification [12] in the urology domain, the recommended list of examinations includes keeping a urination diary, cystoscopy and the use of ultrasound (US) and uroflowmetry, while for complaints involving the urethra, urethroscopy is recommended. These methods allow only indirect assessment of the urethral tissues. Objective evaluation and accurate diagnosis of a disease that does not cause any visual changes, and results from a "diagnosis of exclusion" when using standard instrumental research methods, is important for understanding the pathogenetic aspects of the disease. In this work, we used traditional diagnostic methods, including US and uroflowmetry, and the non-traditional method of cross-polarization optical tomography (CP OCT) to study changes in the functioning of organs and its structure in UPS in comparison with the norm, and assessed the role of background diseases in the development of UPS.

In general, OCT is similar to ultrasonic technique, except for using light instead of sound and is centered on interferometry in the near-infrared range of wavelength (700–1300 nm) [13,14]. It measures the time delay and amplitude of backscattered light. The aim of the OCT technology is to perform a real-time, *in vivo*, optic biopsy, with direct label-free visualization of the histological structure of the human tissues at the level of the general architectonics to a depth of 1.5 mm [15]. High spatial resolution (5-15 μm) and easy performing with minimal expertise are the main advantages of OCT in contrast to US. The endoscopic nature of OCT probes not only enhances patient comfort and safety but also makes it especially suitable for assessing narrow tubular organs as well as using standard guidewires for examining deeply located objects in the body [16].

CP OCT is a functional extension of OCT that enables the detection of changes in the state of polarization of light caused by birefringence and coupling between two polarization states due to scattering in the random media (cross-scattering) [17]. As a result, two types of images are obtained simultaneously: in the initial (co-) polarization and orthogonal (cross-) polarization, which allow assessing isotropic (cells) and anisotropic (collagen and elastic fibers of connective tissue) structures separately [18,19]. This is important in cases when precise observation only connective tissue structures are needed.

The goal of the study was to assess the condition of the tissue in the female urethra in UPS, by using non-traditional methods for thise pathology – transvaginal compression ultrasound (TVUS) and CP OCT.

2. Materials and Methods

2.1. Patients

Fifty five patients with established UPS («UPS» group) received treatment in the urology department of the N.A. Semashko Nizhny Novgorod Regional Clinical Hospital between 2014 and 2019. In these, there were no clinical manifestations of an inflammatory process in the lower urinary tract. In all patients: 1) anamnesis was taken to identify the presence of any previously transferred concomitant pathology; 2) laboratory tests of blood and urine were carried out; 3) a physical examination was performed with the patient positioned on a gynecological chair: the state of the external opening of the urethra was assessed, palpation of the urethra and the walls of the vagina was performed in order to identify the presence of any myofascial aspect in the disease; 4) uroflowmetry was performed; 5) cystoscopy was carried out under anesthesia to exclude the presence of interstitial cystitis. On top of clinical and laboratory evaluation, 30 of the 55 patients underwent TVUS: 24 of them with UPS together with 6 normal controls (women with no complaints regarding the presence of pathology of the genitourinary system) («N» group). White-light cystoscopy in combination with CP OCT study was performed in 47 of the 55 women: in 33 with UPS and 14 with stones of the upper urinary tract but without pyelonephritis, in whom the urethra was accepted as being normal («N» group).

This study was approved by the review board of the Privolzhsky Research Medical University. Informed consent to participate in the study was obtained from the participants.

2.2. Transvaginal ultrasound

TVUS was performed using a Philips Epiq5 system. The sensor was inserted directly into the vagina, allowing visualization of the state of the bladder neck and urethra (assessment of their structure, the condition of their walls, the width of the internal lumen) and detecting abnormalities in the structure of the urethra compared with the norm. This was also the first study in which patients with UPS underwent compression elastography of the adjacent urethral tissues. Compression elastography is a technique that displays the relative deformation of tissues in the form of their color mapping in real time [20]. When the tissue is subjected to an external force (deformation), the harder/denser areas of the tissue exhibit relatively less compression than the softer areas. In our study, on the ultrasound elastographic images, the adjustment scale was set to display the harder areas in blue, with the softer areas appearing in red.

2.3. CP OCT study and image analysis

Time-domain device "Polarization-sensitive optical coherence tomograph OCT-1300U" (BioMedTech LLC, Nizhny Novgorod, Russia) (Fig. 1a), that provides two image acquisition in co- and cross- polarizations was used in the study [17, 21]. The device approved for clinical use (product license №FCP 2012/13479 of 30 May 2012) and has replaceable endoscopic probe (Fig. 1b, 1c). It has the following characteristics: the radiation source is a superluminescent diode, of operating wavelength 1310 nm, spectrum width 100 nm, axial resolution 15 μm, lateral resolution 25 μm and radiation power at the object 3 mW. OCT image size in each polarization is 1.8 × 1.3 mm (width × height), image acquisition time is 2 sec. Due to the presence of a flexible endoscopic probe with an outer diameter of 2.7 mm, the examination of the urethral tissue could be carried out simultaneously with cystoscopy through a standard endoscope. Our group's application of the CP OCT method to the study of the female urethral wall in patients with UPS, is a global 'first' [22].

