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BILE MORPHOMETRIC ANALYSIS IN EARLY DIAGNOSIS OF GALLSTONE FORMATION

Abstract

Study objective. To study the changes in the morphological pattern of bile depending on the age of patients and the possibility of using the information obtained in the early diagnosis of gallstone formation. **Material and methods.** The study enrolled 396 patients with stage I cholelithiasis, group 1 consisted of 125 patients of young adult age (30–44 years), group 2 — 164 patients of middle adult age (45–59 years), and group 3 — 107 patients of late adult age (60–74 years). The studied groups were gender-balanced. In the verification of the diagnosis, in addition to general clinical data, results of the gallbladder ultrasound were used. Multifractional duodenal drainage with the subsequent macroscopic, microscopic, morphometric, biochemical, and physical bile tests was carried out. To assess the morphological pattern and features of the microstructure of bile, crystallogram analysis was carried out. **Results.** According to gallbladder ultrasound, signs of biliary sludge were determined in all patients. The destabilization of bile is evidenced by an increase in cholesterol, a decrease in bile acids, cholesterol-to-bile acid ratio, an increase in its viscosity and surface tension. A morphometric study of bile at the early stage of gallstone disease showed a decrease in the angle of slope of liquid crystal lines, as well as the appearance of optically active inclusions, such as branched dendrites with lamellar branches, tangled fibrous aggregates, shield-shaped aggregates and short branched dendrites. With an increase in the tendency of gallstone formation, the optical activity of microcrystals increases, lamellar druses and branched plateau-like aggregates are determined. **Conclusion.** The crystal-optical method of bile analysis is highly sensitive, but at the same time it is easy to perform and can be widely used in the early diagnosis of CLT. The degree of impairment in the bile microstructure increases with increasing age of patients.

Key words: *early diagnosis of gallstones, lithogenic bile, bile morphometry*

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BV — bile viscosity, CLT — cholelithiasis, BA_B — bile acids, GB — gallbladder, LCL — liquid-crystalline line, ST_B — surface tension of bile, CL_B — cholesterol of bile, CBR_B — biliary cholesterol-to-bile acid ratio

Introduction

Cholelithiasis (CLT) can be attributed to the category of socially significant pathology and, of course, be considered as one of the urgent issues of clinical gastroenterology due to the increase in morbidity with coverage of the most able-bodied part of the population, a pronounced negative impact on social activity and quality of life [1, 2, 3]. Great difficulties in early clinical diagnosis and timely use of preventive measures are caused by nonspecific clinical signs and "silent" course of the disease.

In recent years, the study of the phase composition of various biological media in the body based on the idea of liquid crystals involved in the pathogenesis of a number of diseases has become increasingly

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common [4, 5]. According to few literature data, some biological fluids, in particular, bile, under certain conditions, are able to crystallize with the formation of patterns. The change in morphology of the latter depends significantly on the nature of the pathological process, which allows them to be used for diagnostic purposes [6, 7, 8, 9].

Study Objective

To study the changes in the morphological pattern of bile depending on the age of patients and the possibility of using the information obtained in the early diagnosis of gallstone formation.

Materials and Methods

Three hundred and ninety-six patients with stage I CLT (classification of Central Research Institute of Gastroenterology (CRIG), 2001), secondary to hepatobiliary diseases (functional disorders of the biliary system, chronic non-calculous cholecystitis, non-alcoholic fatty liver and non-alcoholic steatohepatitis) were examined. According to the WHO classification (2012), the 1st group consisted of 125 patients of young adult age (30 to 44 years), 2nd group — of 164 patients of middle adult age (45 to 59 years), and 3rd group — of 107 patients of late adult age (60 to 74 years). Examination of patients was carried out on the basis of informed voluntary consent according to the order No. 390n of the Ministry of Health of the Russian Federation dated April 23, 2012 (registered by the Ministry of Justice on May 5, 2012 under No. 24082), in compliance with ethical principles.

The scope of the study was justified statistically by the sampling frequency using the following formula

$$n=t^2\rho q/\Delta^2,$$

where n is the number of observations in the sample study, ρ is the frequency of the studied event, q is the difference between the conditional number based on which the frequency of the studied event is calculated and the value of this parameter, t is the confidence coefficient, and Δ is the maximum error.

