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INTESTINAL MICROBIOME AND BILE ACIDS METABOLISM IN PATIENTS WITH PRIMARY BILIARY CHOLANGITIS USING MARE'S MILK

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Summary

Introduction. Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis - is an uncommon disease that affects mainly women and characterized by cholestasis, the presence of serum antimitochondrial antibodies (AMA) chronic course with often progresses to the end-stage of liver disease (cirrhosis). The review presents the latest data on the etiopathogenesis of autoimmune liver diseases, including the relationship between the metabolism of bile acids and intestinal microbiome in PBC. Briefly covers the therapeutic approaches for modeling intestinal microbiome

Objectives. To present a review of current data on the pathogenesis of autoimmune liver diseases, the interaction of bile acid metabolism and intestinal microbiome in PBC, as well as therapeutic approaches for correcting intestinal microbiota, including diet changes and using mare's milk.

Search strategy: Search and analysis of scientific publications across the databases and web-resources PubMed, CochraneLibrary, Medline, Embase, ResearchGate were performed. The depth of the search was 20 years, within 2000 - 2018, including the latest publications as of 2019. The review included randomized and cohort studies reportsand systematic reviews. Given the fact that PBC is a rare disease, and there are very few studies on mare's milk (2 publications, 2 ongoing studies), the number of publications on the searched topic is limited. The references in all identified articles were also searched.Excluded: case report format articles, summaries of reports, private messages and newspaper publications. A total of 71 publications were analyzed, of which 41 are included in this review.There were excluded: case-report articles, summaries of reports, personal messages and newspaper publications. A total of 109 publications were analyzed, of which 41 were included in this review.

Results. Bile acids are important signaling molecules involved in the regulation of lipid metabolism in the liver, glucose and maintenance of metabolic and energy homeostasis. The results of multiple trials indicated interactions between the bile and the intestinal microbiota and PBC. There is evidence that the microbiome may play a role in the pathogenesis of PBC. In the absence of bile acids, the bile acid 7α-dehydroxylating bacterial population collapses.

Conclusion. Modulation of the microbiome by pre-/pro-and synbiotics can deliver significant positive hepatic effects without much concern of major side-effects. In light of the search for safe, effective and convenient methods of treatment, adding mare's milk to the treatment of PBC is the dietary approach with high potential for affecting the chain in the pathogenesis of PBC through modeling the composition of the intestinal microbiome. In the presence of the treatment complexity the clinical medicine obtains a remedy in the form of a natural product - mare's milk, the preventive, dietary, and therapeutic effects of which are known for long.

Keywords: *primary biliary cholangitis, bile acids, microbiome, mare's milk.*

Резюме

КИШЕЧНЫЙ МИКРОБИОМ И МЕТАБОЛИЗМ ЖЕЛЧНЫХ КИСЛОТ У БОЛЬНЫХ ПЕРВИЧНЫМ БИЛИАРНЫМ ХОЛАНГИТОМ ПРИ ПРИМЕНЕНИИ КОБЫЛЬЕГО МОЛОКА

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Актуальность. Первичный билиарный холангит (ПБХ), ранее известный как первичный билиарный цирроз, является редким заболеванием, которое поражает главным образом женщин и характеризуется холестазом, хроническим течением, обнаружением специфических антимитохондриальных антител (АМА) и частым прогрессированием до конечной стадии заболевания печени (цирроз печени). В обзоре представлены последние данные об этиопатогенезе аутоиммуных заболеваний печени, в том числе о взаимосвязи метаболизма желчных кислот и кишечного микробиома при ПБХ. Кратко освещены терапевтические подходы моделирования кишечного микробиома

Цель. Представить обзор современных данных о патогенезе аутоиммунных заболеваний печени, взамосвязи метаболизма желчных кислот и кишечного микробиома при ПБХ, а также о терапевтических подходах коррекции микробиоты кишечника, в том числе с помощью кобыльего молока.