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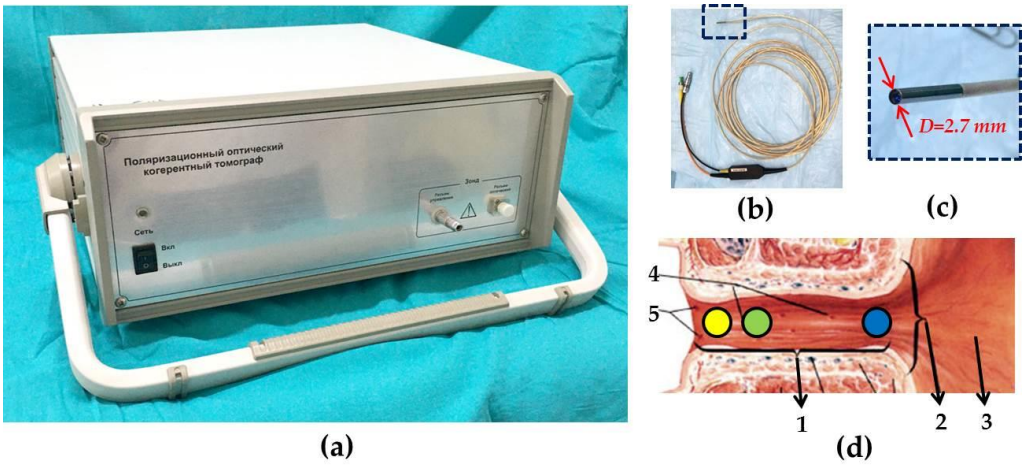

Figure 1. CP OCT device and areas under study shown on a diagram of the female urethra. (**a**) CP OCT device; (**b**) Flexible endoscopic forward-looking CP OCT probe; (**c**) Enlarged tip of the probe from (**b**). (**d**) Drawing of the urethra where it transitions to the bladder. Here, the circles indicate the locations from which CP OCT images were obtained in the proximal (blue), middle (green) and distal parts of the urethra (yellow) [23]. 1 – urethra, 2 - neck of urinary bladder, 3 - triangle of urinary bladder, 4 - lacunae and openings of urethral ducts, 5 - openings of paraurethral Skene's ducts.

From 4 to 13 images were obtained from each patient: of the bladder neck and three regions of the urethra (Fig. 1 d) at the 6 o'clock position corresponding to a conventional clockface; and, if possible, with other additional images of the urethra in the three directions (9h, 12h, 3h of the clockface). In the "UPS"/"N" groups, 169/58 CP OCT images were obtained, of which there were 43/16 CP OCT images of the bladder neck, as the section closest to the urethra and therefore potentially involved in processes occurring in the proximal urethra and 126/42 CP OCT images of the urethra (its proximal 41/14, middle 40/12 and distal 45/16 regions) (Table 1).

Table 1. Distribution of the CP OCT images by patient's groups and parts of the urethra.

Group	Number of patients	Number of CP OCT images	Average number of CP OCT images created from 1 patient	Number of CP OCT images of each location			
				Bladder neck	Distal urethra	Medium urethra	Proximal urethra
UPS	33	169	5.12	43	41	40	45
Norm	14	58	4.14	16	14	12	16
Total	47	227	4.63	59	55	52	61

A visual assessment of the CP OCT images of the bladder neck and urethra was performed by two respondents. The objects of interest were the epithelium and the state of the connective tissue structures of the urethra in patients with UPS, relative to the normal state of these structures. In the epithelium, the thickness was assessed as: normal, thickening (hyperplasia), or thinning (atrophy); in the connective tissue stroma, attention was paid to the presence of any element in the images corresponding to an inflammatory process: 1) lack of clarity of the border between the first (epithelial) and the second (connective tissue) layers, 2) the absence of horizontal ordering of the structures characteristic of the norm, 3) the presence of any indistinctness in their images, which would correspond to cellular tissue infiltration [22,24].

After an independent blind visual assessment of the CP OCT images, the «UPS» group was divided into 2 age subgroups: patients under 50 and those over 50.

3. Results

3.1. The role of background diseases in the development of UPS

An analysis of concomitant pathology in patients with UPS, identified by their history is presented in Table 2.

