ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2023 October 27; 15(10): 1084-1169

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 15 Number 10 October 27, 2023

World Journal of Hepatology

Monthly Volume 15 Number 10 October 27, 2023

Contents

ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Moinak Sen Sarma, MBBS, MD, Associate Professor, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India. moinaksen@gmail.com

AIMS AND SCOPE

The primary aim of *World Journal of Hepatology* (*WJH*, *World J Hepatol*) is to provide scholars and readers from various fields of hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online.

WJH mainly publishes articles reporting research results and findings obtained in the field of hepatology and covering a wide range of topics including chronic cholestatic liver diseases, cirrhosis and its complications, clinical alcoholic liver disease, drug induced liver disease autoimmune, fatty liver disease, genetic and pediatric liver diseases, hepatocellular carcinoma, hepatic stellate cells and fibrosis, liver immunology, liver regeneration, hepatic surgery, liver transplantation, biliary tract pathophysiology, non-invasive markers of liver fibrosis, viral hepatitis.

INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (ESCI), Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 Edition of Journal Citation Reports® cites the 2022 impact factor (IF) for *WJH* as 2.4.

RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

PUBLICATION DATE
October 27, 2023

PUBLISHING PARTNER
Department of Infectious Diseases, the Second Affiliated Hospital of Xi'an Jiaotong University

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION ETHICS
https://www.wjgnet.com/bpg/GerInfo/288

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

POLICY OF CO-AUTHORS
<WebSite>https://www.wjgnet.com/bpg/GerInfo/310</WebSite>

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

PUBLISHING PARTNER's OFFICIAL WEBSITE
http://2yuan.xjtu.edu.cn/Html/Departments/Main/Index_21148.html

E-mail: bpgoffice@wjgnet.com https://www.wjgnet.com

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1084-1090

DOI: 10.4254/wjh.v15.i10.1084

ISSN 1948-5182 (online)

OPINION REVIEW

Dietary salt in liver cirrhosis: With a pinch of salt!

Ramesh Kumar, Sudheer Marrapu

Specialty type: Gastroenterology & hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C, C, C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Ferrarese A, Italy; Laghmani K, France; Sato T, Japan

Received: July 12, 2023
Peer-review started: July 12, 2023
First decision: September 11, 2023
Revised: September 21, 2023
Accepted: October 8, 2023
Article in press: October 8, 2023
Published online: October 27, 2023

Ramesh Kumar, Sudheer Marrapu, Department of Gastroenterology, All India Institute of Medical Sciences, Patna 801507, India

Corresponding author: Ramesh Kumar, MD, Additional Professor, Department of Gastroenterology, All India Institute of Medical Sciences, 4th Floor, IPD Block-C, Patna 801507, India. docrameshkr@gmail.com

Abstract

Patients with liver cirrhosis are advised to limit their sodium consumption to control excessive fluid accumulation. Salt is the most common form in which sodium is consumed daily. Consequently, various recommendations urge patients to limit salt intake. However, there is a lack of consistency regarding salt restriction across the guidelines. Moreover, there is conflicting evidence regarding the efficacy of salt restriction in the treatment of ascites. Numerous studies have shown that there is no difference in ascites control between patients with restriction of salt intake and those without restriction. Moreover, patients with cirrhosis may have several negative effects from consuming too little salt, although there are no recommendations on the lower limit of salt intake. Sodium is necessary to maintain the extracellular fluid volume; hence, excessive salt restriction can result in volume contraction, which could negatively impact kidney function in a cirrhotic patient. Salt restriction in cirrhotic patients can also compromise nutrient intake, which can have a negative impact on the overall outcome. There is insufficient evidence to recommend restricted salt intake for all patients with cirrhosis, including those with severe hyponatremia. The existing guidelines on salt restriction do not consider the salt sensitivity of patients; their nutritional state, volume status and sodium storage sites; and the risk of hypochloremia. This opinion article aims to critically analyze the existing literature with regard to salt recommendations for patients with liver cirrhosis and identify potential knowledge gaps that call for further research.

