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Ya. M. Vakhrushev[1], N. A. Khokhlacheva*[1], T. Yu. Maksimova[2]

[1] — Izhevsk State Medical Academy, Izhevsk, Russia

[2] — City Clinical Hospital No.8 n. a. I.B. Odnopozov, Izhevsk, Russia

STUDY OF PHYSICO-CHEMICAL PROPERTIES OF BILE AFTER CHOLECYSTECTOMY ON CHOLELITHIASIS

Abstract

The objective of the study: To study the physicochemical properties of hepatic bile and the state of lipid metabolism before and after cholecystectomy in cholelithiasis. **Material and methods.** 210 patients with stage I cholelithiasis (comparison group) and 90 patients who underwent cholecystectomy for stage II and III of the cholelithiasis (observational group) were examined. The groups were balanced by gender and age. In verification of the diagnosis, in addition to general clinical data, the results of ultrasound examination of the biliary system were used. A duodenal biliary drainage was carried out followed by a gross and microscopic examination of the hepatic portion of bile, determination of its physical properties and chemical composition. Lipid blood metabolism was studied with an estimate of the atherogenic index. **Results.** Ultrasound signs of biliary sludge were found in 86% of the comparison group patients, and in 37% of patients in the observational group there was a bile duct dilatation. The study of the chemical composition of the hepatic bile of patients in both groups revealed an increase in cholesterol, total sialic acids and total protein, a decrease in bile acids, phospholipids, cholate-cholesterol and phospholipid-cholesterol coefficients. In the study of physical properties, a thickening of bile and an increase in its viscosity were established. Evaluation of the lipid spectrum of blood revealed that lipid metabolism disorders, which are present in patients with cholecystectomy, are preserved after cholecystectomy. **The conclusion.** After cholecystectomy in cholelithiasis treatment, bile remains lithogenic, which is evidenced by its changed physicochemical parameters. Patients after cholecystectomy require preventive care to avoid lithogenic bile formation.

Key words: *cholelithiasis, gall bladder, cholecystectomy, lithogenic properties of bile, lipid metabolism*

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V_b — bile viscosity, CLS — cholelithiasis, GB — gall bladder, AI — atherogenic index, HDL — high-density lipoprotein, LDL — low-density lipoprotein, VLDL — very low-density lipoprotein, TP_b — total biliary protein, ST_b — surface tension of bile, pH_b — pH of bile, SA_b — total sialic acids of bile, TGs — triglycerids, SG_b — specific gravity of bile, PL_b — biliary phospholipids, PLC_b — phospholipid to cholesterol ratio, Ch_b — biliary cholesterol, Ch_{bl} — total blood cholesterol, BAC_b — bile acids to cholesterol ratio, CE — cholecystectomy

Introduction

According to international statistics, cholelithiasis morbidity doubles every ten years [1, 2]. In Russia, cholelithiasis (CLS) is rightfully considered one of the most common diseases with 5 to 40% prevalence depending on the region [3, 4]. Surgery (cholecystectomy, CE) remains the main treatment option, being the most common surgical procedure after herniotomy and appendectomy [5, 6, 7].

* Contacts. E-mail: stoxel@yandex.ru

CE that is indicated and performed on time is considered to improve clinical symptoms and to result in complete recovery of working ability and quality of life [8]. However, gall bladder (GB) removal only relieves the body of the impaired organ, though it does nothing to compensate for complex pathophysiological disorders that are present in patients with CLS. Therefore, it cannot be considered the final stage of treatment [9, 10, 11]. There is a high probability of postcholecystectomy syndrome with such manifestations as cholangiolithiasis and choledocholithiasis that develop in 30% of patients and become the most common cause of recurrent pain and repeated surgeries.

Up to the present day, the pathogenic mechanisms of lithogenesis remain understudied. Preserved dyscholia is considered to be its probable cause [6]. A better understanding of this aspect of the problem would allow expanding prevention options after CE.