Table 2. Concomitant pathology and the source of its occurrence in the group of patients with UPS (n = 55).

№	Organ system with pathology	n-abs. (%)	Genesis of pathology	n-abs. (%)
1.	Gynecological	39 (70.9)	Hormonal	37 (94.8)
			Inflammatory	30 (76.9)
			Surgical interventions on the pelvic organs	12 (30.7)
2.	Respiratory	37 (67.2)	Upper (nose, nasal cavity, pharynx, larynx)	32 (86.4)
			Lower (trachea, bronchi, lungs)	5 (13.5)
			Psycho-emotional sphere	23 (41.8)
3.	Neurological	35 (63.6)	Central nervous system	10 (18.2)
			Peripheral nervous system	42 (76.4)
			Psycho-emotional sphere	23 (41.8)
4.	Urological	24 (43.6)	Inflammatory	10 (41.6)
			Non-inflammatory	17 (70.8)
5.	Gastroenterological	18 (32.7)	Inflammatory diseases of the stomach, duodenum, biliary tract	38 (69.0)
			Bowel disease	21 (38.2)
6.	Cardiovascular	9 (16.3)	Arterial hypertension	5 (55.5)
			Other	4 (44.5)
Total cases of pathology		162		

From Table 2 it follows that the predominant area of comorbidity was gynecological (70.9%). Hormonal abnormalities (94.8%) were found in 24 sexually active women in the pre-menopausal period, as well as in 13 women of the menopausal period; inflammatory diseases of the female genital area of bacterial and viral etiology was also present (76.9%).

Anamnesis of upper respiratory tract pathology, more common in adolescence, was recorded in 67.2% of women, of whom the bulk of patients (64.9%) reported frequent viral diseases or herpes infection.

The premorbid background in patients with UPS was neurological pathology (63.6%), and these are diseases associated with the involvement of the peripheral nervous system and, as is important, with the state of the psycho-emotional sphere.

Each patient suffering from UPS had 2.94 (162/55) cases of comorbidity. Thus, the role of other factors in the presence of foci of chronic infection in the body, and a decrease in immune defense factors, as a comorbid background for the development of UPS, cannot be denied, since the presence in the patients' history of inflammatory diseases of the respiratory tract, gastrointestinal tract, urological and gynecological organs was revealed.

 doi:10.20944/preprints202009.0623.v1

3.2. Results of cystoscopic examination

In 32.7% of cases (18 out of 55), clinical manifestations of UPS was combined with urinary pain syndrome. Low-volume (less than 300 ml) urination was reordered. The number of urinations exceeded 12 per day. Pains over the womb were present. During cystoscopy in patients of the «UPS» group, the bladder mucosa was unchanged — shiny, pale pink, while, in 16 cases (29.0%), there was a slight hyperemia in the bladder neck. A picture corresponding to interstitial cystitis — the presence of glomerulations in the mucous membrane of the bladder after the hydrodistension procedure, was found in 23.6% (n = 13).

3.3. Uroflowmetry results

In 72.7% of cases (40 out of 55), there was a decrease in the urination rate to 13.7±3.2 ml/sec in combination with low-volume urination while the normal values of the urination rate for women are 23–32 ml/sec [25]. The average volume of excreted urine was 172±33 ml.

3.4. Results of transvaginal ultrasound research

The results of TVUS studies showed that in the norma group in women, the urethra looks like a tube with a uniform lumen diameter without dilatations and contractions, which was 4.6±0.6 mm, wall thickness 4.8±1.1 mm. According to research by a group of authors [26] normally, the outer diameter of the urethra is 10.0 mm, the inner lumen of the urethra is closed during TVUS or 0.3 mm. According to the authors [27], who conducted a study with an intraurethral sensor, the thickness of the urethra in the proximal section was normally 3.7 mm.

In women with UPS (n = 24), the structural features of the urethra were revealed: the urethra was funnel-shaped (Figure 2d), opening to the bladder. The internal lumen of the urethra in the proximal segment was expanded to 5.9±2.1 mm. At the same time, 44% of patients had an expansion up to 7.5±0.5 mm, in 56% up to 5.5±0.5 mm. The thickness of the urethral walls in our study averaged 3.6 mm (from 2.4 to 6.0 mm).

