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Assessment of bilirubin concentrations in the bile of patients with obstructive jaundice by Raman spectroscopy

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Raman spectroscopy (RS) is widely used in biomedicine to detect various chemical compounds in substrates, as well as to determine their concentration. In this work, RS was used to detect bilirubin in the hepatic bile of patients with obstructive jaundice (OJ) of different etiologies obtained via a cholangiostomy drainage catheter. The spectral bands of bilirubin (1258–1264 and 1615–1620 cm^{-1}) were identified using the excitation wavelength of 785 nm. It was found that the assessment of bilirubin in bile allows to predict the recovery of excretory liver function and the dynamics of convalescence of patients with OJ after biliary decompression.

Keywords: obstructive jaundice, bile, bilirubin, Raman spectroscopy, decompression of bile ducts.

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Introduction

Obstructive jaundice (OJ) is a syndrome that develops when the outflow of bile through the intrahepatic and extrahepatic bile ducts into the duodenum is impaired [1]. The biliary obstruction may be caused by cholelithiasis, inflammatory processes, benign and malignant diseases of organs of the hepato-pancreatoduodenal zone. Impaired patency of the biliary tract is the cause of the development of hepatocyte dysfunction and leads to disorders of detoxificative, synthetic, excretory and other functions of the liver, as a result of which pathological changes develop in the body, leading to functional and morphological disorders of the liver and other vital organs [2].

Performing surgical interventions with underlying hyperbilirubinemia in patients with OJ increases the risk of intrasurgery and post-surgery complications. Therefore, a two-stage treatment strategy is currently widely used. The primary goal at the first stage of treatment of patients with OJ is decompression of the bile ducts, aimed at preoperative unloading of the biliary system and reducing biliary hypertension [3]. Despite numerous data confirming the effectiveness of antegrade decompression interventions in relieving OJ, restoring the functional state of the liver and reducing the overall complication rate during subsequent surgical interventions [3–6], the issue of widespread use of biliary decompression in the treatment of patients with OJ syndrome remains controversial [7–9].

Indications for preoperative decompression of the biliary system in most cases are based on the absolute levels of bilirubin fractions and liver enzymes in the blood serum. There are several options for surgical aids (endoscopic stenting of the biliary tract, external and external-internal percutaneous drainage of the biliary tract, antegrade prosthetics with a nitinol stent in combination with or without additional external drainage of the biliary system), aimed at ensuring adequate decompression of the biliary system in the form of external drainage of the bile ducts or restoration of the outflow of bile into the duodenum. Each of the above-listed options has its specific advantages and disadvantages. The choice of the optimal method of biliary decompression is individual for each patient and depends on many factors, including patient preference and capabilities of the clinic, and should be made by a multidisciplinary team consisting of gastroenterologists, surgeons, and interventional radiologists [10].

The degree of severity and rate of development of pathomorphological changes in the liver parenchyma depend on the rate and duration of the increase in biliary hypertension, the development of microcirculation disorders and tissue hypoxia, and the presence of inflammation in the bile ducts [11–14]. Liver failure (LF) occurs when one, several, or all liver functions are impaired. Early decompression of biliary hypertension helps in restoring liver function [15]. However, in some clinical situations, despite the installation of drainage systems in the bile ducts, LF may progress. This is due to the fact that metabolic aberrations in the liver parenchyma are not always amenable to rapid recovery and require the use of extracorporeal methods of hemocorrection or, in especially severe cases, immediate intensive therapy for the progressive LF.

Modern diagnostics of LF is based on data from experimental, biochemical, and instrumental research methods, supplemented by clinical observations. Due to the fact that OJ causes severe hemodynamic, metabolic, coagulation and immune changes in the body leading to functional and morphological disorders of the liver, in current clinical practice, many clinical and biochemical parameters, prognostic scales and systems are used to assess the severity of hepatocellular dysfunction [16]. Widely used assessments are Child-Pugh

and MELD (model for end-stage liver disease) scores, serum albumin — bilirubin level (ALBI score), and the clearance test with indocyanine green [17]. However, these criteria for assessing the functional reserves of the liver give an approximate indirect result or are used for extensive liver resections, and this determines the relevance of the problem of developing additional criteria for the objective assessment of liver functional reserves in OJ syndrome.