The Objective of the Study

To evaluate physicochemical properties of liver bile and lipid metabolism before and after cholecystectomy for CLS treatment.

Materials and Methods

A total of 210 patients (comparison group) with stage I (prelithiasis) CLS (according to the classification of the Central Scientific Research Institute of Gastroenterology, 2001) [4] and 90 patients (study group) after cholecystectomy for stages II and III (lithiasis stage) CLS were examined. The groups were balanced in terms of gender and age. The mean age in the study group was (58 ± 6) years. A total of 37 patients in this group were males, and 53 were females. Cholecystectomy had been performed 3 to 8 years ago. The mean age in the comparison group was (54 ± 8) years. A total of 84 patients in this group were males, and 126 were females. Patients were examined only after they signed a mandatory Informed Consent in accordance with the Order No. 390н of the Ministry of Health and Social Development of the Russian Federation of April 23, 2012 (registered by the Ministry of Justice of the Russian Federation on May 05, 2012 under record number 24082). The study received approval from the Ethics Committee of the Federal State Budgetary Institution of Higher Education Izhevsk State Medical Academy. The scope of examination was statistically justified by sample frequency using the Sachs equation. The groups were formed by random and stratified sampling.

Medical history data and biliary ultrasound results that were obtained with S-DN-500 device were used to verify the diagnosis. All patients underwent duodenal intubation followed by gross and microscopic examination of a liver bile portion (C portion). Then its physical properties were determined (specific gravity — SG_b, surface tension — ST_b, viscosity — V_b, acidity — pH_b) and biochemical composition (total bile acids — BA_b, cholesterol — Ch_b, and phospholipids — PL_b [12, 13]). Bile acids to cholesterol ratio (BAC_b) and phospholipid to cholesterol ratio (PLC_b), which are indices of liver lithogenicity, were calculated. Total sialic acids (SA_b) were measured by SialoTest [14], and total protein (TP_b) was measured using a FP-901 (M) analyzer from Labsystems (Finland).

Lipid metabolism was evaluated based on plasma total cholesterol (Ch_{bl}), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TGs). Total cholesterol, HDL and TGs were measured using a FP-901 (M) analyzer from Labsystems (Finland). VLDL and LDL levels were calculated using the following equation: VLDL = TGs / 2, LDL = Total cholesterol — (VLDL + HDL). Based on obtained data, the atherogenic index (AI) was determined using the following equation: AI = Total cholesterol — HDL / HDL.

Results of laboratory tests were compared to data obtained from a control group (50 apparently healthy subjects between the ages of 20 to 40).

Statistical analysis was performed on an AMD Sempron mobile x86 personal computer using Microsoft Excel (MS Windows XP Professional) software and Biostat library. Mathematical tools included conventional methods for relative (P) and mean (M) values, including the determination of errors (±m). In some cases outlying values were excluded. Significance was evaluated using a non-parametric Wilcoxon signed-rank test for samples with normal distribution. The difference is significant at $p < 0.05$.

Correlation coefficient was calculated using Pearson's equation

$$r = \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\sqrt{\Sigma(x-\bar{x})^2\Sigma(y-\bar{y})^2}}$$

where r – correlation index;

x, y – variables;

$\bar{x}, \bar{y}$ – mean variable values.

The correlation is significant at $p < 0.05$.

Results and Discussion

Biliary ultrasound revealed signs of biliary sludge (microlithiasis, nonhomogeneous echo pattern of bile with clots) in the gall bladder of 86% in patients from the comparison group. Thirty-seven percent of patients from the study group (with removed gall bladders) had bile duct dilatation (to 10–12 mm). Gross examination of liver bile portions from all patients showed that it was not liquid, was heterogeneous, and contained flakes; microscopic examination revealed crystals of cholesterol and microliths. Chemical composition of the C portion is provided in Table 1. According to the table, the Ch_b level was significantly elevated, and the levels of BA_b and PL_b that stabilize bile and prevent the settling of cholesterol crystals were decreased. The lithogenicity of bile was confirmed by drastically decreased BAC_b and PLC_b. The SA_b level (an indicator of biliary mucosa inflammation) was elevated. Inflammation leads to slower absorption of proteins that enhance cholesterol nucleation [15], which is evidenced by obligatory presence of these proteins in the core of cholesterol stones [16, 4]. Proteins may also act as a cementing factor in gallstone formation [17].