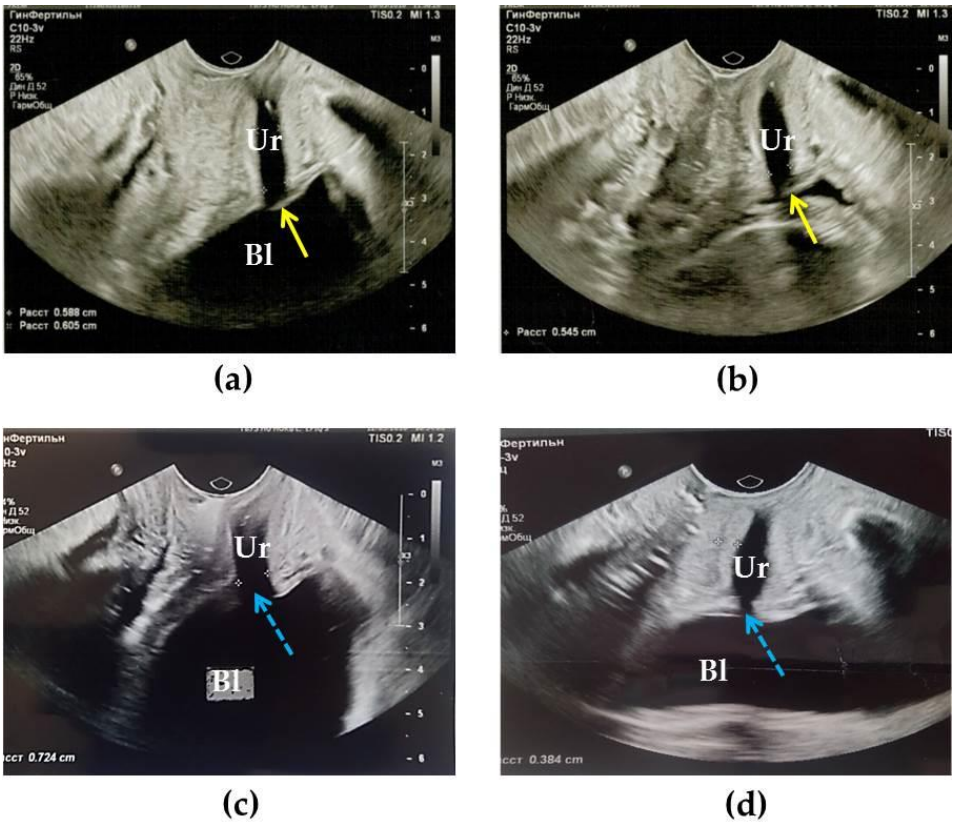

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Figure 2. TVUS of the urethra and adjacent tissues in normal conditions and with UPS. (**a**), (**b**) A healthy woman 30 years of age before (**a**) and after (**b**) urination. The urethral tongue closes the opening to the urethra, as indicated by the yellow arrow; (**c**) Patient K., 30 years old, with a UPS disease duration of more than 10 years; (**d**) Patient Z., 38 years old, over 13 years of illness. In both cases, with UPS, the urethral tongue is indistinguishable; the gaping opening at the transition of the bladder into the urethra is indicated by the blue dashed arrows. Bl - bladder, Ur – urethra.

Thus, in all patients with UPS, an increase in the diameter of the internal lumen of the urethra, especially in the proximal region, was recorded.

In 7 (29.1%) cases pathological changes were recorded in the urethral tongue, a cavernous structure that, as the bladder fills, normally increases in volume due to becoming engorged with blood and, together with the sphincter trigonalis, closes the exit from the bladder into the urethra. With the contraction of the urethra the posterior semicircle of the bladder neck is pressed against the anterior wall of the urethra and this closes its internal opening [28]. In patients with UPS, an absence of urethral tongue visualization, or the absence of its adherence to the entrance to the urethra, was revealed. No residual urine was found in patients with UPS.

Compression elastography of the urethra and adjacent tissues of patients with UPS in the proximal and middle regions showed a significant predominance of areas colored blue, indicating tissue stiffness and rigidity compared to the norm, where no blue color was observed (Fig. 3). Thus, our studies confirm the presence of fibrosis of the tissues surrounding the urethra in UPS.

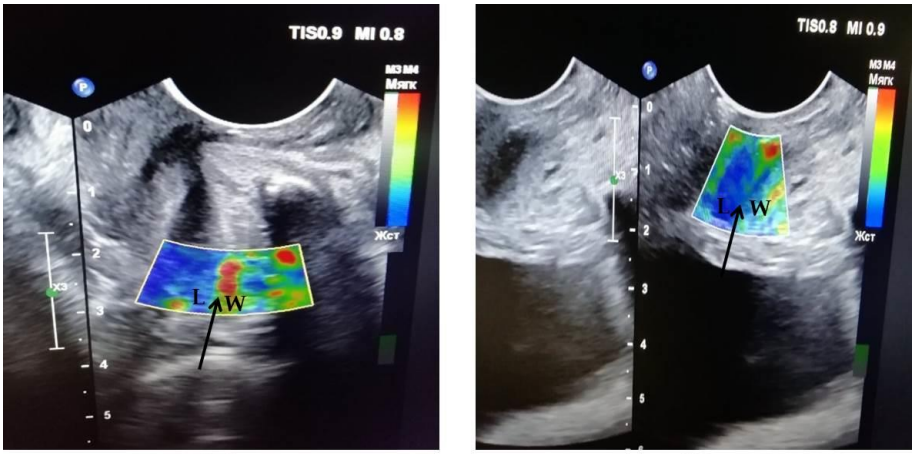

Figure 3. Ultrasound elastometry in the normal condition (**a**) and in UPS (**b**). Normally, the urethral wall is softer (red color) (**a**) than in UPS (predominance of green and blue colors) (**b**). L – lumen of the urethra, W – urethral wall.