Bile, as a direct product of hepatocytes, is a marker of the functional state of the liver. The composition of bile is closely correlated to the clinical severity, progression, and prognosis of the disease, and its components can be reliable diagnostic and prognostic parameters [18]. For example, it is known that when gallstones form in the bile, the concentration of bile acids increases significantly [19] and cholesterol crystals appear [20]. It has also been shown that the composition of bile is closely related to the viability of the liver parenchyma and the prognosis of postoperative complications [21,22]. Changes in bile flowing through the cholangiostomy catheter, indicating the effectiveness of drainage of the biliary system and the therapy provided, are the basis for assessing the effectiveness of treatment and prognosis of the course of the disease. D.Yu. Sosnin and N.A. Zubareva identified a pattern of changes in the composition of bile in the postoperative period, reflecting the development of functional LF [23]. It was found that determining the concentration of cholesterol in bile is not inferior in sensitivity to generally accepted plasma indicators when assessing the degree of liver dysfunction.

One of the functions of the liver is the excretory function, which gives an idea of the general functional activity of hepatocytes. Bilirubin is a tetrapyrrole compound that is synthesized by reticuloendothelial cells of the liver, so its concentration in bile can serve as an important indicator for confirming the excretory function of the liver [24]. In the case of OJ, an increase in pressure in the bile capillaries leads to disorder of microcirculation and blood supply to liver cells, the membranes of the bile ducts and hepatocytes are damaged. The secretion of bilirubin and bile acids may completely stop [25]. Therefore, the content of bilirubin in bile assessed during antegrade decompression of the biliary tract can serve as the basis for rapid and highly sensitive diagnosis of the functional state of the liver parenchyma.

Determination of bilirubin in human bile is not a widespread clinical test, but has great diagnostic potential, for example, in predicting postoperative LF in patients with OJ with a recent experience of hepatectomy [26]. Various analytical methods are currently available for measuring bilirubin and its metabolites in biological fluids (usually in serum, less commonly in urine). Serum bilirubin is determined by the colorimetric method using diazo reagent, the high-performance liquid chromatography (HPLC), the electrophoresis, enzymatic and electrochemical methods, the direct spectrophotometry, etc. [27,28]. All these methods, as a rule, require additional reagents or are performed by specialists in clinical diagnostic laboratories. One of the ways to increase the information content of diagnostic algorithms in modern clinical practice, as well as to develop effective and simple point-of-care technologies, is the use of optical diagnostic methods [29–34].

The Raman spectroscopy (RS) is a vibrational molecular spectroscopy method and provides information about the chemical composition and structure of biological tissues and liquids [35]. Due to the fact that basically most biomolecules have their own typical spectral characteristics of vibrations („molecular fingerprint“), changes in their concentration, chemical transformations, as well as the formation of new compounds due to pathological processes can give characteristic RS [36]. Despite the great interest of researchers in this method, blood is most often considered among the analyzed biological fluids [37–40], and the number of studies on RS of bile is very limited. Tung Duy Vu's group analyzed the Raman spectra of extracted aqueous phases of bile to differentiate between polyps and gallbladder cancer [41].

Thus, the goal of this study is to develop a fairly simple and fast technology for obtaining objective information about the functional state of the liver as a factor determining the progression of LF, which consists of assessing the bilirubin content in bile using RS.

Materials and methods

The study involved 19 patients with OJ syndrome of various etiologies (oncological diseases of the hepato-pancreatoduodenal zone — 15 people, cholelithiasis — 2 people, cyst of the pancreatic head with compression of the common bile duct — 2 people). All patients underwent external percutaneous transhepatic cholangiostomy. Depending on clinical and laboratory parameters and general condition, patients were divided into groups with positive ($n = 16$) and negative ($n = 3$) dynamics of the course of pathological changes in the liver parenchyma. Additionally, the optical characteristics of bile from patients without OJ syndrome ($n = 3$) were studied. Bile in this group of patients was obtained through a drainage catheter from the common bile duct 5 days after surgery for cholelithiasis and can be considered as a bile of apparently healthy volunteers without OJ symptoms. All studies were conducted at the Orel Regional Clinical Hospital and were approved by the ethics committee of the Orel State University (protocol № 28 dated May 31, 2023).

