PERSPECTIVE FOR EVALUATION OF MICRONRNA IN PLASMA AND BILE FOR DIFFERENTIAL DIAGNOSIS OF OBSTRUCTIVE BILIARY TRACT DISEASE

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ABSTRACT

Introduction: Cholelithiasis diagnosed in 10-25% of the adult population. The manifestation of cholelithiasis in the form of acute cholecystitis, cholangitis, jaundice etc. occurs with a probability of 2-3% or more per year depending on the risk factors. Most often, it manifests itself as acute calculous cholecystitis. Up to 20% of such patients have additional calculi in extrahepatic bile ducts requiring simultaneous surgical intervention. Currently, the algorithm for diagnosis of concomitant choledocholithiasis in acute cholecystitis is multilevel and it needs to be simplified and improved. Conventional non-invasive diagnostic techniques have low specificity and sensitivity. A new safe diagnostic test is needed for the diagnosis of choledocholithiasis in cholecystitis in order to improve the results of surgical treatment of patients.

The aim of the study – to determine the prospects for diagnosis of microRNA for differential diagnosis of obstructive diseases of the biliary tract on the basis of the literature data.

Materials and methods: The analysis of the selected international literature in the period of 1991-2018 concerning the studies of microRNA in cholestatic liver diseases was carried out. The search for literature was conducted using Google Scholar and PubMed search engines for the following keywords: microRNA, cholestasis, choledocholithiasis, acute cholecystitis, biliary obstruction in their various combinations.

Review: MicroRNA is a specific post-transcriptional regulator of gene expression in all organs and systems of the body. There are specific types of miRNAs for different tissues. The most studied type of liver miRNA is miR-122. The studies showed better sensitivity and specificity of the detection of miR-122 both in the plasma and in the bile for the diagnosis of pathological liver conditions compared with conventional liver tests (aminotransferase, alkaline phosphatase, gamma-glutamyltransferase). Regarding the cholestatic hepatocyte injury occurring in choledocholithiasis, the following types of miRNA were identified: 122, 21, 29, 125, 222, let-7, 98 etc. Their concentrations in plasma and bile can be used as a diagnostic test for the presence of concomitant benign cholestasis in acute cholecystitis. Moreover, these markers can be used for the differential diagnosis of cholestasis of different etiology.

Conclusions: Quantitative and qualitative characteristics of microRNA in bile and plasma can be used as an additional non-invasive method for diagnosis of cholestasis induced by choledocholithiasis in acute calculous cholecystitis. Further studies need to identify the most optimal algorithm for the administration of microRNA in clinical practice.

KEY WORDS: microRNA, cholestasis, diagnosis, choledocholithiasis, acute cholecystitis, biliary tract obstruction

INTRODUCTION

Cholelithiasis the most common cause of non-malignant hepatobiliary diseases in the general population [1]. About 10-15% of males and 20-25% of females have stones in the biliary tract. Up to 20% of patients with calculous cholecystitis simultaneously have calculi in the choledoch or common bile duct. About 50% of patients with cholelithiasis do not notice any specific symptoms of the disease and can complain only of general weakness and fatigue [1]. The symptoms of the obstructive hepatobiliary disease such as colic, jaundice, etc. [2, 3] develop in 2-4% of patients with gallstones within one year.

Most often, cholelithiasis manifests as acute cholecystitis, which according to modern guidelines requires surgical intervention in the form of cholecystectomy [3]. It is essential that stones are also found in the common bile duct or choledoch in about 20% of patients during the laparoscopic cholecystectomy [4]. In the surgical treatment of acute cholecystitis, the simultaneous removal of calculi from extrahepatic bile ducts, if they are present, can improve the results of the operation and prevent the development of such complications as acute cholangitis and acute pancreatitis in the future. Therefore, the pressing issue is the precise diagnosis of concomitant choledocholithiasis in acute cholecystitis in order to reduce the risk of postoperative complications and decrease overall economic costs for the patient [3].

The conventional diagnostic algorithm consists of a survey and inspection, laboratory tests and instrumental diagnostic techniques (ultrasound, endoscopic retrograde cholangiopancreatography (ERCP), and magnetic resonance cholangiopancreatography). However, ultrasound and tests of such liver markers as aminotransferases, alkaline phosphatase, bilirubin, and gamma-glutamyltransferase are not sufficiently sensitive and specific methods [5, 6]. The use of invasive diagnostic techniques is associated with the risk of complications and they are expensive. Thus, the topical issue is the introduction to the clinical practice of a specific and sensitive marker for the diagnosis of choledocholithiasis in both asymptomatic patients and those who are referred to undergo cholecystectomy.
THE AIM
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MATERIALS AND METHODS
The analysis of the selected international literature in the period of 1991-2018 concerning the studies of microRNA in cholestatic liver diseases was carried out. The search for literature was conducted using Google Scholar and PubMed search engines for the following keywords: cholestasis, choledocholithiasis, microRNA, acute cholecystitis, biliary obstruction in their various combinations.