Key Words: Salt; Cirrhosis; Sodium; Hyponatremia; Ascites; Malnutrition

Core Tip: There are still many inconsistencies in the guidelines regarding salt recommendations for patients with liver cirrhosis. Although controlling ascites is the core tenet of salt restriction, there is insufficient data to back up this assertion. Moreover, the guidelines have no recommendations for minimum salt intake, even though too little salt consumption may have a variety of negative effects on patients with liver cirrhosis. To achieve optimal salt consumption in these patients, several factors need to be considered. This article discusses several important aspects of salt consumption in patients with cirrhosis.

Citation: Kumar R, Marrapu S. Dietary salt in liver cirrhosis: With a pinch of salt! *World J Hepatol* 2023; 15(10): 1084-1090
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1084.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1084

INTRODUCTION

Sodium is essential for fluid balance and cellular homeostasis[1]. Under normal conditions, effective sodium balance, and hence extracellular fluid volume, is maintained by a complex interplay between various systems that regulate renal sodium excretion[2]. For example, a progressive increase in sodium intake activates natriuretic systems while suppressing the sodium retaining systems to maintain an effective sodium balance. However, in patients with liver cirrhosis, portal hypertension-related splanchnic vasodilatation reduces effective arterial blood volume, which in turn activates the sympathetic nervous system, renin-angiotensin-aldosterone system (RAAS) and, in later stages, arginine vasopressin, all of which result in renal sodium and fluid retention and, eventually, ascites[3]. Therefore, limiting sodium intake is suggested for the treatment of ascites in such patients[4]. Salt is the most common source of sodium consumed by humans; hence, the guidelines have focused on dietary salt restriction to achieve sodium limitation.

Data on the effectiveness of salt restriction for the management of ascites are not conclusive[5-7], and there are still many inconsistencies in the guidelines for salt recommendations in patients with liver cirrhosis[8-11]. Although the guidelines have suggested the upper limit, a lower limit for salt consumption was not included. Severe salt restriction could potentially result in hyponatremia and cause volume contraction, which might adversely affect kidney function in a cirrhotic patient. Salt restriction in cirrhosis compromises nutrient intake and has a negative impact on the overall outcomes[12,13]. There is a paucity of evidence regarding the dietary salt recommendation for patients with compensated cirrhosis and decompensated cirrhosis with severe hyponatremia[14,15]. The ability of salt to expand extracellular volume, known as salt sensitivity, varies among individuals; thus, a 'one size fits all' approach to dietary salt recommendations may not be appropriate[16]. Furthermore, a new finding indicating that sodium is also stored in a third compartment (interstitium and endothelial surface layer) in a non-osmotic equilibrium can have significant impact on our understanding of sodium intake and homeostasis in cirrhotic patients[17]. This opinion article covers all the aforementioned issues and emphasizes the knowledge gaps and the need for additional research in the area.

SALT AND CIRRHOSIS: IMPORTANT ISSUES

How much salt should cirrhotic patients consume, and are the current recommendations supported by data?

The recommended limits of salt consumption for cirrhotic patients with ascites varies from 4.6 g to 6.9 g/d, although most guidelines recommend around 5 g of salt, which corresponds to a teaspoon of cooking salt (Table 1). As per current recommendations, salt restriction should be considered for all cirrhotic patients with ascites, including those with refractory ascites. When it comes to the grade of ascites at which salt restriction should start, the International Ascites Club suggests grade 1, and the European Association for the Study of the Liver (EASL) suggests grade 2; however, the American Association for the Study of Liver Diseases does not provide any specific grade of ascites to this purpose[8,9,11]. The goal of salt restriction is to avoid sodium overload. Hence, the net sodium intake must be equal or lower than the sodium excretion; this can be achieved either by lowering the dietary salt intake or by increasing natriuresis using diuretics. For patients with grade 2 or higher ascites, salt restriction alone would be insufficient and diuretic therapy would need to be implemented. The first-line diuretics are often aldosterone antagonists, such as spironolactone, which can be administered alone or in conjunction with a loop diuretic such as furosemide. Restriction of fluid intake is required only in patients with dilutional hyponatremia.