Bile was sampled from patients with OJ syndrome during antegrade decompression of the biliary system using a puncture needle, and then every 3 days from the cholangiostomy catheter: up to 4 samples from each patient. The bile samples were investigated using RS. The volume of the test sample placed in the measuring cell was 5 ml. For each sample, 3 Raman spectra were recorded, which were subsequently averaged.

To record Raman spectra of bile, an experimental system was used, including a 785-LAB-ADJ laser radiation source with a wavelength of 785 nm, a QEPRO-RAMAN

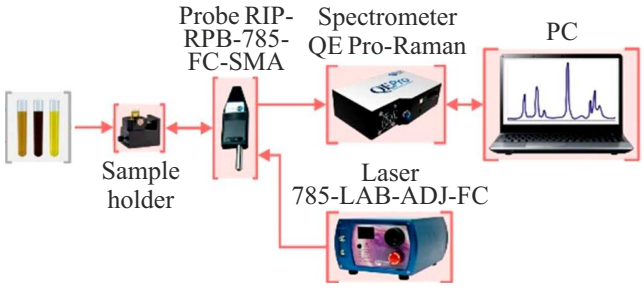

Figure 1. Schematic diagram of the experimental setup for recording Raman spectra of bile.

spectrometer with a spectral range of 780–935 nm, a OOA-HOLDER-RFA Raman Sample Holder for 10×10 mm measuring cells, a RIP-RPB-785-FC-SMA probe for Raman studies with a focal length of 7 mm (Fig. 1, *a*). The assembled setup was calibrated based on the measurement of known Raman spectra of substances, including isopropyl alcohol. It was proven that the assembled setup makes it possible to obtain Raman spectra of samples comparable to literature data.

In order to obtain Raman spectra of bile with a high signal-to-noise ratio, based on preliminary experiments, an exposure time of 90 s was selected. To reduce the contribution of fluorescence intensity to the overall signal and to eliminate photobleaching of the sample, the power of the laser source was set at a level of 16.5 mW.

To subtract the fluorescence background and to isolate Raman peaks, the fluorescence spectrum was built up using the method of polynomial approximation of the obtained data [42]. The degree of the polynomial was taken to be 10. The spectra were smoothed by filtering using the Savitzky-Golay method. To smooth out noise without suppressing Raman peaks, the following filter parameters were chosen: number of sliding window points — 15, polynomial degree — 3. All preprocessing methods and data analysis were implemented in OriginPro 2021 software. The study analyzed part of the spectrum in the range from 1000 to 1800 cm^{-1}. Detection of bilirubin was carried out in the spectral bands of 1258–1264 and 1615–1620 cm^{-1} [43].

Study results and discussion

Fig. 2 shows the results of measuring the bile of apparently healthy volunteers using RS, which were later used as a reference in the studies. The Raman lines of bilirubin are clearly visible in the spectra. Line 1260 cm^{-1} in the Raman spectrum of bile is caused by bending and stretching of the C-H bond. The appearance of a peak in the region of wavenumbers of 1615 cm^{-1} is associated with the bending of the C=H bond in the five-membered carbocycle [44]. The signal intensity (in arbitrary units, arb. units) in the wavenumber region of 1260 cm^{-1} was 1840 ± 160 and in the region of 1615 cm^{-1} it was 1833 ± 77 (average value ±SD).

Fig. 3 shows the Raman spectra of bile in patients with OJ and with different dynamics of the pathological process in the liver. It was found that the RS peaks of bilirubin in bile in all patients do not always have a fixed position and can be within the spectral bands of 1258–1264 and 1615–1620 cm^{-1} . The shift in the amplitude of the RS peak may be associated with the conjugation of bilirubin with glucuronic acid [44] and requires a separate study. When processing statistical data, the maximum amplitude in the above-specified range was selected for each patient.