REVIEW AND DISCUSSION
MICRORNA
MicroRNA (miRNA, miR) is a group of physiologically active non-coding endogenous RNAs of 19-23 nucleotides in length, the main role of which is the post-transcriptional regulation of the expression of certain genes [6, 7]. The mechanism for regulating expression is to bind complementary nucleotide sequences to the matrix RNA blocking the translation in the ribosomes. However, miRNAs can also interact with repressor proteins by blocking them, which, on the contrary, activates the expression of target genes [6, 7]. To date, more than a 1,000 different types of miRNAs have been identified, which can be either organ- and tissue-specific, or universal.

The microRNA in biological fluids can be found in specific transfer vesicles called extracellular vesicles, which, along with the microRNA, transfer other biologically active substances, or it can be bound to transport proteins [8]. On the other hand, there is also cell-free circulating microRNA that has high stability in almost all biological fluids [5, 9, 10]. Currently, methods for precise identification of miRNA in different types of biological fluid (plasma, saliva, bile, etc.) are available with the help of PCR [6]. Concerning liver diseases, the most reasonable is the evaluation of miRNA in plasma and bile collected during gastroduodenoscopy or ERCP.

As a result of pathological processes in cells, the structure of both intercellular and intracellular miRNAs varies considerably [5, 7, 8, 10, 11]. Thus, depending on the etiology of liver damage, the concentrations of various types of miRNA may change, which has high levels of specificity and sensitivity to certain diseases [4, 5, 10, 12, 13, 14, 15]. Concerning liver diseases, the miRNA has the largest evidence base to diagnose and predict the success of therapy in oncological processes [5, 7]. However, the study of miRNAs in acute and chronic cholestatic liver diseases is promising.

MICRORNA AS A MARKER FOR HEPATOCYTE INJURY
Cholangiocytes are cells forming the walls of the biliary tree of the liver and the external biliary tract, account for 3-5% of the total number of hepatocytes, but they, in turn, synthesize up to 40% of the daily amount of bile [5, 7, 16]. If the hepatobiliary system is obstructed due to the presence of stones in the extrahepatic biliary tract, cholangiocytes first experience morphological signs of oxidative stress due to the direct toxic effects of bile [5]. As an adaptive reaction, certain types of miR may alter their synthesis activity to activate intracellular antioxidant and regulatory systems [5]. The role of miRNA as a regulator of adaptive gene expression was confirmed on the model of experimental atresia of extrahepatic bile ducts in the integrative study of Bessho K. et al. [17]. Such regulation provides an adequate proliferative response to cholestatic injury enabling to maintain the normal morphology of the hepatobiliary tree during short-term cholestasis [16, 17]. Unfortunately, the adaptive capacity of cholangiocytes is lost in long-term cholestasis and fibrosis may progress. Thus, changes in the concentration of miRNAs can be considered as the process of cell adaptation to certain homeostatic conditions, not just as a marker of cytotoxic damage. Determining the changes in the structure and concentration of certain miRNAs can be a powerful diagnostic test for a significant number of liver and biliary tract diseases.

Yamaura Y. et al. in their study showed that a different pathophysiological reaction occurs in cholangiocytes depending on the etiology of cholestasis (toxic or mechanical), which is accompanied by the change in the concentration of various profiles of plasma miRNA [6]. Two models of cholestatic liver damage were used in mouse study: introducing a-naphthyl isothiocyanate (ANT) and applying a ligature to the bile duct. Further, changes in the profiles of plasma miRNA were recorded. As a result, changes in the concentration of different types of miRNAs were found depending on the type of cholestatic damage, indicating a high specificity of the marker [6]. Such results confirm the potential of the miRNA as a marker for the differential diagnosis of diseases of the hepatobiliary tree.