Several studies, including randomized controlled trials (RCTs), have examined the role of salt restriction in patients with cirrhosis and ascites[5,6,7,12,13,18]. The amount of salt restriction ranged from a salt-free diet to 7 g of salt per day. The majority of these studies are old with varying methodology and sub-optimal quality, making it challenging to draw clear conclusions. Some of these studies found a faster elimination and better control of ascites with strict salt restriction [5,6]. Some studies found no difference in ascites control between patients with and without salt restriction[12,18]. Furthermore, two recent RCTs found that a salt-unrestricted diet (5-6.5 g/d) was superior to a salt-restricted diet (5 g/d) in resolving ascites in a larger proportion of patients (45% *vs* 16%) and in reducing the need for large volume paracentesis [7,13]. Authors proposed that worsening hyponatremia caused by salt restriction can weaken the effects of diuretics and reduces renal blood flow, both of which worsen ascites[7]. This idea is further supported by research showing that the use

Table 1 Recommendations for salt consumption in patients with liver cirrhosis

Scientific society/Guidelines	Recommended salt intake	Patient group
EASL practice guidelines (2010)[8]	4.6-6.9 g/d	Cirrhosis with grade II or more ascites
AASLD practice guidelines (2021)[9]	5.1 g/d	Cirrhosis with ascites
British Society of Gastroenterology (2021)[10]	5–6.5 g/d	Cirrhosis with ascites
International Ascites Club (2003)[11]	5.2 g/d	Cirrhosis with ascites

AASLD: American Association for the Study of Liver Diseases; EASL: European Association for the Study of the Liver.

of hypertonic saline solutions in conjunction with diuretics enhance fluid mobilization in patients with cirrhosis and refractory ascites[19]. The proposed mechanisms for these advantageous effects of salt loading include an increase in intravascular volume, an osmotic shift of fluid from the tissues, an increase in renal blood flow and a decrease in sympathetic tone. For the management of ascites in cirrhosis, each of these pathways is important. Therefore, although managing ascites is the fundamental premise of salt restriction in cirrhosis patients, there is conflicting evidence regarding this claim.

What adverse effects might cirrhosis patients experience with a salt-restricted diet?

The significant adverse events with salt restriction reported by various studies include hyponatremia, reduced caloric intake, higher risk of renal impairment, hepatic encephalopathy and mortality[5,6,7,12,13]. In a randomized trial, Reynolds *et al*[5] concluded that unrestricted salt intake decreased the likelihood of hyponatremia and azotemia. The risk of hyponatremia is significantly increased by concurrent use of diuretic medication. Severe salt restriction makes the food unpalatable and alters dietary patterns, which might promote protein-calorie malnutrition and increase mortality risk[4,20,21]. Notably, malnutrition and sarcopenia are already prevalent in patients with advanced cirrhosis[22,23]. In a study of cirrhotic patients with ascites requiring repeated paracentesis, salt restriction without nutritional support resulted in a 3.9-fold higher risk of mortality within one year, compared to that of unrestricted sodium intake with nutritional support [13]. In the small intestine, sodium absorption facilitates the absorption of chloride, amino acids, glucose and water[24]. Thus, severe salt restriction may affect the absorption of these substances, contributing to malnutrition.

