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METABOLISM OF BILE WITH RESPECT TO ETIOLOGY OF GALLSTONE DISEASE — SYSTEMATIC REVIEW

Abstract: Based on current literature authors reviewed information on bile metabolism, i.e. production and chemical compounds, synthesis of bole acids, their hepato-intestinal circulation with respect to etiology of cholelithiasis — its epidemiology and clinical aspects.

Key words: bile, gallstone disease, cholelithiasis, etiology.

INTRODUCTION

Bile is a result of osmotic filtration and is permanently released by hepatocytes into biliary canaliculi, about 500–600 ml/24 hrs. This so called canalicular fraction is about 80% of daily production. The remaining volume of the bile arises from epithelium lining intrahepatic biliary tracts (cholangiocytes). Bile of the liver is relatively isotonic in comparison to the blood serum. Entire concentration of substances dissolved in the hepatic bile is 30–40 g/l.

Bile is a complex liquid containing 95% of water and dissolved substances i.e. salts of biliary acids (80%), phospholipids — mainly lecithin (16%), free, non-esterified cholesterol (4%), also others: conjugated bilirubin, proteins, electrolytes, mucus, medications and their metabolites [1].

It is worth to mention that lithogenity may increase percentage of non-esterified cholesterol till 8–10% of the total amount of dissolved substances.

The next compound of the bile are biliary pigments: bilirubin conjugated with glucuronic acid (in the form of mono- and diglucuronates) and biliverdin. Bilirubin is product of hemoglobin degradation, released from damaged erythrocytes. It arises in reticulo-endothelial system of the liver, bony marrow and spleen. Such bilirubin is referred to as free (non-conjugated), it is poorly water soluble; its solubility increases that to conjugation with blood serum albumin. In the liver free bilirubin is conjugated with glucuronic acid, what increases its water-solubility.

Bile components:

Water — 95%		
Lipids	Sterols	cholesterol (95% out of all sterols of the bile) remaining sterols
	Phospholipids	mainly phosphatydylocholin (lecithin)
	Bile acids	primary: cholic acid, chenodeoxycholic acid secondary: deoxycholic acid, lithocholic acid tertiary: ursodeoxycholic acid, suphofofitocholans
Proteins	Albumins	
	Immunoglobulins	IgG, IgM
	Apolipoproteins	AI, AII, B, CI, CII Transferin α_2-macroglobulin
	other proteins	EGF, insulin, haptoglobin, CCK, amylase, lisosomal hydrolase
Ions		sodium, potassium, calcium, magnesium, phosphorus, zinc, iron, manganium, molibden, strontium, cuprum
Bile pigments		bilirubin, biliverdin

SYNTHESIS OF BILE ACIDS

Bile acids arise in the liver and are end-products of cholesterol metabolism. Synthesis of bile acids is the main physiologic mechanism thank to which organism is able to remove excess of cholesterol.

Bile acids can be divided into primary, i.e. cholic and chenodeoxycholic acids, and secondary. Primary bile acids are synthetized in the liver during a multienzymatic process in which non-water soluble, electrically neutral particle of cholesterol is transformed into ionized water-soluble particle of bile acid. Initial stage of bile acid synthesis is addiction of hydroxide group to cholesterol in 7α position. This reaction is dependent on 7α-hydroxylase. Before the bile is secreted both acids are conjugated in hepatocytes partially with glycine (70%) and partially with taurine (30%, what increases their polarity and water solubility. Because mentioned acids exist in a bile as salts of different cations, i.e. sodium, potassium, they are also referred as bile acids.

Next they are actively secreted on the biliary pole of the hepatocyte into biliary canaliculi, and then become bile components, to travel together to the intestine. In a distal fragment of ileum and in the large colon aerobic bacteria using enzymatic de-conjugation (removal of glycine and taurine residues) and dehydroxylation (removal of hydroxide group from 7α position) transform cholic acid

into deoxycholic and chenodeoxycholic into lithocholic respectively. Such complex substances are referred to as secondary bile acids. Part of the undergoes further modification under the influence of intestinal flora or after resorption in the liver to so called tertiary bile acids, which are represented i.e. by ursodeoxycholic acid (7α-epimer of chenodeoxycholic acid), or sulphofitocholan which is derivative of lithocholic acid. Lithocholic acid is poorly water soluble and mainly removed with feces.

Thank to bile acids some water-insoluble substances i.e. cholesterol, metabolites of hormones and medications can be transported in the water environment of the bile. In the small intestine bile acids facilitate emulgation of fats, i.e. triacyloglycerols, what results in a better exposure to pancreatic lipases. Fatty acids facilitate intestinal absorption of vitamins fat-soluble (A, D, E, K).