3.5. Results of CP OCT study

CP OCT images of all sections of the female urethra in norm are structural. In co-polarization images (Fig. 4 a1–d1), the epithelium is clearly visualized in all areas of interest, its border contrasting with the underlying mucous layer. The signal from the connective tissue in the cross-polarization images (Fig. 4 a2–d2) of medium intensity, has a horizontal orientation; in the middle and distal segments of the urethra, single, gland-like lacunas with clear contours can be determined. In cross-polarization, the OCT signal is determined mainly by the collagen fibers of the connective tissue layer; therefore, only this layer of the urethral wall is clearly visible in such images, and the epithelium and muscles are not visualized.

In the normal group, there were no changes in the visible thickness in the zones of interest (Fig. 4 a1–d1). However, in women over 50 years old, a tendency of the epithelium to atrophy was revealed, which can be explained by the influence of hormonal changes. The connective tissue

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stroma generated approximately the same signal level in the cross- channel, without any extensive dark or bright areas and occupied 40–50% of the entire image height (Fig. 4 a2–d2).

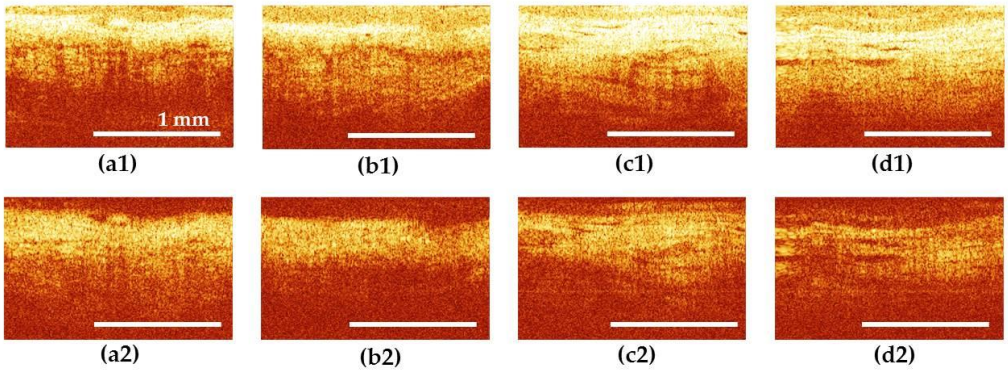

Figure 4. CP OCT images of the bladder neck (**a**) and three segments of normal urethra (**b–d**): (**b**) Proximal; (**c**) Middle; (**d**) Distal. The first row shows co-polarization images, the second row shows corresponding cross-polarization images.

Visual analysis of CP OCT in the UPS group revealed that, in terms of the characteristics of the epithelium and connective tissue, the proximal part of the urethra was more similar to the bladder neck than to the middle and distal parts of itself. Examples are shown in Figures 5 and 6.

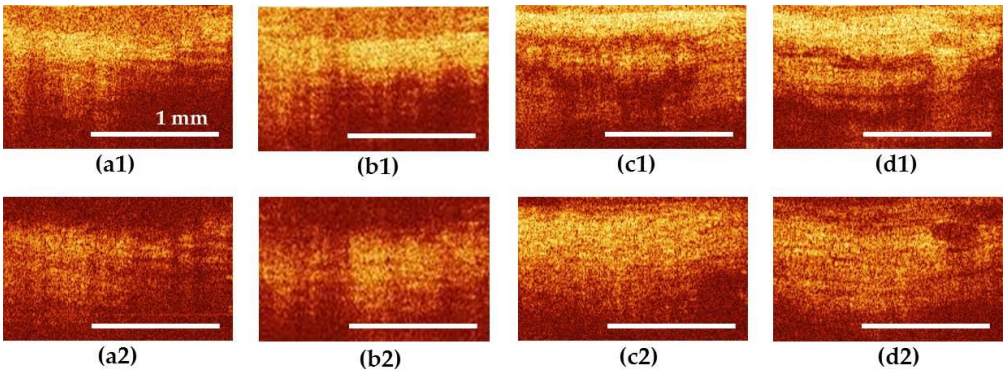

Figure 5. CP OCT images of the bladder neck (**a**) and three segments of the urethra (**b-d**) in patient I., 22 years old with UPS lasting 5 years. (**b**) Proximal; (**c**) Middle; (**d**) Distal parts of the urethra. The first row shows co-polarization images, the second row shows corresponding cross-polarization images.