Another issue concerns compliance with a salt-restricted diet. The average salt consumption by the general population is significantly above the levels recommended by the World Health Organization. A recent systemic review reported that most European countries consume between 4.2 g and 18.5 g of salt per day per capita[25]. Salt intake in India and China is about 10 g/d[26,27]. A cross-sectional survey indicated that only about a third of cirrhotic patients were compliant with salt restriction, with an additional 45% incorrectly stating that they were compliant[28]. Another potentially significant but unappreciated issue with low salt consumption, particularly with concurrent diuretic medication, is the development of hypochloremia, *i.e.*, a low level of serum chloride. Serum chloride, the most important anion in the blood, has received less attention in cirrhosis patients, even though hypochloremia has been recognized as an important prognostic marker in patients with advanced cirrhosis. According to the findings of two recent studies, hypochloremia may be an even better predictor of mortality in patients with decompensated cirrhosis than serum sodium[29,30]. Chloride reabsorption in the renal tubule constitutes a crucial process for the auto-regulation of the acid-base balance as well as the electrochemical equilibrium. Moreover, hypochloremia causes activation of the RAAS and the upregulation of NaCl channels in the distal convoluted tubules, which can aggravate sodium retention and contribute to diuretic resistance[31].

Thus, existing evidence tends to suggest that severe salt-restricted diets (< 5 g/d) may not significantly improve ascites control and could even lead to complications. Considering the excessive salt consumption by the general population, a moderate salt restriction, with daily salt intake of no more than 5–6.5 g may be advisable for such patients. This translates to a 'no added salt diet' with avoidance of high sodium containing meals. Table 2 provides a list of foods with high sodium content so that doctors can counsel patients on their consumption. Also, one must be mindful while recommending salt restriction in advanced cirrhosis patients with hyponatremia as such patients generally have relative hypovolemia where salt restriction could cause volume contraction and renal dysfunction.

Is unrestricted salt intake justified for patients with compensated cirrhosis?

For patients with preascitic compensated cirrhosis, guidelines do not recommend dietary salt restriction. Nonetheless, a study has found that even compensated cirrhosis patients retain sodium when faced with high salt intake[14]. However, compensatory activation of atrial natriuretic peptide and inhibition of the RAAS result in a new steady state of sodium balance in such patients, which tends to prevent ascites. Nevertheless, Jalan *et al*[15] found that the degree of portal hypertension had a significant impact on the sodium handling capacity in patients with compensated cirrhosis. In fact, it is now believed that clinically significant portal hypertension (CSPH) is the main driver of decompensation in cirrhotic patients[32]. Compensated cirrhotic patients with baseline hepatic venous portal gradient > 20 mmHg had a 47% risk of decompensation in a mean duration of just 1.6 years, compared to < 10% over 4 years when it is < 10 mmHg[33,34]. Therefore, it is reasonable to assume that the compensatory natriuretic mechanism might be overwhelmed with the rising portal pressure, and as a result, sodium retention with high dietary salt intake may result in decompensation in the form of ascites in compensated cirrhosis patients. Hence, until further data emerge, salt restriction may be considered in compensated cirrhotic patients with CSPH. This extrapolation, however, needs to be tested in a controlled trial.

Table 2 Food items with a high content of sodium

Food category	High sodium-content food[a]
Cereals, breads, and, grains	Biscuits, pancakes, pizza, sandwiches, burgers, bread with salted tops, potato crisps, and salty snack foods
Vegetables, fruits, and soups	Canned vegetables and vegetable juices, pickles, commercially prepared pasta and tomato sauces, canned soup, and cup-noodles
Dairy products	Butter milk, processed and cottage cheese
Meat products and eggs	Smoked, salted or canned meat, fish or poultry, omelettes
Fats, desserts and sweets	Salted butter or margarine, soy sauce, bottled salad dressings, instant pudding and cake, ketchup
Legumes and nuts	Salted nuts and beans

[a]The sodium content can vary significantly between similar types of foods. Therefore, the nutrition label on the products should be checked to identify foods high in sodium.

What are the implications of high salt intake for cirrhotic patients and the general population?