Examination of the physical properties of bile revealed elevated SG_b, V_b and ST_b as well as decreased pH_b in both groups (Table 2). The thickening of bile and increased viscosity reduces the solubility of various components in bile, and in particular, it enhances the settling of cholesterol crystals [18, 19, 20, 21].

The absence of significant differences between physicochemical parameters of bile in the comparison and study groups indicates that the composition of liver bile does not change essentially after CE and bile remains prone to lithogenesis.

A correlation analysis (Table 3) established a negative correlation between the specific gravity, viscosity, surface tension, total protein, sialic acids level, and lithogenicity indices in both groups. The correlation between the acidity of bile and lithogenicity

Table 1. *The results of a chemical study of hepatic bile*

Parameter	Control (n=50)	Observational group (n=90)	Comparison group (n=210)	p_1	p_2	p_{12}
Cholesterol, mmol/l	3,63±0,06	13,74±0,46	16,38±0,54	$5{,}0\times10^{-27}$	$1{,}3\times10^{-22}$	0,07
Bile acids, mmol/l	20,76±0,20	13,84±0,52	15,44±0,59	$4{,}9\times10^{-13}$	$3{,}1\times10^{-13}$	0,40
Phospholipids, mmol/l	0,39±0,00	0,19±0,01	0,21±0,02	$9{,}9\times10^{-27}$	$1{,}0\times10^{-21}$	0,69
Cholate-cholesterol ratio, UOM	6,14±0,10	1,06±0,05	1,14±0,05	$6{,}5\times10^{-28}$	$1{,}3\times10^{-22}$	0,99
Phospholipid-cholesterol ratio, UOM	0,11±0,002	0,02±0,002	0,02±0,002	$8{,}4\times10^{-31}$	$9{,}0\times10^{-23}$	0,06
Total protein, g/l	3,50±0,03	12,71±0,29	14,51±0,28	$4{,}2\times10^{-28}$	$1{,}3\times10^{-22}$	0,4
Sialic acids, mmol/l	1,85±0,09	3,92±0,11	4,24±0,08	$3{,}6\times10^{-25}$	$7{,}7\times10^{-19}$	0,06

Note: n — the number of observations; p_1 — reliability of differences in the comparison group relative to the control group; p_2 — reliability of differences in the observation group relative to the control group; p_{12} — the reliability of the differences between the observation group and the comparison group

Table 2. *The results of the study of the physical properties of the bile portion «C»*

Parameter	Control (n=50)	Observation group (n=90)	Comparison group (n=210)	ρ_1	ρ_2	ρ_{12}
Specific gravity, UOM	1010,22±0,18	1031,71±0,92	1029,52±0,58	5,8×10^{-27}	1,4×10^{-22}	0,06
Viscosity, UOM	2,52±0,02	6,64±0,40	6,82±0,15	5,1×10^{-28}	1,3×10^{-22}	0,06
Surface tension, mkN/m	22,05±0,14	40,15±0,66	41,84±0,54	2,6×10^{-27}	1,3×10^{-22}	0,07
Acidity, UOM	7,62±0,06	10,96±0,21	10,33±0,17	4,3×10^{-14}	7,4×10^{-21}	0,22

Note: n – the number of observations; ρ_1 _ the reliability of differences in the comparison group relative to the control group; ρ_2 — the reliability of differences in the observation group relative to the control group; ρ_{12} — the reliability of the differences between the observation group and the comparison group

Table 3. *Correlation between lithogenicity indices and physicochemical parameters of hepatic bile in cholelithiasis*