Figure 5 shows an example of patient I., 22 years old with UPS lasting 5 years. Epithelial hyperplasia is visible in the bladder neck and the proximal urethra, the border of the epithelium with the underlying connective tissue layer is blurred, indicating the presence of inflammatory processes in these tissues (Fig. 5 a1, b1). The signal from the connective tissue structures in cross-polarization has a noticeable local decrease in intensity caused by the shadows of dilated blood vessels and by tissue edema (Fig. 5 a2, b2). In the middle and distal parts of the urethra, by contrast, thinning of the epithelium is noticeable (Fig. 5 c1, d1), while in the middle part, the border with the underlying connective tissue layer is clear (Fig. 5 c1). The connective tissue layer is thickened (Fig. 5 c2, d2) and looks more homogeneous in structure (Fig. 5 c2) than in the non-pathogenic case (Fig. 4 c2, d2). In this subgroup of patients, a thickening of the connective tissue layer in cross-polarization to occupy over 60% of the image height was observed in 44.4% (32 CP OCT images out of 72). In this case, an increase in the OCT signal was observed in all the images.

Figure 6 shows an example of patient E., 60 years old with UPS lasting 5 years. In the bladder neck and proximal urethra (Fig. 6 a1, b1), as well as in the rest of the urethra (Fig. 6 c1, d1), the

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epithelium is atrophic; in places where it is partially preserved, the border of the epithelium with the underlying connective tissue layer is blurred (Fig. 6 a1, d1). The signal from connective tissue structures in cross-polarization is weak, presumably due to severe tissue edema (Fig. 6 a2–c2). In the distal urethra, on the other hand, the connective tissue layer exhibits cross-scattering, but appears homogeneous in structure (Fig. 6 d2) compared to normal (Fig. 4 d2). In this subgroup of patients, thickening of the connective tissue layer in cross-polarization to over 60% of the image height was observed in 46.7% (28 CP OCT images out of 60): 71.4% of them with an increase in the OCT signal (20 of 28), while 28.6 % (8 out of 28) showed a weakening of the signal.

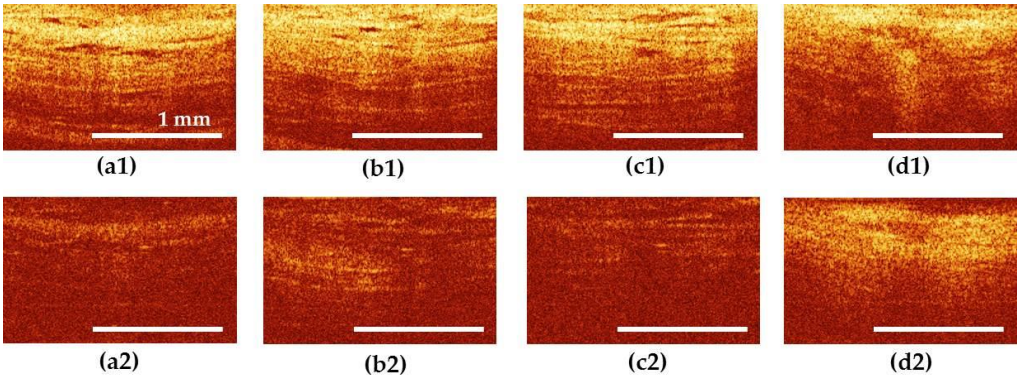

Figure 6. CP OCT images of the bladder neck (**a**) and three segments of the urethra (**b-d**) in patient E., 60 years old with UPS lasting 5 years. (**b**) Proximal; (**c**) Middle; (**d**) Distal parts of the urethra. The first row shows co-polarization images, the second row shows corresponding cross-polarization images.

The results of the incidence of the bladder neck + proximal conditions are presented in Table 3. 132 CP OCT images obtained at the '6 o'clock position' from 33 patients were analyzed.

Table 3. State of the epithelium of the bladder neck and the proximal region of the urethra compared with the epithelium of the middle and distal regions at the '6 o'clock position' in patients with UPS, depending on age.