Regulation of synthesis of bile acids is determined by many factors, i.e. functional condition of hepatocytes, cholesterol availability (precursor particle), and also by amount of salts of biliary acids migrating back to the liver by the help of hepato-intestinal circulation.

HEPATOINTESTINAL CIRCULATION OF BILE ACIDS

In the lumen of small intestine bile acids enable emulgation of lipids and absorption of fat soluble vitamins. Conjugation of bile acids with glycine or taurine in the liver changes their physicochemical features, causing fact that they are not absorbed in the duodenum nor in the jejunum. Thus their concentration remains relatively high in upper small intestine until the moment when digestion and absorption of fats is completely finished.

Most (95%) of bile acids, which get with bile to intestines is actively absorbed in the peripheral ileum and after binding with proteins returns back to the liver through portal circulation, to be actively released to the bile. So, newly synthetized bile acids of the liver account for about 5% of the total amount of their number and compensate loss of these acids which were not absorbed in the intestine but removed with the feces.

The cycle of hepato-intestinal circulation of bile acids repeats 6–10 times per day. Total amount of bile salts in the body reaches 2–4 g, and the amount necessary to a human during 24 hrs. to digest the fats is around 15–20 g. This is why their permanent circulation is necessary. Intestinal absorption of bile acids may be disabled in the following situations: state after resection of small intestine, Leśniowski-Crohn's disease, after application of cholestyramin (ion-exchange resin which binds salt of bile acids). In such situation interruption of the hepato-i testinal circulation of bile acids occur and increase of their removal with feces, what causes increase of hepatic synthesis of bile acids *de novo* from cholesterol.

CHOLELITHIASIS

Diseases of biliary tracts existed in humans for many years. The proves are gallstones found in mummified cadaver of young priestess living in XI century BC, and the first description of the jaundice caused by cholelithiasis, made by Soranos from Efez in IInd century [2, 3]. A very precise description of a case of cholelithiasis was given by pathologist Antonio Benivenius in 1507 [4]. Through ages the diseases of biliary tracts and their complication were one of the most common causative factors of death of patients, because although properly diagnosed still in XVIIIth century, they were inappropriately treated. Efficacious tactic in treatment of the diseases of biliary tracts is based on surgical procedures, but patients and medical doctors had to wait until second half of XIXth century, when Lawson Tait in 1879 in his hospital in Spark Hill in Birmingham popularized cholecystotomy, it means surgical removal of the gallstones without removing the gallbladder [5]. Critical date in gallbladder surgery was 1882, when on July 15th Karl von Langenbuch made in Berlin first in the world cholecystectomy, it means surgical removal of the gallbladder together with the gallstones [6]. Through the next 100 years this operation was a standard of surgical procedure in case of cholelithiasis. In 1987 Philippe Mouret, a surgeon from Lyon — carried out for the first time laparoscopic cholecystectomy [7, 8]. It is worth to mention that first laparoscopic cholecyctectomy in Poland was carried out by prof. Marek Krawczyk in 1991.

Undertaken attempts of conservative treatment changed throughout the years, but as widely known there is no reliable, secure and effective method for inoperative treatment of cholelithiasis.

EPIDEMIOLOGY

Cholelithiasis is a disease which is relatively common in well-developed countries. Epidemiologic studies in USA show that cholelithiasis involves 6,3 millions of males and 14,2 millions of females at the age between 20 and 74 [9]. One of the widest European epidemiological studies of the cholelithiasis was an Italian study of MICOL (Multicentric Italian Study on Cholelithiasis). The research covered almost 30 thousands of individuals at the age between 30 and 69. In a screening carried out on the studied group they found cholelithiasis in 18,8% of the females and 9,5% of the males [10]. Obtained epidemiologic data from Mediterranean region do not differ significantly from the rest of the Europe, where frequency of the disease is estimated on 10–15% of the males and 25% of the females [11]. The highest indexes were observed in Indians Pima in Northern America, in citizens of US of Mexican origin, the lowest in people of Bantu and Masai in Africa, where this disease practically does not occur [12, 13].

Despite most of people suffering from cholelithasis is clinically inarticulate, symptomatic cholelithiasis is considered to be the most frequent and most expensive disease in the United States [14].