Стратегия поиска: проведен поиск и анализ научных публикаций в базах данных и web-ресурсах PubMed, CochraneLibrary, Medline, Embase, ResearchGate по ключевым словам. Глубина поиска составила 20 лет, с 2000 по 2018 годы, включая последние публикации 2019 г. В обзор были включены отчеты о рандомизированных и когортных исследованиях и обзорные статьи. С учетом того, что ПБХ относится к редким заболеваниям, а исследований по кобыльему молоку крайне мало (2 публикации, 2 продолжающихся исследования), количество публикаций по искомой теме ограничено. Изучались также цитируемые ссылки в найденных релевантных источниках. Исключены: статьи формата «кейс-репорт», резюме докладов, личные сообщения и газетные публикации. Всего было проанализировано 71 публикаций, из них 41 публикация включены в данный обзор.

Результаты: Согласно современным представлениям, желчные кислоты являются важными сигнальными молекулами, участвующими в регуляции липидного обмена в печени глюкозы и поддержании метаболического и энергетического гомеостаза. Результаты многочисленных испытаний показали взаимодействие между желчью и кишечной микробиотой и ПБХ. В отсутствие желчных кислот популяция бактерий, участвующих в 7α-дегидроксилировании желчных кислот, значительно снижается.

Выводы: моделирование кишечного микробиома с помощью пребиотиков, пробиотиков и синбиотиков при ПБХ может давать значительные положительные эффекты на функции печени без особых побочных эффектов. В свете поиска безопасного, эффективного и удобного дополнительного метода лечения кобылье молоко приобретает высокий потенциал в плане воздействия на одно из звеньев патогенеза ПБХ путем моделирования композиции кишечного микробиома. На фоне сложности лечения клиническая медицина приобретает средство в виде натурального продукта – кобыльего молока, профилактические, диетические, лечебные эффекты которого известны давно.

Ключевые слова: *первичный билиарный холангит, желчные кислоты, микробиом, кобылье молоко.*

Түйіндеме

БІРІНШІЛІК БИЛИАРЛЫ ХОЛАНГИТ АУРУЫНА ШАЛДЫҚҚАН НАУҚАСТАРДЫҢ САУМАЛ ПАЙДАЛАНУ БАРЫСЫНДАҒЫ ӨТ ҚЫШҚЫЛЫ ЖӘНЕ ІШЕК МИКРОБИОМ МЕТАБОЛИЗМІ

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Өзектілігі. Бұрын бастапқы билиарлы цирроз деп аталатын біріншілік билиарлы холангит сирек кездесетін ауру болып табылады.Негізінен әйелдерге әсер етіп, холестазбен сипатталады, созылмалы ағымда өтеді сондай – ақ антимонитохондрияға қарсы антиденелер (АМА) анықталуымен сипатталады. Шолуда бауыр аутоиммунды ауруларының этиопатогенезі туралы, соның ішінде біріншілік биллиарлы холангит - де өт қышқылдарының метаболизмі мен ішек микробиомасы арасындағы байланыс туралы соңғы мәліметтер келтірілген. Ішек микробиомасын модельдеуге арналған терапиялық тәсілдерге қысқаша шолу жасалады.

Мақсаты. Бауырдың аутоиммунды ауруларының патогенезі, өт қышқылы метаболизмі мен ішек микробиомының біріншілік биллиарлы холангит - мен байланысы, сонымен қатар ішек микробиоты, оның ішінде саумалмен емдеудің емдік тәсілдері туралы ағымдағы мәліметтерге шолу жасау.

Іздеу стратегиясы: PubMed, CochraneLibrary, Medline, Embase, ResearchGate веб-ресурстарындағы ғылыми жарияланымдарды кілт сөздер бойынша іздеу және талдау. Іздеу терең дігі 2000 жылдан 2018 жылға дейін 20 жыл болды, оның ішінде 2019 жылы шыққан соңғы басылымдар. Шолуда рандомизацияланған және когорттық зерттеулер туралы есептер және шолу мақалалары болды. Біріншілік биллиарлы холангит - сирек кездесетін ауру екенін және бие сүтіне қатысты зерттеулердің өте аз екенін ескерсек (2 жарияланым, 2 зерттеу), қажетті тақырыптағы жарияланымдар саны шектеулі. Анықталған сілтемелер табылған тиісті дереккөздерде де

қарастырылды. Қамтылмаған: мақалалар форматындағы мақалалар, баяндамалардың қысқаша мазмұны, жеке хабарламалар және газет басылымдары. Барлығы 71 жарияланымға талдау жасалды, оның 41-і осы шолуға енгізілді.