Depending on the form of spectral curves of bile obtained on the day of decompression of the biliary tract, patients with positive dynamics of recovery according to clinical and laboratory parameters and assessment of the general condition by the attending physician were divided into three groups. Laboratory parameters of the studied patients over time are summarized in the table. Due to the large scatter of data and the asymmetry of the law of their distribution on different days of research, hereinafter the information is presented in the format of Me [Q1–Q3], where Me is median, Q1 is the first quartile , Q3 is the third quartile.

In the first group (6 patients), on the first day of observation, almost no bilirubin peaks were detected in bile (Fig. 3, *a*) or their amplitude was extremely low: 167 [71–212] (1258–1264 cm^{-1}) (Fig. 3, *d*) and 385 [146–414] (1615–1620 cm^{-1}) (Fig. 3, *g*). Laboratory data indicated hepatosuppression on the background of biliary blockage. Typically, bile samples from such patients were colorless or light yellow. An increase in pressure in bile ducts results in disorder of the secretion of hepatocytes, because they have to overcome greater resistance to release secretions into the biliary capillaries. With OJ, there is an obstruction to the outflow of bile from the liver acini. As pressure increases in the biliary capillaries, microcirculation and blood supply to liver cells are disordered, and the membranes of the bile ducts and hepatocytes are damaged [45]. The secretion of bilirubin and bile acids can completely stop, which is observed in the Raman spectra of bile from patients in the first group. After decompression

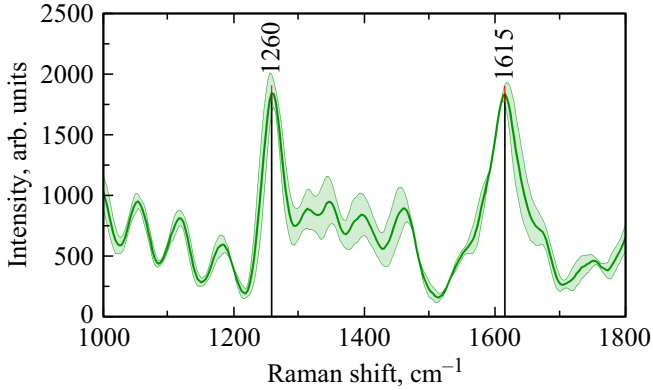

Figure 2. Bile spectra of patients without OJ syndrome.

Laboratory parameters of patients without OJ syndrome and patients with OJ syndrome with different courses of the postoperative period

Parameter	Protein, g/l	Urea, μmol/l	Creatinine, μmol/l	Bilirubin total, μmol/l	Bilirubin direct, μmol/l	ALAT, IU	ASAT, IU	ALP, u/l
Apparently healthy volunteers (without OJ syndrome)								
5th day	79 [77; 81]	5.8 [4.7; 6.1]	74 [70; 78]	15 [14; 15]	4 [3; 4]	28 [27; 29]	24 [23; 25]	192 [188; 197]