ETIOLOGY OF THE CHOLELITHIASIS

From a definition — cholelithiasis is a condition where concretions are placed in the gallbladder (cholecystolithiasis) and/or in the biliary tracts (choledocholithiasis). Creation of the concretions is genetically and environmentally determined. The frequency of the cholelithiasis increases with ageing. Morbidity in the females is 2–3 times bigger than in the males. The next, important risk factor is obesity. In many screening tests it was shown the linear dependence between body mass index (BMI) and the frequency of the disease [15–18]. The stages which predispose to bile retention and creation of the concretions in the gallbladder are: diabetes, injuries of the spinal cord with autonomic nervous system dysfunction, and complete parenteral nutrition [19].

CLINICAL ASPECTS

Cholecystolithiasis is usually asymptomatic (80%), and in 1–4% yearly becomes symptomatic [20, 21]. In about 12% of cases cholecystolithiasis coincidences with the choledocholithiasis [22].

The most typical sign of gallstone disease is a biliary colic, which may exist in 10–30% of diseased patients [22]. Preferably it is followed by acquisition of a fatty meal and is associated with sharp agonizing pain in right hypochondriac region, and central epigastrium, radiating to right scapular region. Colic is commonly accompanied by emesis, always by nausea. In a typical, non-complicated colic there is no increase of the body temperature. Pain in the colic is a typical visceral pain and is caused by contractions of the gallbladder and the biliary tracts freed by irritation resulted from passage of the gallstone. It is associated with increase of the pressures in the gallbladder and biliary tracts [23]. Pain fades within few hours.

The main complication of the gallstone disease are: acute cholecystitis, inflammation of the biliary tracts and acute pancreatitis, hepatic abscesses, mechanical jaundice and liver cirrhosis, first of all cancer of the gallbladder.

Cholecystitis is mostly forwarded by cholelithiasis. It may be recognized when after hepatic colic attack symptoms of pain and rebound tenderness last more than 6 hrs. within the right hypochondriac region (positive signs of Chełmoński and Murphy), with muscle defense, fever and leukocytosis. The most frequent is aseptic inflammation, which is a reaction of the gallbladder wall to biochemical

changes of the bile, irritation of the gallstones, increase of the pressure and expansion of the gallbladder wall when the natural flow of the bile is closed.

A particular form of cholecystitis is a hygroma of the gallbladder, which forms when with obstructed cystic duct there is no sign of infection of its content nor necrosis of the wall of gallbladder. In such case the content of the gallbladder is so called "white bile", which develops in response to increased secretion by mucosa muco-serous liquid accompanied by reabsorption of the bilirubin and bile salts. The gallbladder significantly increases because of fluid accumulation. For the most part is slightly painful and easy palpable during physical examination. When content of the gallbladder is infected and empyema may develop. The gallbladder is then painful and guarded by muscular defense. Fever is usually hectic. The signs of local peritonitis in the right hypochondriac region prove necrotic cholecystitis or subsequent perforation of the gallbladder. Perforation may occur into peritoneal cavity (greater sac) or space limited by neighboring organs. In this last case a local empyema may develop with local peritonitis limited to right upper quadrant of the abdomen.

In case of formation of the fistula between gallbladder and the lumen of neighboring organ, most frequently the duodenum — the content of the gallbladder together with the gallstones empties into the lumen of gastrointestinal tract. It leads to regression of the signs of cholecystitis and general recovery of the patient. Although later mechanical obstruction may develop, and its causative factor is gallstone causing obstruction of ileum.

A consequence of chronic prolonged presence of gallstones within the gallbladder is its chronic inflammation. Because of the chronic inflammation the wall of the gallbladder becomes thicker, fibrous and hardens, while the gallbladder becomes smaller (cirrhotic).

The cholelithiasis and chronic inflammation are per se associated with a risk of cancer of the gallbladder [24, 25]. Nowadays it widely postulated that asymptomatic cholelithiasis is not absolute indication for operative treatment.

The treatment of choice of the symptomatic, non-complicated cholelithiasis is a surgical excision of the gallbladder together with concretions.

PATOPHYSIOLOGY OF CHOLESTEROL GALLSTONE FORMATION

Gallstones can be subdivided following their chemical composition: this is why we can distinguish cholesterol gallstones (over 50% of their mass is consisted of cholesterol), mixed (20–50% content of cholesterol) and pigment (they contain less than 20% of cholesterol and the main compound is calcium bilirubinate) [26]. Following the epidemiologic studies in Europe and America cholesterol and mixed concretions dominate, pigment concretions are more common in Asian countries, in Europe they can be found extremely rare [27–29].

The source of cholesterol for the organism is its intake with food and hepatic synthesis *de novo* from acetyl coenzyme-A. Cholesterol is eliminated from the body through its secretion to the bile as free element or conjugated with salts of bile acids.