Studies from different countries found that dietary salt intake in the general population was approximately 10 g/d[25-27]. Directly or indirectly, high salt consumption adversely affects multiple organs in the body and may have some serious implications in patients with liver cirrhosis. Consuming excessive amounts of salt has been associated with oxidative stress, insulin resistance, vascular endothelial damage, sympathetic nerve sensitization, alteration of gut-microbiome and an increased risk of cancer (Figure 1). There is a strong positive association between dietary salt intake and cardiovascular diseases[35]. Therefore, the World Health Organization has recommended a daily salt intake of less than 5 g/d, which is approximately 2 g of sodium, for the general population[36]. According to this viewpoint, the dietary salt recommendation for advanced cirrhosis patients is similar to that of the normal healthy population. In a population-based study, subjects with intermediate salt intake (6–10 g/d) and high salt intake (> 10 g/d) were found to have a higher risk of hepatocellular carcinoma with a multivariable hazard ratio (HR) of 1.49 and 1.9, respectively, compared to those with low salt intake (< 6 g/d)[37]. A recent cohort study from Iran reported that high dietary intake of salt (9.5–15 g/d) increases the rate of mortality in patients with cirrhosis (HR 2.26). Moreover, moderate salt restriction (3–5 g/d), as compared to salt elimination, decreases the risk of death (HR 0.72)[38].

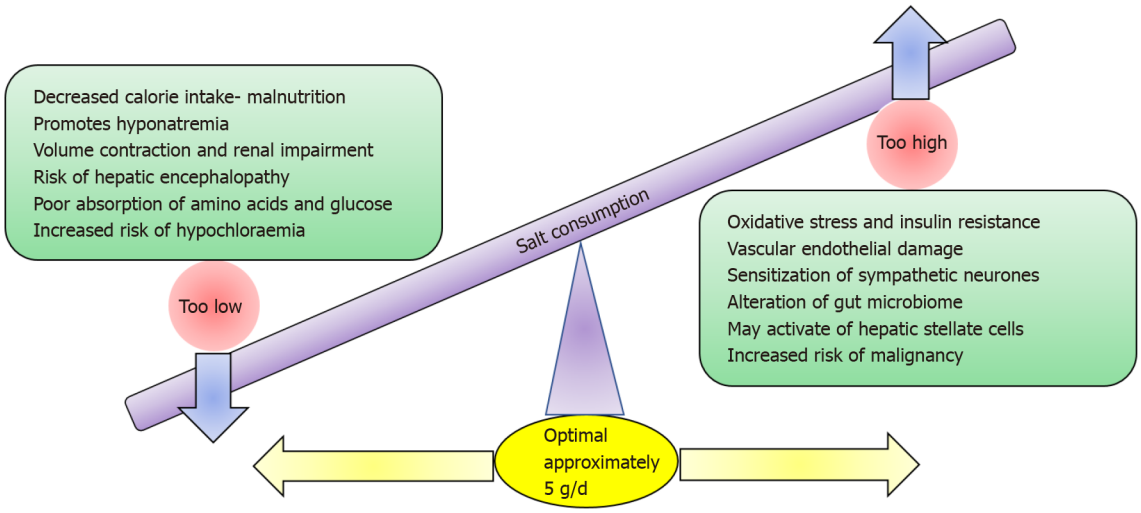
What could be the implications of salt sensitivity and third space sodium storage in patients with liver cirrhosis?

The ability of salt to expand extracellular volume, known as salt sensitivity, varies among individuals. It is estimated that around 26% of normotensive and 51% of hypertensive persons are salt sensitive[39,40]. The remaining individuals are salt resistant – their hemodynamic parameters are likely to be unaffected due to change in dietary salt consumption. Although the relevance of salt sensitivity in cirrhotic patients has never been assessed in the context of dietary salt intake, extrapolation of data from the general population suggests that between half and three-quarters of patients would be salt resistant. It would be safe to assume that a major restriction in dietary salt consumption at the cost of nutritional compromise would be undesirable in a large number of patients. Therefore, instead of having a 'one size fits all' salt recommendation for cirrhotic patients, the salt sensitivity of individual patients should also be considered.