Group 1 patients (with OJ syndrome, with positive dynamics of the pathological process in the liver)								
day 1	69 [68; 71]	6.4 [5.2; 7.6]	94 [92; 109]	232 [194; 250]	84 [65; 104]	333 [269; 407]	150 [113; 203]	649 [609; 666]
day 4	69 [67; 71]	4.9 [4.7; 5.0]	76 [75; 87]	149 [136; 180]	61 [59; 92]	255 [211; 300]	119 [93; 134]	404 [384; 446]
day 7	67 [64; 69]	4.7 [4.3; 5.4]	78 [76; 83]	126 [112; 165]	45 [33; 82]	155 [130; 208]	88 [84; 99]	326 [305; 361]
day 10	69 [66; 72]	3.7 [3.4; 4.6]	85 [77; 93]	80 [73; 135]	37 [28; 66]	186 [106; 276]	112 [78; 147]	296 [279; 312]
Group 2 patients (with OJ syndrome, with positive dynamics of the pathological process in the liver)								
day 1	68 [66; 69]	5.1 [5.1; 9.8]	99 [89; 114]	395 [259; 436]	232 [173; 259]	329 [261; 362]	244 [206; 264]	349 [222; 555]
day 4	66 [65; 67]	4.4 [4.0; 5.0]	79 [79; 90]	111 [90; 214]	68 [52; 89]	109 [34; 196]	88 [23; 146]	292 [231; 454]
day 7	72 [68; 76]	4.7 [4.3; 7.0]	76 [73; 88]	125 [107; 142]	40 [36; 45]	441 [129; 748]	112 [87; 174]	550 [320; 853]
day 10	68 [64;72]	4.9 [3.6; 6.3]	85 [78; 91]	105 [79; 112]	48 [46; 53]	169 [115; 226]	127 [90; 153]	373 [224; 672]
Group 3 patients (with OJ syndrome, with positive dynamics of the pathological process in the liver)								
day 1	70 [69; 74]	9.0 [8.0; 11.5]	96 [83; 181]	160 [124; 367]	42 [39; 50]	250 [241; 278]	146 [138; 174]	229 [229; 234]
day 4	67 [66; 69]	7.4 [6.0; 8.7]	95 [94; 108]	153 [108; 160]	23 [21; 37]	245] [234; 319]	89 [85; 242]	170 [168; 171]
day 7	65 [64; 73]	4.6 [4.2; 4.9]	80 [79; 83]	42 [31; 57]	15 [9; 16]	43 [39; 70]	60 [51; 79]	165 [164; 188]
Patient 1 (with OJ syndrome, with negative dynamics of the pathological process in the liver)								
day 1	67	8.1	134	534	310	320	274	627
day 4	63	9.3	156	503	265	312	256	608
day 7	61	10.7	211	510	273	310	260	603
day 10	58	12.1	300	482	251	287	226	574
Patient 2 (with OJ syndrome, with negative dynamics of the pathological process in the liver)								
day 1	78	7.4	93	396	241	210	186	623
day 4	72	8.0	142	380	257	217	193	605
Patient 3 (with OJ syndrome, with negative dynamics of the pathological process in the liver)								
day 1	66	13.2	126	417	368	428	376	712
day 4	62	11.4	119	341	295	382	312	673