Cholesterol is practically water-non-soluble. Its solubility in bile is resulted from interactions with other particles [30]. Cholesterol carriers are micelles and lecithin vesicles.

The lecithin vesicles are spherical, unilamellar, bilayerous structures — 40–100 nm. Apart from the cholesterol and phospholipids they contain small amounts of salts of bile acids. More than 95% of phospholipids are diacyl phosphatydylcholins (lecithins). Their polar cholic residua are directed to water phase, and the chains of lipid acids till internal phospholipid bilayer, where they create hydrophobic milieu for cholesterol particles. Lecithin vesicles are thermodynamically metastabile and undergo conversion into other lipid aggregates during balance achieving by the bile present in the paths and gallbladder.

The particles of bile acids conjugated with tauryn or glycine are amphipathic, what means that they possess both hydrophilic and hydrophobic (lipophile) domains. In water solution, salts of bile acids exist as monomers. With achieving a certain concentration, so called critical micellar concentration (CMC) the particles (monomers) of the bile acids automatically create aggregates in the bile, named micellae, in which the hydrophilic poles are directed to the outside — into water environment, while hydrophobic "tails" composed of fatty acids to the interior. This is how the contact of hydrophobic domains with water is minimized, and the interior of the micelle forms an "enclave", where water-insoluble substances, i.e. cholesterol and fat-soluble vitamins are maintained in a fluid state. Process of aggregation of bile acids into micellae is gradual and begins at the value of MCM about 2 mmol/L [1].

Concluding, cholesterol is bile-soluble, because it is an element of such micellae.

The bile, as mentioned above, is consisted of simple micellae (composed of bile acids only), discoid in shape with diameter about 3 nm and mixed micellae (composed of bile acids and lecithin) — 4–8 nm in diameter. These last dissolve averagely 3 times more cholesterol that simple micellae. Only the micellar solutions of cholesterol can be described as thermodynamically stabile.

Lipids, i.e. cholesterol, phosphatydylcholine (lecithine) and bile salts are secreted to bile using ATP-dependent proteins carriers (ABC transporters) [31].

After secretion into the bile, cholesterol and phospholipids form lecithin vesicles, and the salts of bile acids aggregate into simple micellae. In normal condition, when the amount of bile salts is appropriate all listed above lipid aggregates are transformed during their transition with bile to the vesicle into mixed micellae [31]. This is how cholesterol remains stable in a solution during transport through the biliary system (unsaturated bile).

If the bile contains more cholesterol, that it can be dissolved in the form of mixed micellae, it means that it is supersaturated with cholesterol. Then nucleation

phenomenon occurs, which is associated with aggregation and fusion unilamellar vesicles supersaturated with cholesterol into bigger multilamellar structures (around 500 nm in diameter), known as liposomes or liquid crystals. These vesicles can be seen in polarization light microscope as bifragile droplets resembling Maltese cross. The process of nucleation is followed by coming into being the crystals of cholesterol monohydrate, so crystallization. More, the gallbladder secretes mucous glycoproteins, which form the matrix for precipitation of the aggregates of cholesterol crystals into gallstones.

In 60-ties Admirand and Small (1968), and earlier Isaksson (1951) suggested that cholesterol gallstones resulted from excessive secretion of cholesterol through the liver into the bile, what resulted from liver disturbances (hepatocytes) or problems in other points of hepato-intestinal circulation (i.e. in the intestine) [30, 32].

It is postulated nowadays that formation of cholesterol gallstones is caused by simultaneous action of many factors, which show different level of importance, leading together to formation of nuclei (nucleation), cholesterol monohydrate crystallization and as a consequence creation of gallstones [1, 33, 34]. These basic pathophysiologic factors can be presented as the following events:

- Supersaturation (oversaturation) of bile with cholesterol due to its hepatic hypersecretion and/or decrease of secretion of salts of bile acids.
- Decrease of motor activity of the gallbladder leading to bile retention (biliary stasis) and acceleration of cholesterol crystallization within the bile
- Increase of mucous secretion in the gallbladder [34].

Admirand and Small defined the lithogenous bile as the bile which contains excess of the cholesterol, it means more that it can be normally found within simple and mixed micellae or lecithin vesicles. They estimated values of cholesterol solubility using diagram of phase balance [30].

Based on these conceptions, in patients with gallstones, a lithogenous bile was generally found (apart from the non-cholesterol lithiasis), while in healthy individuals bile was unchanged or non-saturated. It is however partially true, because in healthy individuals during fasting state, supersaturated bile can exist (Northfield *et al.* 1975) [35].