Recently, a third compartment (skin interstitium and endothelial surface layer) of sodium storage sites, in which sodium can accumulate in a non-osmotic equilibrium and hence without concurrent water retention, have been identified [41,42]. This can have implications for sodium homeostasis, osmoregulation and the hemodynamic response to salt intake, all of which are relevant to patients with cirrhosis. In the third space, sodium can be osmotically inactivated following binding to negatively charged glycosaminoglycans[42]. Due to the changes in the dynamics of the interstitium, alterations in glycosaminoglycans and endothelial damage in cirrhotic patients, the amount of dietary salt intake can affect sodium homeostasis. Moreover, high interstitial sodium concentrations stimulate lymph angiogenesis *via* vascular endothelial growth factor-C, which helps in the mobilization of excess fluid from the skin *via* lymphatics[43]. Therefore, it would be interesting to see whether a salt-restricted diet worsens pre-existing lymphatic dysfunction in patients with advanced cirrhosis[44].

What are the practical issues with ensuring a pre-defined sodium consumption?

Measuring sodium intake in individuals is challenging as sodium is so widespread in food items. Even with extensive food labelling, it is often difficult to quantify the sodium content of food. Commonly used approaches include 24-h urine sodium measurement, 24-h dietary recall and food questionnaires[45]. Dietary sodium intake can be calculated by dividing the urine sodium excretion by 0.9, based on the assumption that 10% of sodium intake is lost through sweat and feces, and thus urinary excretion accounts for 90% of intake. Therefore, 24-h urine collection is considered the most reliable method. However, a systematic review found that measured urinary sodium varied as widely as 76%–122% of ingested sodium amount, making it a 'not so reliable' test[46]. Thus, most of these methods would only provide a rough estimate of sodium consumption. As moderate salt restriction might make foods unappealing and affect overall nutrition, some strategies need to be adopted in order to ensure adequate nutrient intake. One of the strategies is to partially substitute sodium with potassium or other minerals, such as calcium or magnesium[47]. However, there are concerns about possible negative effects of such a replacement, such as hyperkalemia with potassium-based salt, especially in



DOI: 10.4254/wjh.v15.i10.1084

Figure 1 Adverse consequences of too little and too high salt consumption. Salt consumption < 5 g/d appears to have no advantage on the control of ascites. Severe salt restriction (< 3 g/d) as well as very high salt consumption (> 10 g/d) can produce many deleterious consequences in cirrhosis patients. The World Health Organization has recommended a daily salt intake of approximately 5 g/d in the general population which is similar to the recommendation for cirrhosis patients with ascites.

cirrhotic patients with renal impairment or those taking potassium sparing diuretics. Additionally, flavors and sensory experiences can be imparted using herbs, spices and yeast extract. When used as salt alternatives, they have demonstrated good customer acceptance[48].

CONCLUSION

Inconsistencies in the recommendations for salt intake and conflicting evidence regarding the effectiveness of salt restriction for controlling ascites in cirrhotic patients necessitate further research. Presently, the term 'salt restriction' for cirrhotic patients appears to be a misnomer, given that the salt recommendation for normal populations is also the same. However, it is necessary to carefully evaluate the efficacy of varied levels of salt intake at various stages of cirrhosis, including those who also have concurrent hyponatremia. Studies on salt restriction must consider the patients' salt sensitivity, nutritional status, volume status, sodium storage sites and hypochloremia risk. Innovative ideas in this area would be to evaluate the efficacy and safety of low-sodium salt substitutes (such as potassium-based salt) and find ways to make low-sodium foods more palatable (by utilizing herbs, spices and yeast extract, *etc.*) to ensure appropriate nutrition.

Until further data emerge, it seems appropriate for cirrhotic patients with ascites to consume 5–6 g of salt per day, which would mean avoiding foods with added salt. To increase adherence, prevent malnutrition and avoid harmful effects of excess salt consumption, it is crucial to educate patients about the recommended salt limit. A formal consultation with a nutritionist may be sought. It is necessary to set a lower limit for salt consumption as too much salt restriction has just as many negative effects as too much consumption. Finally, personalized salt management, depending on the sodium balance, nutritional status and volume status of the patient, may be required for some of these patients.