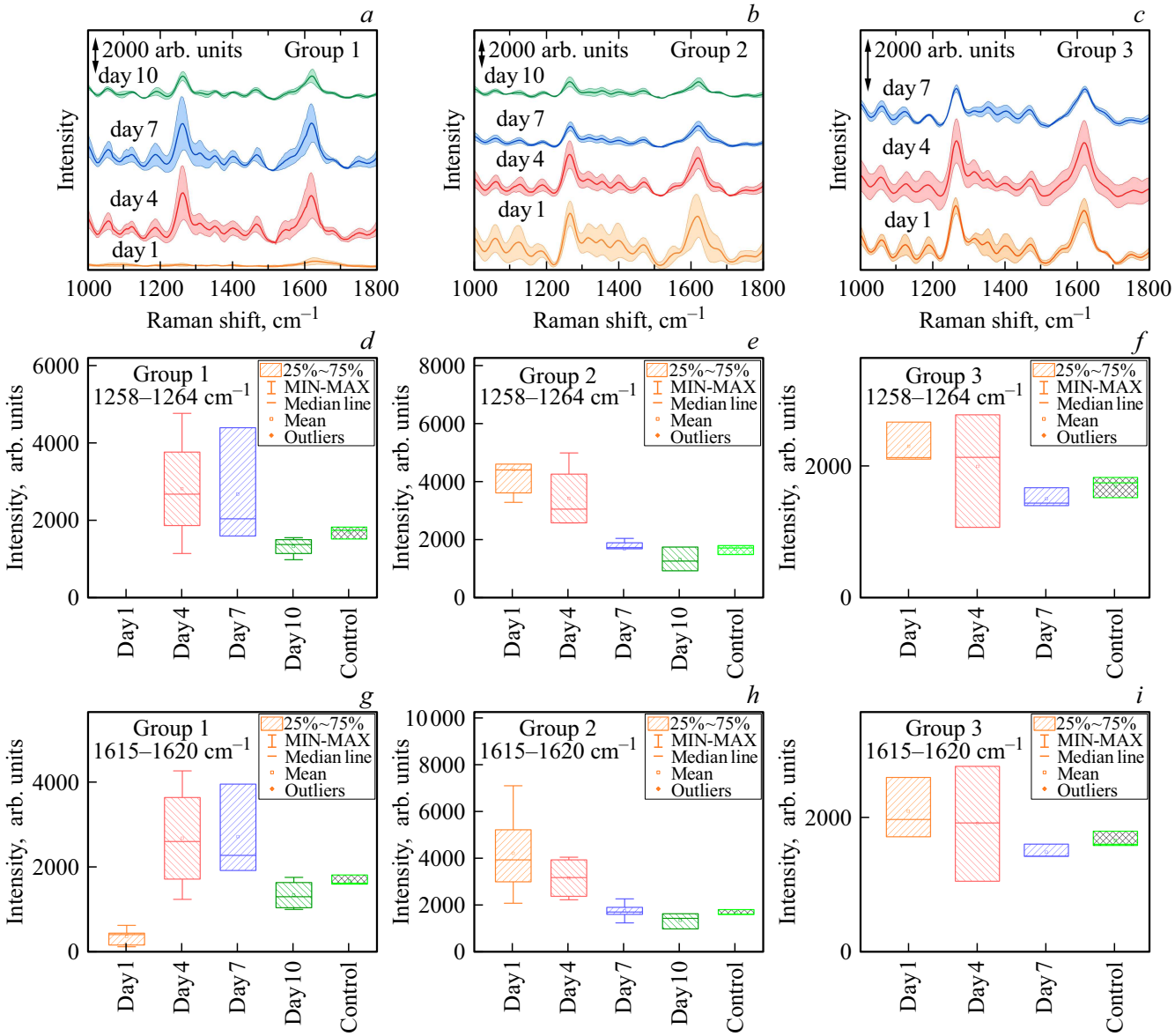

Figure 3. Raman spectra of bile from patients with positive dynamics of the pathological process in the liver (*a*–*c*), dynamics of changes in the intensity of Raman peaks of bilirubin in the region of wavenumbers of 1258–1264 cm^{-1} (*d*–*f*), dynamics of changes in the intensity of Raman peaks of bile in the region of wavenumbers of 1615–1620 cm^{-1} (*g*–*i*).

of the biliary system, the pressure in the liver stabilizes and the outflow of bile is restored. Some patients in this group experience a compensatory increase in the level of bilirubin in bile to a level of more than 3000: on the fourth day of the study 2669 [1861–3753] (1258–1264 cm^{-1}) and 2583 [1699–3616] (1615–1620 cm^{-1}), on the seventh day 2063 [1600–4312] (1258–1264 cm^{-1}) and 2256 [1901–3930] (1615–1620 cm^{-1}) with gradual normalization of bilirubin levels by the tenth day of the study to a level of about 1300 in both ranges: 1371 [1139–1495] (1258–1264 cm^{-1}) and 1284 [1027–1619] (1615–1620 cm^{-1}) (Fig. 3, *d* and *g*). At all time points of the study, the laboratory data of the patients showed a gradual decrease in the level of parameters critical for assessing the functional state of the liver, allowing to proceed to the next stage of surgical tactics by the fourth day for urgent indications, and to the treatment as planned by the tenth day.

In the second group (7 patients), laboratory signs of severe cholestasis were observed with impaired functional state of the liver and very high amplitudes of bilirubin in bile, recorded on the day of antegrade decompression (Fig. 3, *b*): 4435 [3586–4663] (1258–1264 cm^{-1}) (Fig. 3, *e*) and 3990 [3000–5120] (1615–1620 cm^{-1})(Fig. 3, *h*). The bile of this group of patients had a darker color. We assume that because of stop of bile flow into the duodenum, hepatocytes actively produce bile secretion into the biliary capillaries until their