Supersaturation of bile with cholesterol (oversaturation) binds with the presence of cholesterol excess in comparison to phospholipids and bile acids concentration. This is why based on the current literature one can describe three main anomalies of secretion (Holzbach and Kibe 1985) [36]:

Type 1 — shortage of lipid carriers of cholesterol in bile, caused by absolute decrease of bile acids and/or phospholipids secretion;

Type 2 — increased secretion of cholesterol (hepatic hypersecretion), so absolute increase of cholesterol secretion into the bile, as it is observed in obesity

Type 3 — connection of phospholipids/ bile salts shortage with excessive secretion of cholesterol

The excess of cholesterol secreted into the bile by hepatocytes, causes its supersaturation, despite normal secretion and level of salts of bile acids. Cholesterol hypersecretion is found in obesity. It results from increased activity of enzyme: hepatic reductase 3-hydroxy-3-methyl-glutarate-coenzyme A (HMG-CoA-reductase), with a consequence of increased production and secretion of cholesterol into the bile [37, 38].

The normal cholesterol secretion in the presence of decreased amount of bile acids, due to shortage of their hepato-intestinal return or secretion volume, leads to hypersaturation of bile with cholesterol.

Despite the fact that cholesterol gallstones arise in the presence of lithogenous bile, supersaturation itself is not sufficient factor for lithogenesis. Most of individuals with supersaturated bile do not develop lithiasis, because the time necessary for nucleation, cholesterol crystallization and growth of the gallstone is longer than time of bile retention in the gallbladder. Bile retention is the next gallstone disease predisposing factor. Risk of cholelithiasis increases in all conditions associated with shortage of gallbladder emptying. These conditions are: pregnancy [39], complete parenteral nutrition [40]. During the pregnancy the volume of the gallbladder while fasting state and residual capacity increase with level of serum progesterone concentration, which through inhibition of contractility of smooth muscle exacerbates gallbladder emptying. In patients with parenteral nutrition one can observe decrease of cholecystokinin release [41]. Similar phenomenon exists in interdigestive state, when lack of meal stimulation slows down the motor activity of the gallbladder. Disturbances with gallbladder emptying are relatively common in suffering from cholelithiasis. Functional ultrasonography tests showed that patients with gallstone disease have increased residual and fasting state capacity of the gallbladder in comparison to the control. Complete emptying did not differ in both groups studied, however partial emptying during interdigestive state was short [42].

The dependence between the motor activity of the gallbladder and the duodenum during interdigestive state is very important. It was observed that gallbladder empties partially also in this period (about 20–30% of output volume in comparison to 70–80% decrease of the volume after meal) and then refills. It occurs within 1–2hrs intervals and is associated with intestinal cycle of Migrating Myoelectric or Motor Complex (MMC) accompanied by simultaneous increase of motilin in blood serum [43, 44]. MMC and its relationship with gallbladder motor activity was briefly described in [45]. During period of hunger the bile is slowly concentrated in the gallbladder, what is counteracted by temporary motor activity of the gallbladder coordinated with phase III of MMC [46]. Bile condensation results in consequences as: increased solution of cholesterol (and phospholipids) in micellae. Because phospholipids ae more soluble in micellae than cholesterol,they use to migrate from vesicles into micellae. The remaining vesicles rich in cholesterol reveal a tendency to create nuclei — nucleation of cholesterol crystals.

Following this thesis, decreased frequency of phase III of MMC and lack of gallbladder contractility during interdigestive state seen in patients with cholelithiasis promotes bile condensation, crystallization, and gallstone formation [47].

Suppression of CCK release by somatostatin or its analogues administered to the diseased because of acromegaly, significantly increases the risk of cholesterol cholelithiasis development through reduction of gallbladder contractility [48]. Similar effect of decrease of gallbladder contractility was achieved during studies on mice with deletion of CCK-1 receptor coding gene [49].

Bile contains few proteins, which promote crystallization and gallstone development, i.e. mucus glycoproteins — mucine. Excess in mucine secretion was observed in animal models of cholethogenesis (Carey and Cahalane 1988) [34], Lee 1981 [50, 51]. Following some reports saturation of bile with cholesterol cause probably through the prostaglandin mechanism , increased secretion of mucus by epithelial cells of the gallbladder wall [51, 52].

As a result of increased synthesis and mucine secretion above critical level, epithelium of the gallbladder covers with mucine gel. This gel sticks lecithin vesicles overloaded with cholesterol, what causes that this gel becomes a factor causing nucleation and gallstone formation [53].

CONFLICT OF INTERESTS

None declared.

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