Group formation was carried out using random and stratified sampling procedure among patients who were hospitalized in the internal medicine and gastroenterology departments of hospital No. 8 of Izhevsk.

In the verification of the diagnosis, along with anamnestic and general clinical data, the results of ultrasound examination of the gallbladder were taken into account. All patients underwent multifractional duodenal drainage followed by macroscopic, microscopic, morphometric, biochemical, and physical examination of bile.

To identify signs of bile destabilization in bile portions B and C, the total concentration of bile acids (BA_B) and cholesterol (CL_B) was determined [10], the biliary cholesterol-to-bile acid ratio (CBR_B), which is an index of bile lithogenicity, was calculated. The study of the surface tension of bile (ST_B) and bile viscosity (BV) was carried out using the method developed by T. L. Redinova for saliva [11] and adapted by us for bile.

The method of wedge-shaped dehydration was used to study the morphological pattern of gallbladder bile. Three μl of bile portion B was applied in the form of droplet on the surface of ungreased polished quartz glass, was dried in a desiccator with desiccant, placed in a dry-air cabinet at 35 °C for 2–2.5 hours. The formation of structures occurred due to evaporation from the edges of the sample and primarily appeared in the peripheral areas. Sample review (crystallograms) was made by simple polarization in transmitted light using a Leica DM 2500 (lens × 3.5) microscope with a Leica DFC 420 digital camera and Leica applications software. The main morphokinetic changes in the sample begin in the first hours and occur by the end of the first day [12], so the sample was studied 3 hours and 24 hours after its preparation. Using ImageJ (Free Ware) program, the features of bile microstructure were determined, and the images were input into a computer, analyzed and processed using the original computer program VIDEO test.

The results of laboratory and instrumental examination of patients were compared with the data in the control group, which consisted of 50 healthy individuals aged 20 to 50 years who had no complaints about the gastrointestinal tract.

The obtained data were analyzed using SPSS statistical processing program. The data are presented as mean values (MV) with the determination of their errors (±m). P-value was assessed by Student's t-test in the normal distribution of the sample.

Results and Discussion

In all patients, ultrasound examination of the gallbladder revealed signs of biliary sludge (microlithiasis, putty bile), bile microscopy in 72.6% revealed crystals of cholesterol and calcium bilirubinate which is evidence of the I (precalculous) stage of CLT.

The results of the biochemical analysis of bile in observed patients are especially noteworthy (*Table)*. One hundred percent showed signs of destabilization of bile portions B and C. The decrease in BAs_B concentration, which are stabilizers of the colloidal state of bile, subsequently leads to precipitation of CL_B, supersaturation of bile, bile propensity to gallstone formation, as evidenced by a significant decrease in the biliary cholesterol-to-bile acid ratio (CBR_B), which is an index of bile lithogenicity. Obviously, with age, changes in all of the above parameters progress, which means that the risk of gallstone formation also increases.

Of particular interest is the fact that in young adulthood (group 1), lithogenic properties of bile are mainly associated with elevated cholesterol levels, in late adulthood (group 3) — with a reduced level of bile acid pool, and in middle adulthood (group 2) these changes are approximately balanced. Our results are consistent with the literature data indicating that in case of the propensity to cholelithiasis in young people the metabolism of exogenous cholesterol significantly slows down, in the elderly, the activity of 7-alpha-hydroxylase involved in the synthesis of bile acids from cholesterol decreases [12, 13].

Test of the physical properties of bile revealed elevated BV and ST_B levels in both portions, which is in agreement with few literature data [15, 16, 17]. The inspissation of bile and increase in its viscosity reduces the solubility of various components including the deposition of cholesterol crystals, agglomeration and nucleation of bile. Bile lithogenicity determined by its physical characteristics increases in older age groups.

It is known that the process of lithogenesis occurs in three stages: saturation, crystallization, and growth [13, 15, 18]. The decisive factor is the stage of bile supersaturation with cholesterol, which commences when solubilization of all cholesterol by vesicles becomes impossible. Supersaturated vesicle is very unstable, and it aggregates forming liquid crystals (liposomes). After that, the nucleation of cholesterol crystals occurs with the deposition of cholesterol monohydrate crystals (solid crystals) which are the center of crystallization [8, 19], a key link and the basis for the formation of gallstones [20].