Thirteen different types of miRNA were identified in the study by Schaap F. G. et al., which were significantly associated with extrahepatic cholestasis [18]. It is of interest to note that the concentration of some types of miRNAs may increase, and of others, on the contrary, decrease [18, 19]. However, the most studied type of liver miRNA is miR-122, which accounts approximately for 70% of all liver miRNAs [5, 6, 15]. This type of miRNA plays an important role in the synthesis of cholesterol by hepatocytes [9, 15]. A hyperexpression of a large number of liver genes occurs and hepatocytes increase the production of cholesterol when this type of miRNA is blocked [15]. It is also important to note that secretion of both bilirubin and miR-122 into bile are interrelated processes [20]. Thus, in the study by Verhoeven C. J. et al., mutual positive correlation between the levels of bilirubin and miR-122 in bile was established at different pathological states of the liver [20].

There are data suggesting a significantly higher sensitivity and specificity of miR-122 for the diagnosis of hepatocyte injury due to primary sclerosis of the biliary tract, sclerosing cholangitis, polycystic liver, extrahepatic obstruction,
Taking into account pathophysiological processes, the concentration of miR-122 increased significantly within 48 hours after the ligation of the bile duct [11]. In another study of the toxic injury of cholangiocytes, the concentration of miR-122 increased significantly in the period from 6 to 24 hours [14]. The data are confirmed by other studies by Th'ng F. et al. and Shifeng H. et al. in patients with cholelithiasis [9, 12, 23]. An increase in the concentration of miR-122 in the study group of patients with cholelithiasis was shown, regardless of the presence of symptoms as compared with the controls in both studies [12, 23]. The sensitivity of the diagnostic technique for cholelithiasis by determining the level of miR-122 in plasma reached 77.4%, and the specificity was 96.4% [12]. Moreover, the study by Th'ng F. et al. showed that choledocholithiasis can be differentiated from cholecystitis using miRNA [9]. Thus, patients with choledocholithiasis had statistically more significant increase of miR concentration in plasma compared to patients with cholecystitis [9]. This is primarily due to the degree of obstruction of the hepatobiliary tree, which is usually more pronounced in choledocholithiasis compared with cholecystitis. The miR concentrations were positively correlated with the severity of the inflammatory process in the liver [9].

It is of interest to note that it is potentially possible to determine the type of pathophysiological liver damage by determining the concentrations of different types of miRNAs. Thus, chronic injury of hepatocytes (but not cholangiocytes) with fibrosis may lead to the increase in the concentration of miR-122 in plasma, while the concentration of miR-122 in bile may decrease. On the other hand, miR-122 is excreted in both blood and bile in an increased amount in the presence of acute cytotoxic process in the liver. If independently the cholangiocyte is initially damaged, the miR-122 levels may slightly vary, unlike miR-222, the concentration of which increases in plasma and decreases in bile [20].

A specific marker for cholangiocytes is microRNA-222 [20]. Thus, its expression is 17 times more intense in cholangiocytes compared with stromal hepatocytes of a healthy liver. Cholangiocytes are primarily injured in choledocholithiasis, which leads to a disturbance of the barrier function of the outer membrane and the release of miR-222 in both bile and blood [20].

MicroRNA-21 also plays an important role in the pathogenesis of cholestatic liver injury [18]. In the study by Kennedy L. L. et al., the experimental ligation of the common bile duct led to the increased miR-21 concentration in bile [24]. Taking into account pathophysiological processes, miR-21 stimulates the proliferation of cholangiocytes and their fibrotic transformation [16]. Moreover, inhibition of miR-21 in mice was associated with a decline in local concentrations of proinflammatory factors in the liver and a decrease in the severity of cholestatic fibrosis one week after ligation [24]. Kishimoto T. et al. showed the possibility to distinguish between healthy patients, ones with benign biliary diseases and those with malignant liver neoplasms with the help of miR-21 [25]. Presumably, miR-21 plays a significant role in the proliferation of cholangiocytes, which is significantly more intense in malignant processes than cholestasis. MicroRNA-7a-1 has antagonist properties with type 21, and vice versa, its level decreases in bile with cholestatic liver injury [16].

MicroRNA-98 and let-7 have suppressive properties for the expression of a large number of proinflammatory liver cytokines [16]. In the study by Glaser S. et al. on the model of cholestatic liver injury in mice, it was found that cholestatic inflammation of cholangiocytes activates the synthesis of the neuroendocrine hormone secretin, which in turn reduces the levels of let-7 and 125b [19]. The result is an increase in the expression of a large number of cytokines, mainly nerve growth factor and vascular endothelial growth factor, which, together with hypersecretion of miR-21, stimulate proliferative degeneration of hepatocytes [16, 19, 25]. MiR-143 and miR-218 have similar properties, which are activated due to cholestatic hepatocyte injury [10].