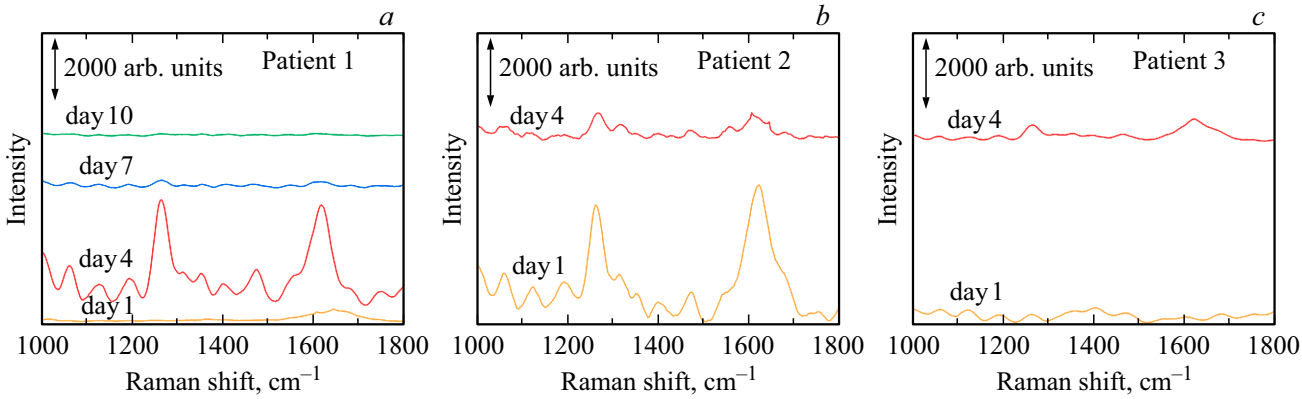

Figure 4. Raman spectra of bile from patients with negative dynamics of the pathological process in the liver.

membranes are damaged under the action of the increasing biliary decompression. This assumption is confirmed by the fact that after decompression of the biliary tract, this group of patients as early as by the seventh day of the dynamic examination had bilirubin spectrum intensity in the range of values observed in apparently healthy volunteers: 1767 [1685–1883] (1258–1264 cm^{-1}) (Fig. 3, *e*) and 1750 [1611–1856] (1615–1620 cm^{-1}) (Fig. 3, *h*), and laboratory parameters decreased to a level allowing radical surgical planning for urgent indications. By the tenth day of the study, the amplitudes of spectral lines of bilirubin also decreased to a level of about 1300 in both ranges: 1294 [965–1767] (1258–1264 cm^{-1}) (Fig. 3, *e*) and 1422 [972–1615] (1615–1620 cm^{-1}) (Fig. 3, *h*) with a tendency to laboratory parameters decrease allowing for planned radical surgery.

The third group included patients (3 people) with an initially relatively satisfactory condition based on the totality of the results of a biochemical blood test and a general assessment of the patient's condition by a doctor. Such clinical situations are associated with an incomplete or partial blockage of the biliary tract, when, on the background of cystic neoplasms or incomplete stricture of the tumor tissue, a certain amount of bile continues to flow beyond the blockage into the duodenum. This is sufficient to maintain normal hepatocyte function, however, it does not exclude the presence of biliary hypertension. The third group of patients was examined three times (Fig. 3, *c*), because the stabilization of the test results occurred quickly, and the patients were discharged or transferred to other departments for further therapy. On the seventh day of studies of such patients, the amplitudes of the spectral lines of bilirubin were: 1429 [1393–1638] (1258–1264 cm^{-1}) (Fig. 3, *f*) and 1424 [1419–1603] (1615–1620 cm^{-1}) (Fig. 3, *i*). Laboratory indicators for this period of time allow planning radical surgery.

Fig. 4 shows the results of RS of bile from patients with negative dynamics of the pathological process in the liver. The results of the study for each patient are presented separately. Patient 1 was admitted to the hospital with a severe form of impaired excretory function of the liver with the absence of bilirubin peaks in the spectral characteristics of bile (Fig. 4, *a*). In the process of performing antegrade biliary interventions, the excretory function was restored for some time, however, by the seventh day, the Raman spectra again appeared without obvious bilirubin peaks. It is worth noting that the color of this patient's bile did not differ from the bile spectra of patients who showed positive dynamics of recovery, which predetermines the issue of the possibility of visual assessment of bilirubin content in bile. Patient 2 was admitted to the hospital with compensation for the excretory function of the liver, the spectral characteristics of bile contained high peaks of bilirubin, but by the fourth day the patient did not show positive dynamics in the normalization of the spectra and was transferred to the intensive therapy ward (Fig. 4, *b*). Patient 3 was admitted to the hospital with severe suppression of the excretory function of the liver with the absence of bilirubin peaks in the spectral characteristics of bile (Fig. 4, *c*), and responded poorly to minimally invasive interventions aimed at decompressing the biliary system; the patient was also transferred to the intensive therapy ward. The dynamics of changes in laboratory parameters in these three patients indicated an unsatisfactory response of the liver parenchyma to antegrade biliary decompression and the need for active use of extracorporeal detoxification methods [46].