Bile crystallography is a research method based on the ability of some crystal-forming substances to

Table. *Physical and chemical properties of bile tested*

Parameter	control (n = 50)	group 1 (n = 125)	group 2 (n = 164)	group 3 (n = 107)
CL_B (mmol/l):				
Portion B	7.56 ± 0.07	27.76 ± 2.14*	29.96 ± 2.45*	19.96 ± 2.15*
Portion C	3.63 ± 0.06	14.99 ± 2.16*	14.87 ± 1.33*	8.41 ± 1.54*
BA_B (mmol/l):				
Portion B	54.33 ± 0.14	49.35 ± 2.17*	48.93 ± 2.67*	26.02 ± 1.34*
Portion C	20.76 ± 0.20	18.01 ± 2.23	17.37 ± 2.31	10.43 ± 2.01*
CBR_B (U):				
Portion B	7.15 ± 0.07	2.77 ± 0.04*	1.63 ± 0.07*	1.3 ± 0.07*
Portion C	6.14 ± 0.10	1.45 ± 0.04*	1.16 ± 0.07*	1.2 ± 0.07*
BV (U):				
Portion B	2.74 ± 0.20	3.78 ± 0.29*	4.15 ± 0.3*	4.41 ± 0.56*
Portion C	2.52 ± 0.02	3.22 ± 0.03*	3.81 ± 0.19*	4.23 ± 0.8*
ST_B:				
Portion B	22.31 ± 0.15	22.95 ± 2.15	24.16 ± 2.03	28.34 ± 1.16*
Portion C	22.05 ± 0.14	24.99 ± 1.19*	25.96 ± 1.19*	28.04 ± 2.41*

Note: n is the number of observations; * P < 0.05, compared to control

form different structures. The shape of the crystal is a natural system of indication for the chemical composition of the biological fluid [4].

In the control group the morphometric study of gallbladder bile microstructure revealed the presence of widely branched crystals which were analyzed on the basis of liquid crystal lines (LCL) (Figure 1A). The angle of slope for LCL was (98.97 ± 2.92)°.

The morphological pattern of bile in patients with stage I of CLT was dependent on age. The group 1 of patients (Figure 1B) also was characterized by the presence of widely branched crystals, but there were a decrease in the slope angle of LCL down to (46.16 ± 3.67)° (p in relation to the control was < 0.01) and a fuzzy structure.

In addition, optically active inclusions reacting to polarized light appear in the initial stage of CLT. A lower degree of destabilization of bile colloidal structure (group 1) is characterized by the appearance of crystals with low optical activity (Figure 2: A, B), the so-called cholesterol monohydrate crystals. The most typical were branched dendrites with lamellar branches (in 49 patients — 39.2%) and tangled fibrous aggregates (in 62 patients — 49.6%).

The increase in biochemical signs of bile instability (group 2) led to the formation of crystals with high optical activity (Figure 3: A, B), the so-called large spherolites. Such characteristic structures as shield-shaped aggregates (in 52 patients — 31.7%) and short branched dendrites (in 96 patients — 58.5%) were shown.