Subgroup of patients by age, years	Number of patients	Number of CP OCT images	Hyperplasia of the bladder neck + proximal urethra	Atrophy of the bladder neck + proximal urethra	Total matches	% of changes in the bladder neck + proximal urethra of the total number of patients
≤49	18	72	4	4	8	44.4% (8/18)
50≥	15	60	7	7	14	93.3% (14/15)
Total (n=33)	33	132	11	11	22	68.8% (22/33)

It was revealed that changes in the epithelium of the bladder neck and proximal urethra — hyperplasia or atrophy, which differed from the middle and distal segments of the urethra — coincided in 22 cases out of 33, representing 68.8%. Hyperplasia was identified in 34.4% of cases (n = 11) as well as atrophy in 34.4% of cases (n = 11). It is noteworthy that in women over 50 years of age (n = 15), changes in the analyzed area were more common — 93.3%, compared with women of reproductive age (n = 18) — 44.4%. It can be surmised that hormonal levels undoubtedly play a role in changing the state of the tissues of the bladder neck and urethra.

 doi:10.20944/preprints202009.0623.v1

Of the 11 cases of hyperplasia detected in the proximal urethra, only in the case of the epithelium was there also thickening in the middle and distal urethra. In other situations, atrophy was recorded — 4 cases, while, in 6 the epithelium was of normal thickness. In the presence of atrophy in the proximal urethra (n = 11), atrophy was recorded in the underlying regions — 5 cases, while the epithelium was of normal thickness in 6 cases.

Thus, the CP OCT method allowed us non-invasively to determine the state of the epithelium and connective tissue structures of the bladder neck and urethra *in vivo*. It was shown that with UPS, the structure of the tissues in most cases is changed. In this case, the proximal fragment of the urethra with UPS undergoes changes identical to those of the bladder neck.

4. Discussion

UPS is still a pathology in which the diagnosis is formulated as a "diagnosis of exclusion". Despite significant global use of OCT in many fields of medicine [29–33], in urology, ours was the first use of this technique for examining the urethra [22]. This paper shows that the introduction of new technology — CP OCT —in conjunction with TVUS allows verification of tissue changes and assessment of the structures of the connective tissue matrix of the lower urinary tract at the level of their architectonics.

According to TVUS, in our study, women with UPS had an enlarged internal lumen of the urethra in the proximal segment — on average to 5.9±2.1 mm. According to the literature, with an intraurethral ultrasound study performed on sectioned material, the inner diameter of the proximal segment of the urethra at distances of 10, 15 and 20 mm from the neck was 3.73 mm; 4.18 mm; and 2.64 mm, respectively [27]. In another study, when measuring the internal diameter of the urethra using TVUS in women with urinary incontinence [34], the diameter in the middle third of the urethra in the control (healthy) group of patients was 4.7±1.1 mm. Thus, we have recorded an increase in the diameter of the internal lumen of the proximal urethral segment in all patients with UPS. Normally, upon initiation of urination, the mechanism for opening the funnel-shaped depression in the bladder neck is associated with contraction of the muscles of the deep triangle and of muscles located anterior to the internal opening of the urethra, as well as with the simultaneous contraction of the longitudinal muscle fibers of the urethra [28]. This means, we can assume the presence of insufficiency of these muscle groups in UPS.

Trophic disorders recorded by CP OCT in the epithelium of the urethral neck and the proximal segment of the urethra were more common in women over 50 years of age — in 93.3%, indicating their dependence on the patient's hormonal background. The hormonal dependence of a number of urinary disorders is explained in [35,36]. In these works, it was shown that in the deep layers of the mucous membrane of the urethra there is a powerful venous plexus, and that this has a large number of anastomoses with the venous uterovaginal plexus. At the same time, the works of Petros P. and Everaerts W. [37] indicated that the epithelium of the urinary system (urothelium) acts as a mechanoreceptor, using its sensitive nerve endings, and that it controls the activity of the afferent nerves, so this may contribute a pathogenetic component of chronic pelvic pain, and of urethral syndrome in particular.

Using the CP OCT method, we have previously shown that the thickness of the tissue of the urethral membrane in women is dependent on age [38]. The work reported that, with UPS, there are corresponding tendencies towards thinning of the epithelium and an increase in the thickness of the connective tissue matrix of the bladder neck, as occurs in women without pathology of the urological sphere, but that these processes proceed at a higher rate.

The recorded changes in the thickness of the epithelium are undoubtedly associated with the state of the connective tissue matrix of the subepithelium of the structural components. The compaction of the walls of the urethra and surrounding tissues that we have revealed using elastometry data, as well as in our earlier CP OCT data on the state of the connective tissue matrix of the urethra during UPS [22], indicate the presence of fibrosis processes both within the wall of the urethra and around it, the cause of which, at present, is not clear. Our studies have previously shown that the state of the urethral tissues in UPS is not normal, with changes in the urethral tissues

occupying an intermediate place between the norm and the changes seen in chronic bacterial inflammatory processes [22].