FOOTNOTES

Author contributions: Kumar R designed the manuscript, collected data, and wrote the manuscript; Marrapu S collected data, and wrote the manuscript.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest for this article.

Country/Territory of origin: India

ORCID number: Ramesh Kumar 0000-0001-5136-4865; Sudheer Marrapu 0009-0002-8209-8648.

S-Editor: Liu JH
L-Editor: Webster JR
P-Editor: Liu JH

REFERENCES

1 **Bernal A**, Zafra MA, Simón MJ, Mahía J. Sodium Homeostasis, a Balance Necessary for Life. *Nutrients* 2023; **15** [PMID: 36678265 DOI: 10.3390/nu15020395]
2 **Bie P**. Mechanisms of sodium balance: total body sodium, surrogate variables, and renal sodium excretion. *Am J Physiol Regul Integr Comp Physiol* 2018; **315**: R945-R962 [PMID: 30110176 DOI: 10.1152/ajpregu.00363.2017]
3 **Cárdenas A**, Arroyo V. Mechanisms of water and sodium retention in cirrhosis and the pathogenesis of ascites. *Best Pract Res Clin Endocrinol Metab* 2003; **17**: 607-622 [PMID: 14687592 DOI: 10.1016/s1521-690x(03)00052-6]
4 **Haberl J**, Zollner G, Fickert P, Stadlbauer V. To salt or not to salt?-That is the question in cirrhosis. *Liver Int* 2018; **38**: 1148-1159 [PMID: 29608812 DOI: 10.1111/liv.13750]
5 **Reynolds TB**, Lieberman FL, Goodman AR. Advantages of treatment of ascites without sodium restriction and without complete removal of excess fluid. *Gut* 1978; **19**: 549-553 [PMID: 680588 DOI: 10.1136/gut.19.6.549]
6 **Gauthier A**, Levy VG, Quinton A, Michel H, Rueff B, Descos L, Durbec JP, Fermanian J, Lancrenon S. Salt or no salt in the treatment of cirrhotic ascites: a randomised study. *Gut* 1986; **27**: 705-709 [PMID: 3522371 DOI: 10.1136/gut.27.6.705]
7 **Gu XB**, Yang XJ, Zhu HY, Xu BY. Effect of a diet with unrestricted sodium on ascites in patients with hepatic cirrhosis. *Gut Liver* 2012; **6**: 355-361 [PMID: 22844565 DOI: 10.5009/gnl.2012.6.3.355]
8 **European Association for the Study of the Liver**. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
9 **Biggins SW**, Angeli P, Garcia-Tsao G, Ginès P, Ling SC, Nadim MK, Wong F, Kim WR. Diagnosis, Evaluation, and Management of Ascites, Spontaneous Bacterial Peritonitis and Hepatorenal Syndrome: 2021 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology* 2021; **74**: 1014-1048 [PMID: 33942342 DOI: 10.1002/hep.31884]
10 **Aithal GP**, Palaniyappan N, China L, Härmälä S, Macken L, Ryan JM, Wilkes EA, Moore K, Leithead JA, Hayes PC, O'Brien AJ, Verma S. Guidelines on the management of ascites in cirrhosis. *Gut* 2021; **70**: 9-29 [PMID: 33067334 DOI: 10.1136/gutjnl-2020-321790]
11 **Moore KP**, Wong F, Gines P, Bernardi M, Ochs A, Salerno F, Angeli P, Porayko M, Moreau R, Garcia-Tsao G, Jimenez W, Planas R, Arroyo V. The management of ascites in cirrhosis: report on the consensus conference of the International Ascites Club. *Hepatology* 2003; **38**: 258-266 [PMID: 12830009 DOI: 10.1053/jhep.2003.50315]