Нәтижелер: заманауи тұжырымдамаларға сәйкес, өт қышқылдары бауырдағы, глюкозадағы липидтер алмасуын реттеуге және метаболикалық және энергетикалық гомеостазды қолдауға қатысатын маңызды сигналдық молекулалар болып табылады. Көптеген сынақтардың нәтижелері өт пен ішек микробиотасы мен біріншілік биллиарлы холангит арасындағы өзара әрекеттесуді көрсетті. Өт қышқылдары болмаған кезде өт қышқылдарының 7α-дегидроксилденуіне қатысатын бактериялардың саны айтарлықтай азаяды.

Қорытынды: ішек микробиомасын пребиотиктер, пробиотиктер және синбиотиктердің көмегімен біріншілік биллиарлы холангит көмегімен модельдеу кез-келген арнайы жанама әсерлерінсіз бауыр қызметіне айтарлықтай оң әсерін тигізеді. Қауіпсіз, тиімді және ыңғайлы қосымша әдісті іздеу аясында сүт ішек микробиом құрамын модельдеу арқылы біріншілік биллиарлы холангит патогенезіндегі байланыстардың біріне әсер ету тұрғысынан үлкен әлеуетке ие болады. Емдеудің күрделілігі аясында клиникалық медицина алдын-алу, диеталық және емдік әсері бұрыннан белгілі болған табиғи сүт – саумалмен емдеуді ұсынады.

Негізгі сөздер: *біріншілік өт жолдары холангит, өт қышқыл, микробиом, саумал.*

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Introduction

A medical care data analysis shows that autoimmune diseases and their treatment are considered as the complex problem of modern medicine. The severe course of the disease, treatment failure in most cases, and an unfavorable prognosis put these diseases among the most urgent problems of modern medicine. According to WHO, an increase in morbidity and mortality due to autoimmune diseases has been observed in the world in recent decades.

Autoimmune liver diseases such as autoimmune hepatitis (AIH), primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC)) considered as complex problems. PBC, formerly known as primary biliary cirrhosis - is a rare disease that affects mainly women. This ubiquitous autoimmune liver disease is characterized by cholestasis, the presence of serum antimitochondrial (AMA) or specific antinuclear antibodies (ANA) and a histological picture of chronic non-purulent granulomatous lymphocytic inflammation of the small bile ducts [1]. PBC has a chronic course and often progresses to the end-stage of liver disease (cirrhosis) with its complications, such as portal hypertension, liver failure, hepatic encephalopathy [7,13,3].

The treatment goal is to prevent the terminal stage of liver cirrhosis and relieve concomitant symptoms. Conventional drug therapy aims to slow the progression of the disease and includes both drugs approved for use according to the indications (ursodeoxycholic (UDCA) and obeticholic acids (OCA)) and those used beyond approved indications (fibrotic acid derivatives, budesonide) [7, 13].The human enteric microbiome is highly complex and has more than 150 times more genes within it than its host. The host and the microbiome have a commensurate relationship that can evolve over time. The typically symbiotic relationship between the two can become pathogenic. The liver receives a significant amount of its blood supply from the splanchnic circulation, and thus is exposed to bacteria and bacterial products from the intestinal microbiome. Reduced bile acids levels in the gut are associated with bacterial overgrowth and inflammation. This review will focus on the role of the microbiome and bile acids in the development of PBC and one of the therapeutic approaches to modulate the microbiome - bile acid axis through dietary product – mare's milk.