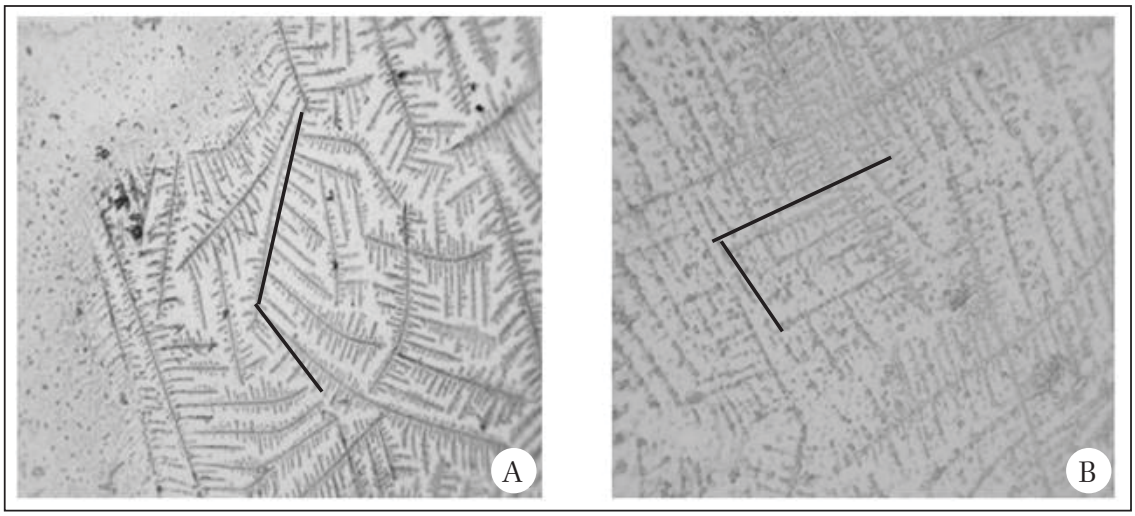

Figure 1. *Bile crystallograms: A – bile of healthy individuals; B – bile of the group 1 patients*

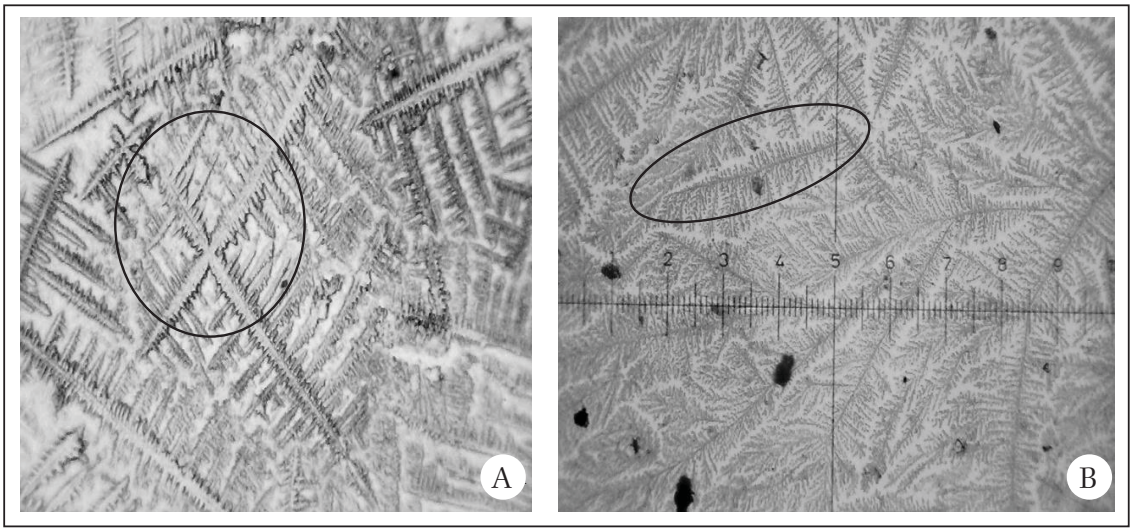

Figure 2. *Bile crystallograms (group 1): A – branched dendrites with lamellar branches; B – tangled fibrous aggregates*