Bessho K. et al. conducted a study assessing the response of cholangiocytes in extrahepatic bile ducts to experimental cholestasis [17]. As a result, increased expression of miR-29b in cholangiocytes was recorded [17]. Interestingly, intrahepatic hepatocytes respond to cholestasis by activating not the expression of 29b subtype, but of miR-29a [13, 26]. In this regard, further study of the 29b subtype as a specific marker of injury of extrahepatic bile ducts is necessary, which may have a special diagnostic value in choledocholithiasis, regardless of concomitant conditions.

However, it is important not only to define the profile of the miRNA but also to specify the reference limits for different types of disease. Thus, it has been shown that types 517a, 892a and 106a of miRNA may be elevated in both choledocholithiasis and ischemic injury of hepatocytes (liver transplantation) [22, 25]. It is of interest that the increase in concentrations is more significant in ischemic injury [22, 27]. Malignant cholestatic neoplasms of the liver are also accompanied by a more significant change in the miRNA concentrations in both bile and plasma compared with benign cholestasis [5, 10]. These data are the basis for the differential diagnosis of the etiology of obstruction, in the case of ambiguous results of conventional approaches. There are methods for quantifying the miRNAs without their qualitative evaluation for the diagnosis of certain states. Thus, in the study by Severino V. et al. a quantitative analysis of extracellular vesicles (which is the type of transport for miRNA and other biologically active compounds) in plasma and bile was performed in malignant and benign cholestasis [28, 29]. The result was an increase in the concentrations of extracellular vesicles in both plasma and bile in each type
of cholestasis. It should be noted that the quantitative index allowed differentiating between benign and malignant cholestasis regardless of qualitative evaluation. Quantitative analysis is much more accessible for daily clinical use and does not require significant economic costs [29]. Moreover, it was determined that the analysis of bile and not plasma was significantly more specific (100% for bile versus 63% for plasma) for the diagnosis of cholestasis [29]. This is explained by the inflammatory process largely in cholangiocyte and not in hepatocyte, which makes bile the most sensitive acceptor of cell degradation markers [8]. The data are confirmed by another study, which states that the concentration of specific hepatic miRNAs can be 20 times higher in bile than in plasma [20]. Thus, quantitative and qualitative tests of bile microRNA during ERCP may raise the diagnostic value of the approach in questionable results regarding the degree of cholestasis.

THERAPEUTIC POSSIBILITIES OF MICRORNA

Many studies demonstrate the important regulatory role of miRNA [15, 18, 20, 25, 28]. Such regulatory properties give the microRNA a high therapeutic potential. Currently, there are studies confirming the possibility of using miRNA as a therapeutic agent [28]. Thus, injections of miR-122 in patients with cholangiocarcinoma also significantly reduced the intensity of the inflammatory process and suppressed the proliferation of the tumor [15]. Inhibition of miR-122 in hepatitis C can reduce the level of viremia and improve the functional state of the liver [15].

Concerning cholestatic injury, it was shown that blocking the miR-21 gene in mice significantly reduced the intensity of hepatocyte necrosis due to ligation of the common bile duct [28]. This may indicate that miR-21 is among the first to respond to acute cholestasis and triggers pathological changes in the liver. In other studies, Yang Y. L. et al., Tiao M. M. et al. obtained similar results due to the inhibition of miR-29a activity [26].

CONCLUSIONS

Concomitant choledocholithiasis in acute calculous cholecystitis requires surgical treatment along with cholecystectomy. The diagnosis of cholestasis induced by choledocholithiasis in acute cholecystitis is a complex and cost-ineffective task often requiring the use of invasive ERCP. Basic biochemical tests, such as aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, have low specificity and sensitivity in relation to the diagnosis of choledocholithiasis in acute cholecystitis.

The microRNA can be used as an additional method for diagnosing choledocholithiasis in cholecystitis if conventional approaches do not provide comprehensive information on the state of the hepatobiliary system of a patient at the preoperative stage. Simultaneous quantitative and qualitative test of the microRNA profiles in bile and plasma has a combination of high sensitivity and specificity in the diagnosis of benign cholestasis in acute calculous cholecystitis. Moreover, the test enables a differential diagnosis with other cholestatic diseases, mostly of a neoplastic nature. Further studies of miRNAs in both plasma and bile are required to create a greater evidence base and identify the most specific type of microRNA for acute benign cholestasis, namely choledocholithiasis.

REFERENCES


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