Overview of the pathogenesis

It is currently known that the disease develops as a result of infections that cause immune system disorders in hereditarily predisposed individuals when so-called immune complexes (from antibodies, viruses, etc.) are formed and deposited in tissues and lead to tissue damage.

The causes of autoimmune diseases are not fully explained [7,13]. In addition to the immune system, a hereditary predisposition, environmental factors (stress, malnutrition, UVD, etc.), as well as hormonal levels, play its role in these diseases development, as long as the women are more susceptible to autoimmune diseases. If several organs or systems were involved in the process, then such diseases called "systematic" autoimmune diseases.

There is likely a complex sequence of events that occur in the developments of autoimmune liver diseases [3]. A genetic predisposition to auto-immunity in combination with exposure to environmental triggers (Table 1) leads to the development of CD4 T helper cells which are activated to recognize auto-antigens.

Table 1. **Environmental Risk Factors associated with development of autoimmune liver injury.**

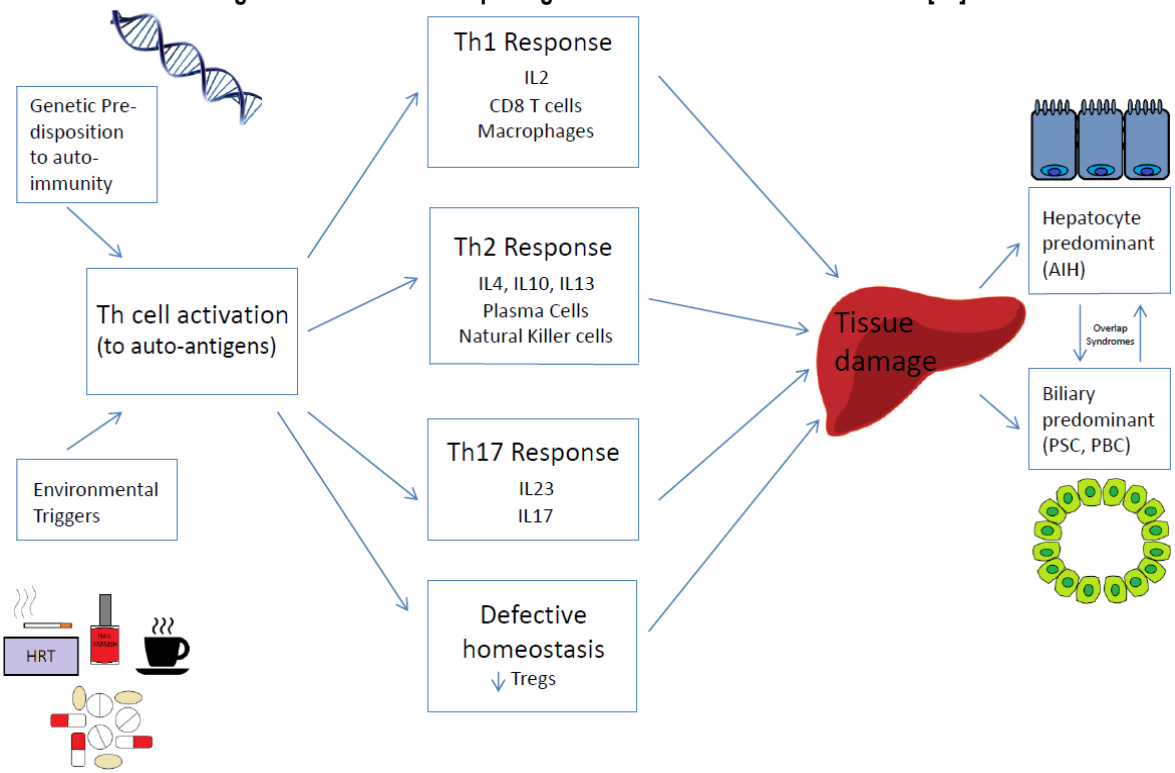
	Factors associated with the disease
Autoimmune hepatitis	Nitrofurantoin; Minocycline; Hepatitis A infection;
Primary biliary cirrhosis	Smoking; HRT; Frequent urinary infections; Nail varnish; Hair dye; Cholestasis of pregnancy; Industrial waste sites; Paracetamol use;
Primary Sclerosing cholangitis	Inflammatory bowel disease; Smoking (protective); Coffee; Hormonal contraception; Diet