Parameter	Specific gravity	Viscosity	Surface tension	Total protein	Sialic acids
Cholate-cholesterol ratio	r=-0,36	r=-0,26	r=-0,43	r=-0,44	r=-0,31
ρ	0,08	9,70x10^{-17}	1,23x10^{-44}	1,84x10^{-47}	0,08
Phospholipid-cholesterol ratio	r=-0,33	r=-0,31	r=-0,41	r=-0,48	r=-0,35
ρ	0,03	0,001	0,0001	0,0004	0,0001

Note: r — correlation; ρ — reliability of correlation

Table 4. *Indicators of lipid blood metabolism*

Parameter	Control (n=50)	Observation group (n=90)	Comparison group (n=210)	ρ_1	ρ_2	ρ_{12}
Cholesterol, mmol/l	5,22±0,07	5,75±0,15	5,75±0,18	0,03	0,36	0,11
Very low-density lipoproteins, mmol/l	0,40±0,00	0,90±0,05	0,76±0,02	1,1×10^{-18}	1,2×10^{-17}	0,003
Low-density lipoproteins, mmol/l	3,34±0,07	3,97±0,14	4,05±0,18	0,001	0,02	0,23
High-density lipoproteins, mmol/l	1,38±0,01	0,85±0,02	0,92±0,01	3,5×10^{-22}	1,1×10^{-27}	0,3
Triglycerides, g/l	0,83±0,02	1,97±0,10	1,91±0,18	3,3×10^{-19}	2,1×10^{-19}	0,07
The coefficient of atherogenicity, UOM	2,62±0,04	5,77±0,21	5,32±0,19	7,8×10^{-21}	1,8×10^{-22}	0,07

Note: n – the number of observations; ρ_1 _ reliability of differences in the comparison group relative to the control group; ρ_2 — reliability of differences in the observation group relative to the control group; ρ_{12} — the reliability of the differences between the observation group and the comparison group

Table 5. *Correlation between lipid metabolism indices and bile lithogenicity indices*

	High-density lipoproteins	Low-density lipoproteins	Triglycerides	The coefficient of atherogenicity
Cholate-cholesterol ratio	r=0,39	r=-0,07	r=-0,34	r=-0,32
ρ	$2,22\times10^{-16}$	0,02	0,0004	$2,22\times10^{-13}$
Phospholipid-cholesterol ratio	r=0,41	r=-0,14	r=-0,31	r=-0,39
ρ	0,0003	0,08	0,08	0,008

Note: r — correlation; ρ — reliability of correlation

indices was positive. The results of correlation analysis in the comparison and study groups were unidirectional.

Therefore, bile becomes more prone to lithogenesis when it is thicker (higher SG_b, V_b, ST_b) and upon progression of inflammation in biliary ducts (elevated SA_b and TP_b).

Taking into account the high proportion of cholesterol in gallstones and in accordance with the modern theory of CLS pathogenic mechanisms, impairment of lipid metabolism plays an important role as a cause of lithogenesis. Table 4 shows that similar changes in blood lipids were observed in both groups: decreased HDL as well as increased VLDL, LDL and TGs with corresponding significant increase in AI. The absence of significant differences between the parameters in the study and comparison groups indicates that lipid disorders present in patients with CLS persist after CE.

Table 5 shows that lipid disorders play an essential role in the formation of lithogenic bile. Thus, bile lithogenicity increases with the reduction of non-atherogenic cholesterol fractions (HDL) and an increase in atherogenic fractions (LDL and TGs) in the blood. Therefore, the higher blood AI, the higher is bile lithogenicity.

Conclusion

In summary, we can say that after CE for CLS, bile remains lithogenic, which is evidenced by its changed physicochemical parameters and persisting lipid disorders. Since there is no “storage reservoir” for bile (like the gall bladder), there is a risk of calculi formation in biliary ducts.

Our data suggest that patients after cholecystectomy require preventive care to avoid lithogenic bile formation.

Conflict of interests

The authors declare no conflict of interests.

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