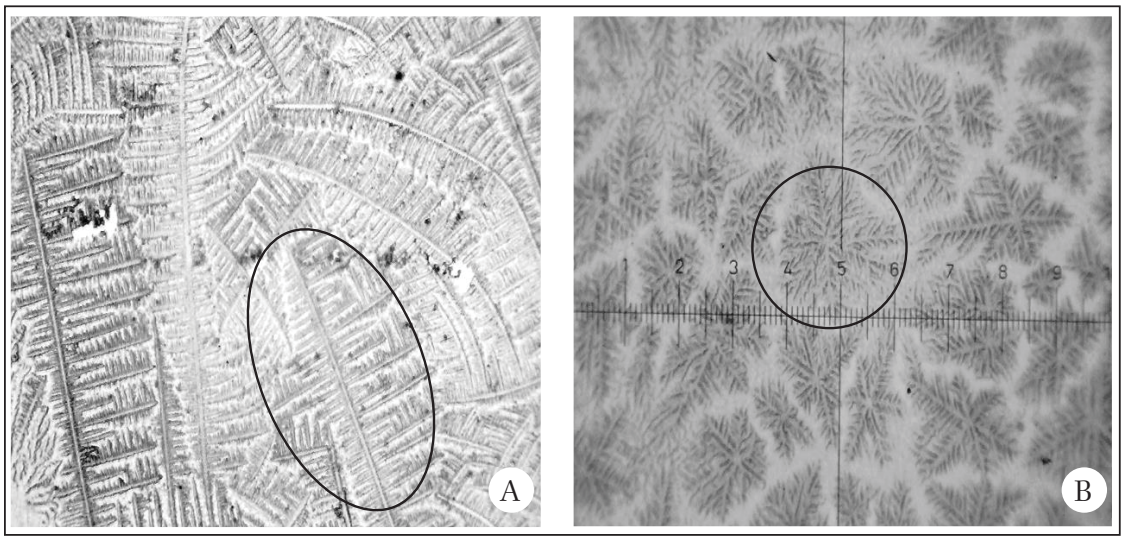

Figure 3. *Bile crystallograms (group 2): A – shield-shaped aggregates; B – short branched dendrites*

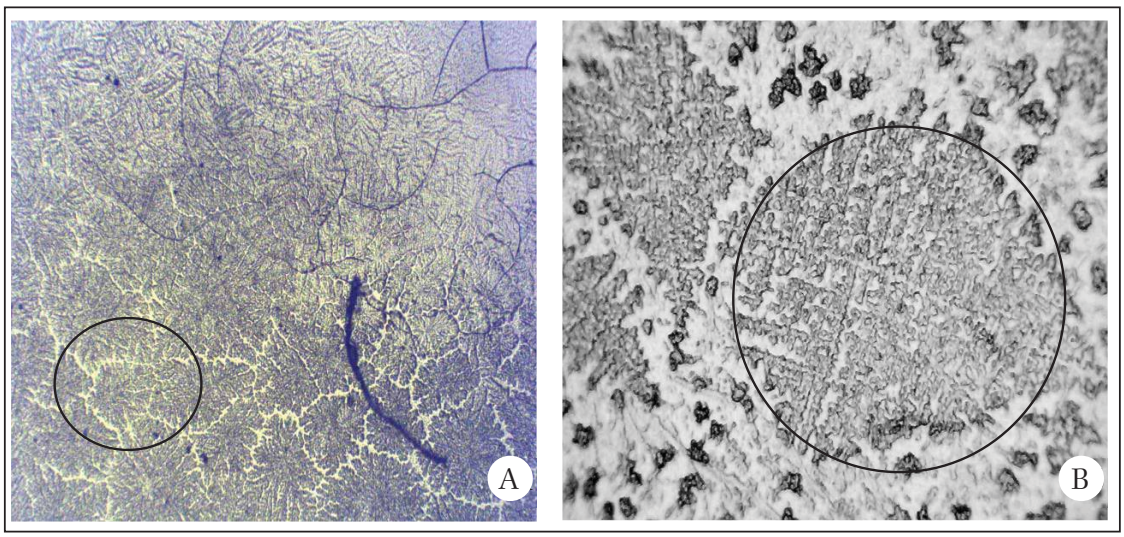

Figure 4. *Bile crystallograms (group 3): A – lamellar druses, B – branched plateau-like aggregates*

The morphological pattern of bile in group 3 patients is characterized by the presence of microcrystals (Figure 4: A, B): lamellar druses and branched plateau-like aggregates (in 42 patients — 39.2% and 51 patients — 47.6%, respectively) (Figure 4) were found approximately with the same frequency.

Our crystal-optical studies indicate that the found crystal-optical morphotypes have a selective morphology in accordance with the age and the degree of bile lithogenicity. With an increase in bile lithogenicity, there is an increase in optical activity of crystals [21, 22], its tendency to precipitate cholesterol with the formation of liquid crystal structures [6, 7, 8].

Thus, polarization microscopy reflecting the spatial supramolecular structures of the biological fluid [4] reveals deeper changes in bile structure in contrast to the biochemical method, which determines only the quantitative content of the main bile components.

Conclusion

1. The crystal-optical method of bile examination is highly sensitive, but at the same time it is easy to perform and can be widely used in the early diagnosis of CLT.

2. Branched dendrites with lamellar branches and short branched dendrites in bile are transitional

forms (promicrolites) and precede the formation of microlites — microcrystals in the form of lamellar druses and branched plateau-like aggregates.
3. The degree of impairment in the bile microstructure increases with increasing age of patients.

Conflict of Interests

The authors declare no conflict of interests.

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