This can lead to a variety of immune responses, all of which can lead to tissue damage; the Th1 response involving IL-2 in the differentiation of CD8 cytotoxic T cells and macrophages; the Th17 response involving IL-17 and IL-23; the Th2 response involving IL-4, IL-10 and IL-13 as well as differentiation of B cells into plasma cells resulting in immunoglobulin production as well as activation of monocytes and Natural Killer (NK) cells;

Defective cell homeostasis results in increased cell senescence and defective apoptosis. Decreased numbers of circulating Tregs affects all of these pathways [3].

A combination of these immune responses leads to tissue injury, although it is not certain exactly what factors are involved in the development of biliary or hepatic-predominant disease (Figure 1).

Figure 1. Overview of the pathogenesis of autoimmune liver disease [21].



Metabolism of the bile acids by the gut microbiota

The intestinal microbiome plays an important role in the etiopathogenesis of PBC by regulating metabolism and immune responses. Bile acids are important molecules involved in the regulation of metabolismof lipids in the liver, glucose and maintenance of metabolic and energy homeostasis. Enterohepatic circulation of bile acids is central to the absorption of nutrients and their metabolic regulation. The research results indicate the relationship of bile acids, intestinal microbiome and liver diseases. A disturbance of the metabolism of bile acids is associated with the development of non-alcoholic fatty liver disease, diabetes, hepatic encephalopathy and cholestatic diseases. [6, 23, 10, 24, 7, 4, 19, 10,11].

Intestinal microbial enzymes contribute significantly to the metabolism of bile acids through deconjugation and dehydroxylation reaction with the formation of unconjugated bile acids and secondary bile acids [6, 23, 10, 24, 7, 4, 19, 10]. These microbial enzymes (which include bile salt hydrolase (BSH) and bile acid-inducible enzymes (BAI)) are important for bile acids homeostasis.The study of the interaction between bile acids and gut microbiota in the context of liver disease is essential because the human liver is the only organ in the body that produces all 14 enzymes required for de novo synthesis of the primary bile acids [8, 27].

The main function of bile acids is to assist the absorption of dietary lipids and lipid-soluble nutrients. However, they are now recognized as signalling molecules through activation of receptors like farnesoid X receptor (FXR) or G protein-coupled receptor (TGR5). Therefore, they may modulate lipid, glucose, energy and drug metabolisms as well as their own biosynthesis [22, 23]. The part of the bile acids that escape the enterohepatic

circulation (200 to 800 mg daily in humans) passes into the colon where they undergo bacterial metabolism. These bacterial conversions appear very early in life as 16 different bile acids were identified in meconium [22, 24]. The main bile salt conversions in the human gut include *deconjugation, oxidation and epimerization of hydroxyl groups at C3, C7 and C12, 7-dehydroxylation, esterification and desulfation* (Table 2) [30, 27] and lead to the presence of over 20 different secondary bile acids in adult human feces.

Table 2.

Bacterial genera of the gut microbiota involved in bile acids metabolism [22].

Reactions	Bacterial genera
Deconjugation	*Bacteroides, Bifidobacterium, Clostridium, Lactobacillus, Listeria*
Oxidation and epimerization	*Bacteroides, Clostridium, Escherichia, Egghertella, Eubacterium, Peptostreptococcus, Ruminococcus*
7-dehydroxylation	*Clostridium, Eubacterium*
Esterification	*Bacteroides, Eubacterium, Lactobacillus*
Desulfatation	*Clostridium, Fusobacterium, Peptococcus, Pseudomonas*