Changes in the state of the connective tissue can lead to a decrease in the sensitivity of the stretch receptors at the base of the bladder, affecting the functionality results [37], in particular, influencing the uroflowmetry data that we obtained. The results of the uroflowmetry allow us to assume the presence of functional disorders of the urethra in women with UPS. Taking into account the indices of the normal values of the urination rate for women, which are 23–32 ml/sec [26], our results of uroflowmetry showing 13.7±3.2 ml/sec are likely to be associated with anatomical changes that are not detected in standard clinical studies, or with dysfunctional and/or obstructive urination due to an overactive urethra. However, it is known that the presence of symptoms of urinary disorders is not a reliable marker of pathological processes [26]. We are continuing our research in this direction.

There is reason to believe that the cause of the development of chronic inflammatory processes in UPS is located in the tissues of the urethra and, accordingly, this serves as an additional stimulus for the occurrence of disorders of the microcirculation, innervation, and functioning of the urethra, indirectly influencing the appearance of pain. Our anamnestic data on the presence of a prevailing gynecological pathology of inflammatory genesis suggest that the cause of such changes in the tissues of the bladder and urethra may be viral-bacterial associations in the tissues of the organs of the gynecological sphere. This aspect requires more detailed study. At present, the effect of the translocation of microorganisms in the tissues of the urinary system, vagina, and intestines has been proven in cases of upper urinary tract infection [39], although research in this area is ongoing. Analyses of the composition of the microflora of urine and of the large intestine in cases of infection of the lower urinary tract have also indirectly confirmed the presence of a translocation mechanism in microorganisms [39].

It is known that the close anatomical connection of the bladder, urethra and vagina provides associated functional mechanisms for the urination process. A component of this mechanism is illustrated by the fact that in the distal urethra the circular fibers of the striated sphincter are transformed into loop structures, the ends of which are woven into the framework of the anterior vaginal wall [35]. According to the anamnesis, hormonal disorders, inflammatory diseases and surgical interventions on the pelvic organs, which could result in dysfunction of the muscles of the urethra and vagina, were found in 70.9% of patients with UPS who were interviewed. At the same time, it is known that functional disorders, on their own, can generate pain [40]. The results of our study indicated that a reason for the development of pain and chronic dysuria in patients with UPS may be failure of the structures of the internal urethral sphincter. This sphincter is formed by the muscles of the external muscular layer of the bladder that pass into the urethra in the bladder neck region, forming spiral structures, occupying about 20% of its length. In the present study on TVUS, 29.1% of women were found to have an insufficiency of structures, namely the urethral tongue, in the area of this sphincter. This fact requires further research.

Thus, it has been shown that there are many factors that cause persistent long-term pain in the urethral region, or that contribute to the intensification of pain, some of which have yet to be studied. Given the non-obviousness of the causes of UPS, new research protocols and additional imaging and diagnostic methods are required for a comprehensive examination of such patients, without focusing only on their pathologies in the urological field.

5. Conclusions

For the first time in the case of UPS, the layered structure of the urethral wall was investigated *in vivo* using CP OCT to assess some of the pathogenetic aspects of the development and progression of this disease. The CP OCT method covers the range of possibilities of traditional cystoscopy and allows information to be obtained about the state of the urethral tissues that cannot be adequately assessed during cystoscopic examination alone. The predominant changes in the tissues of the urethra are fibrosis of the subepithelial structures and trophic changes in the epithelial layer. In 68.8% of cases, the “behavior” of the tissues of the proximal segment of the urethra coincided with changes

in the bladder neck. The importance of the *in vivo* acquisition and operative analysis possible with CP OCT in combination with TVUS data in patients with UPS is beyond doubt.

Deep objective analysis of tissues can reveal the basis of pathogenesis. Real-time visualization of structural changes in the tissues of the urethra (epithelium, connective tissue, muscle layer, vasculature, paraurethral glands) is important because it influences the final diagnosis, understanding of the pathogenesis of the disease and treatment tactics. An analysis of the comorbidities of patients with UPS showed that inflammatory gynecological diseases can become a premorbid background/one of the triggering mechanisms for the development of UPS.

Author Contributions: Conceptualization, O.S. and E.K.; methodology, E.T.; software, M.M. and V.L.; investigation, O.S., A.K., M.M., S.Z. and E.T.; data curation, V.L.; writing—original draft preparation, O.S., A.K. and E.K., S.Z.; writing—review and editing, O.S., A.K. and E.K.; funding acquisition, O.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Russian Foundation for Basic Research, grant number 19-07-00395.

Acknowledgments: The authors would like to thank Dr. Grigory V. Gelikonov and Dr. Valentin M. Gelikonov (Institute of Applied Physics of the RAS, Russia) for the provision of the CP OCT device and its technical support during the research.

Conflicts of Interest: The authors declare no conflict of interest.

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