It is worth noting that the obtained values are lower than those recorded for apparently healthy volunteers. This may be due to incomplete recovery of the liver excretory function in patients with OJ syndrome.

Currently, a comprehensive assessment of the functional state of the liver is based on clinical and laboratory data, based on the information about the duration and severity of ochrodermia and/or itching of the skin, the presence of low-grade fever, increased blood levels of bilirubin and its fractions, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyltransferase (γ-GT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), cholesterol, bile acids, phospholipids, β -lipoproteins, 5-nucleotidase [25,47]. At the same time, very little

attention is paid in the literature to the significance of metabolic disorders in hepatocytes and the state of their product — bile — in OJ. In turn, changes in the composition of bile may be indicative of the effectiveness of various methods of biliary decompression, including by assessing the recovery of the excretory function of the liver.

Based on the results obtained by RS of bile it can be said that normalization of Raman peaks in both regions of study (with a given configuration and operating modes of the diagnostic setup) to intensities in the range of 1300–1600 correlates with the clinical and laboratory data on recovery of the liver function and general condition of patients. Normalization of the intensity of the Raman spectral lines of bilirubin can be taken into account in the decision to prepare the patient for the second stage of OJ treatment: lithoextraction in the case of cholelithiasis or tumor removal if the OJ syndrome has an oncological etiology. The diagnostic criterion developed in the study can be used in a comprehensive assessment of LF in patients with OJ, which will allow doctors to timely adjust treatment algorithms in the postoperative period.

The prospect of this study is to refine the methodology, including the use of surface-enhanced Raman spectroscopy (SERS) [48,49] of bile samples. This will allow studying the concentration of other substances in bile, including bile acids and cholesterol, which characterize the detoxification and synthetic functions of the liver. Also, the results obtained may be useful in studies of bilirubin content in drainage fluid [50] to develop predictors of complications in patients with liver transplantation.

Conclusions

The most important issue in modern surgery is the development of objective methods for predicting the course of the postoperative period in patients with OJ against the background of antegrade biliary decompression, which will allow timely adjustment of treatment regimens, including establishing indications for repeated surgical interventions, including extracorporeal methods of hemocorrection, etc. Currently, the decision on the duration of biliary decompression is made based on standard biochemical tests, however, the best solution would be to assess the period of recovery of liver functions, including the excretory function.

The experimental system presented in this study showed sufficient effectiveness for assessing the bilirubin content in bile. The choice of the laser wavelength of 785 nm is justified by reducing the influence of fluorescence of bile components, including bilirubin itself, on the recorded Raman spectra. The use of longer wavelength radiation for multicomponent hydrated media for this purpose is difficult, because the absorption of exciting radiation by water will have a significant impact on the measurement results, which may resulted in overheating and even boiling of bile samples. The proposed configuration (laser radiation source, probe, detector) and system operating modes (exposure time, laser power), as well as post-processing algorithms in the range from 1000 to 1800 cm^{-1} with minimal influence of background fluorescence of samples allow recording bilirubin RS peaks even in patients with low levels of this pigment in bile.

The method for assessing the recovery of the liver excretory function in patients with OJ syndrome based on the amplitude of bilirubin Raman lines is relatively simple and does not require additional reagents. In contrast to other research methods, the result can be obtained in the operating room and at the patient's bedside, used on the day of taking the test to assess the functional state of the liver and additional diagnostics of LF in the case of OJ.

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Compliance with ethical standards

All studies were conducted in accordance with the principles of biomedical ethics formulated in the Declaration of Helsinki 1964 and its subsequent updates, and approved by the local bioethical committee of the Orel State University (protocol № 28 dated May 31, 2023) (Orel).

Conflict of interest

The authors declare that they have no conflict of interest.

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