The current publication by Kakiyama et al. (2013) provides interesting data in which dysbiosis is occurring in patients with cirrhosis in part due to low bile acid input into the gut [27, 28]. This data suggests that in the absence of bile acids, the bile acid 7α-dehydroxylating bacterial population collapses. Two observations point to this conclusion. First, total bile acids in feces of patients with advanced cirrhosis decreased roughly 5-fold and the ratios of deoxycholic acid/ cholic acid (DCA/CA) and lithocholic/chenodeoxycholic acid (LCA/CDCA) decreased significantly. Second, there is a significant positive correlation between the presence of members of the *Clostridium* cluster XIVa and DCA and LCA concentration. Members of *Clostridium* cluster XIVa, which includes the bile acid 7α-dehydroxylating bacteria, decrease in the intestines as cirrhosis severity advanced. Taken together, these data show a direct relationship between the bile acid pool size and the relative abundance of *Clostridium* cluster XIVa [18, 21].

There are several studies about the gut microbiome in patients with PBC are worth reviewing.In the trial by Tang et al, 37 patients were followed over time to investigate the effect of UDCA-treatment on the microbiome. Similar to PSC, the gut microbiome in PBC patients is characterized by reduced bacterial alpha diversity, and large differences in beta diversity and several genera, including *Haemophilus, Veillonella and Streptococcus*. Interestingly, several genera, including *Haemophilus and Streptococcus*, were affected by UDCA treatment in the followup cohort. In addition, *Veillonella* abundance was reduced after treatment in patients with an adequate UDCA-response, while *Veillonella* increased during UDCA treatment in patients with an inadequate response [36]. This could suggest that microbial markers (like e.g., *Veillonella* abundance) could be used as potential therapeutic or prognostic biomarkers, but this would have to be investigated in dedicated trials and validated in the future [39].

Several of the findings by Tang et al replicate results from an earlier study (Lv L-X, Fang D-Q, Shi D, et al.) of the microbiome in 42 early-stage PBC patients, which also showed that several genera depleted in the gut of PBC patients were negatively associated with markers of liver injury and inflammation [37]. In addition, *Veillonella* has also been reported to be increased in the salivary microbiome of both PBC (n = 39) and autoimmune hepatitis (AIH, n = 17) patients, and correlated positively with IL-1β, IL-8 and immunoglobulin A [1].

Overall, it is probably reasonable to conclude that there are major alterations of the gut microbiome of patients with cholestatic liver diseases. Both conditions (PSC and PBC) show enrichment of specific taxa, e.g. *Streptococcus, Haemophilus and Veillonella* [39].

Therapeutic approaches to modulation of the microbiota

The gut microbiome can be modulated in different ways and targeting intestinal dysbiosis has been investigated as a way to cure liver disease. Therapeutic approaches can be divided into antibiotics, pre-/pro-/synbiotics, dietary changes and fecal microbial transplantation (Table 3) [29]. Prebiotics are nondigestible carbohydrates that promote beneficial changes in the activity and composition of gastrointestinal microflora. Probiotics are living microorganisms (bacteria, fungi) that present a health benefit for the host. Synbiotics contain both prebiotics and probiotics [15, 31, 14]

Absorbable antibiotics should not be used to target the microbiome, whereas non-absorbable antibiotics, e.g. rifaximin are well suited to do so but most likely exert many effects independent from their bactericidal action. In most cases, absorbable antibiotics cause a lasting disruption to the composition of the gut microbiota, which then opens the doors to antibiotic resistance, as well as fungal and pathogen overgrowth (e.g. *Clostridium difficile*) with increased risk of morbidity and mortality [33].

Therefore, the use of pre-, pro- and/or synbiotics has long been advocated. Currently more than 500 clinical trials are registered at nih.gov., clearly underlining the interest in this field [29].

Many years ago, prebiotics, such as *inulin*, were shown to reduce hepatic lipogenesis and serum triglycerides in humans [12] attributable to their fermentation by gut microbiota and the associated increase in short chain fatty acids, such as propionate in the colon and portal vein [16].

Modulation of the microbiome by pre-/pro-and synbiotics can deliver significant beneficial hepatic effects without much concern of major side-effects. In contrast, the safety and efficacy of fecal microbial transplantation, or adsorbents, are less clear [29].

Table 3.

Potential therapies for dysbiosis [15, 31].

Therapy	Effect on intestinal microbiota	Examples
Prebiotic	Complex carbohydrates; digested by colonic microbes to form short-chain fatty acids and lactate, which stimulate the growth of beneficial bacteria	Fructo-oligosaccharide (FOS) Galacto-oligosaccharide (GOS) Lactulose Inulin
Probiotic	Living microorganisms that confer a health benefit on their host through antimicrobial effects, enhancement of mucosal barrier integrity, and immunomodulation	*Lactobacillus GG (LGG) Lactobacillus casei Lactobacillus plantarum Lactobacillus johnsonii Bifidobacterium lactis Saccharomyces boulardii* VSL#3
Synbiotic	Contain prebiotic and probiotic; augment the activity and prolong the survival of potentially beneficial probiotics	*Bifidobacterium* þ FOS Protexin *Lactobacillus* þ inulin
Antibiotic	Antimicrobial effects; changes in bacterial populations and composition; alterations in bacterial metabolic function and virulence	Rifaximin Norfloxacin Neomycin Metronidazole
Fecal microbiota transplant	Colonization resistance (limiting the colonization of pathogens); modulation of bacterial metabolic function	
Dietary changes	Contribution to a higher microbial diversity	A diet rich in fermented milk, vegetables, cereals, coffee, and tea

A diet rich in fermented milk, vegetables, cereals, coffee, and tea contributes to a higher microbial diversity in patients with cirrhosis [5].

In light of the search for safe, effective and convenient methods of treatment, mare's milk acquires a high potential for affecting the chain in the pathogenesis of PBC through modeling the composition of the intestinal microbiome.

Mare's milk – new opportunities

Mare's milk contains about 40 biological components necessary for the human body: amino acids, fats, enzymes (lysozyme, amylase), micro-elements (calcium, sodium, potassium, phosphorus, iron, magnesium, copper, iodine, sulfur, cobalt, zinc, bromine) and vitamins (A, C, B1, B2, B6, B12, E, H, PP, beta-carotene, folic acid) in optimally balanced proportions. A high percentage of nutrients, including vitamins, amino acids, promote immunomodulation, increasing the adaptogenic properties of the body [12]. Valiev A. (2001) demonstrated the effect of essential fatty acids of mare's milk on immunocompetent cells and non-specific resistance after 6 weeks from the start of mare's milk inclusion into the diet[13]. The main immunoglobulin in mare's milk is the secretory IgA [14]. Human and mare's secretory IgA's homology was previously demonstrated by cross-reactions using human anti-IgA antiserum. mare's milk has a powerful detoxification effect, mare's microflora mutagens action, replenishes essential nutrients complex and removes toxins from the body. Milk has a certain degree of antimicrobial effect against opportunistic and pathogenic fungi, bacteria and viruses due to its own microflora [14,2,16]. mare's milk is rich for active substances, natural enzymes that help regulate the intestinal flora, limit the growth of unwanted bacteria and increase the growth of bifidobacteria and lactic acid bacteria. Besides this, mare's milk composition provides with immunoglobulins A, M, and G, which act as potentially pathogenic microorganisms marker, so eases the protection task [14]. High antimicrobial activity of mare's milk is associated with lysozymes, immunoglobulins, lactoperoxidase, and lactoferrin. When ingested in the digestive tract, lysozyme has a strong normalizing effect on the composition of the microbial flora of the mouth and intestines. Breast milk's lysozyme is 100 times more active than cow's milk lysozymes. It not only inhibits the growth of pathogenic flora but also promotes the growth of bifidoflora in the intestines of infants.

The chemical profile of mare's milk is well described [12,16]. Significant quantitative differences were observed between mare's milk, cow's and human milk. These differences include a higher fat and lactose content, but less protein and minerals in mare's milk compared to cow's. However, its protein, sugar and mineral content is similar to human milk, which makes its use as an infant formula more attractive compared to a cow's milk [16]. Mare's milk has a lower concentration of casein than cattle milk but contains twice as much serum. Although the chemical composition of mare's milk is widely available, it is little known of the mare's milk compounds biological activity [16].

The main antimicrobial activity of mare's milk is associated with the serum fraction. Indeed, some components such as lysozyme immunoglobulins, lactoperoxidase, and lactoferrinare well known for their significant antimicrobial effect [16].

Other components, such as complex oligosaccharides can prevent the adhesion of pathogens to the intestinal mucosa and digested only by specific bacteria, such as *lactobacilli or bifidobacteria* which gives them prebiotic properties. The prebiotic property of *Lactobacillus pentosus MMP4* in vivo isolated from mare's milk is described. The ability to inhibit clinically pathogenic microorganisms such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Salmonella* has been identified [9].

Salmonella enterica serovar Typhimurium (Salmonella typhimurium) is the main food pathogen and is the most common salmonella serovar in Europe along with Enteritidis serovar. The antimicrobial activity of *Lactobacillus rhamnosus* strains isolated from mare's milk, growth

inhibition of *Salmonella typhimurium LT-2, Shigella sonnei, Listeria monocytogenes and Escherichia coli O 157* with comparable levels of ampicillin were evaluated, which indicates a favorable aspect of the FSMM15 strain as probiotic strain [2,16].

Clinical trials of using mare's milk are presented to a lesser extent. The effect of the use of mare's milk on the severity of atopic dermatitis, intestinal microbiome and immunological parameters in patients with atopic dermatitis is described: the SCORAD index decreased by 30%, the level of bifidobacteria increased from 4.6% to 11.9%, immunological parameters, with the exception of C- reactive protein has not changed [18].

The Pubmed database contains research data that shows the efficiency and safety of mare's milk for patients with inflammatory bowel diseases [19,28]. Several other clinical trials in Kazakhstan are currently being conducted on the effectiveness of mare's milk for patients with psoriasis (NCT03594877), non-alcoholic fatty liver disease (NCT03664596).

The ongoing clinical study (NCT03665519) aims to increase the effectiveness of PBC therapy targeting on the therapeutic approaches to modulate the microbiome - bile acid axis through dietary product – mare's milk. In the presence of the treatment complexity, the clinical medicine obtains a remedy in form of a natural product - mare's milk, the preventive, dietary, and therapeutic effects of which are known for long.

Conclusion

There are complex interactions between the liver, bile acids and the gut microbiome. Bile acid pool size has recently been shown to be a function of microbial metabolism of bile acids in the intestines. Recent studies have shown potential mechanisms explaining how alterations in the microbiome affect bile acid pool size and composition.

As we enter the era of personalized medicine it will be important to consider the role of microbiota composition in determining individual efficacy and safety of several drugs [26]. A better understanding of the host-microbial interactions in cholestatic diseases may therefore greatly improve clinical care in these conditions.

Modulation of the microbiome by pre-/pro-and synbiotics, diet changes can deliver significant beneficial hepatic effects without much concern of major side-effects.

The use of mare's milk in human consumption is not new, despite it being concentrated in certain regions of the world (especially Central Asia), where it is mainly consumed in the form of kumys. However, scientific discoveries about compositional characteristics have induced interest in mare's milk in other countries of the world [17]. Aspects that deserve to be highlighted and which support recommendations for using mare's milk in human daily ration are its similarity to human milk, high test qualities, the richness in probiotics, the balance between casein and whey proteins, the presence of bioactive compounds and the nutritional quality of the lipid fraction. In fact, the results obtained in studies have proven the functionality of this milk as a food and suggest that itsinclusion into human diets can bring several beneficial effects to health[41].

Thus, adding mare's milk to the treatment options of PBC is the dietary approach with high potential for affecting the chain in the pathogenesis of PBC through modeling the composition of the intestinal microbiome.

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