12 **Bernardi M**, Laffi G, Salvagnini M, Azzena G, Bonato S, Marra F, Trevisani F, Gasbarrini G, Naccarato R, Gentilini P. Efficacy and safety of the stepped care medical treatment of ascites in liver cirrhosis: a randomized controlled clinical trial comparing two diets with different sodium content. *Liver* 1993; **13**: 156-162 [PMID: 8336527 DOI: 10.1111/j.1600-0676.1993.tb00624.x]
13 **Sorrentino P**, Castaldo G, Tarantino L, Bracigliano A, Perrella A, Perrella O, Fiorentino F, Vecchione R, D' Angelo S. Preservation of nutritional-status in patients with refractory ascites due to hepatic cirrhosis who are undergoing repeated paracentesis. *J Gastroenterol Hepatol* 2012; **27**: 813-822 [PMID: 22142548 DOI: 10.1111/j.1440-1746.2011.07043.x]
14 **Wong F**, Liu P, Blendis L. Sodium homeostasis with chronic sodium loading in preascitic cirrhosis. *Gut* 2001; **49**: 847-851 [PMID: 11709521 DOI: 10.1136/gut.49.6.847]
15 **Jalan R**, Hayes PC. Sodium handling in patients with well compensated cirrhosis is dependent on the severity of liver disease and portal pressure. *Gut* 2000; **46**: 527-533 [PMID: 10716683 DOI: 10.1136/gut.46.4.527]
16 **Ishimwe JA**, Dola T, Ertuglu LA, Kirabo A. Bile acids and salt-sensitive hypertension: a role of the gut-liver axis. *Am J Physiol Heart Circ Physiol* 2022; **322**: H636-H646 [PMID: 35245132 DOI: 10.1152/ajpheart.00027.2022]
17 **van IJzendoorn M**, van den Born J, Hijmans R, Bodde R, Buter H, Dam W, Kingma P, Maes G, van der Veen T, Zijlstra W, Dijkstra B, Navis G, Boerma C. An observational study on intracutaneous sodium storage in intensive care patients and controls. *PLoS One* 2019; **14**: e0223100 [PMID: 31581250 DOI: 10.1371/journal.pone.0223100]
18 **Descos L**, Gauthier A, Levy VG, Michel H, Quinton A, Rueff B, Fermanian J, Fombonne E, Durbec JP. Comparison of six treatments of ascites in patients with liver cirrhosis. A clinical trial. *Hepatogastroenterology* 1983; **30**: 15-20 [PMID: 6339343]
19 **Licata G**, Tuttolomondo A, Licata A, Parrinello G, Di Raimondo D, Di Sciacca R, Cammà C, Craxì A, Paterna S, Pinto A. Clinical Trial: High-dose furosemide plus small-volume hypertonic saline solutions vs. repeated paracentesis as treatment of refractory ascites. *Aliment Pharmacol Ther* 2009; **30**: 227-235 [PMID: 19438847 DOI: 10.1111/j.1365-2036.2009.04040.x]
20 **Eghtesad S**, Poustchi H, Malekzadeh R. Malnutrition in liver cirrhosis:the influence of protein and sodium. *Middle East J Dig Dis* 2013; **5**: 65-75 [PMID: 24829672]
21 **Sam J**, Nguyen GC. Protein-calorie malnutrition as a prognostic indicator of mortality among patients hospitalized with cirrhosis and portal hypertension. *Liver Int* 2009; **29**: 1396-1402 [PMID: 19602136 DOI: 10.1111/j.1478-3231.2009.02077.x]
22 **Cheung K**, Lee SS, Raman M. Prevalence and mechanisms of malnutrition in patients with advanced liver disease, and nutrition management strategies. *Clin Gastroenterol Hepatol* 2012; **10**: 117-125 [PMID: 21893127 DOI: 10.1016/j.cgh.2011.08.016]
23 **Kumar R**, Prakash SS, Priyadarshi RN, Anand U. Sarcopenia in Chronic Liver Disease: A Metabolic Perspective. *J Clin Transl Hepatol* 2022; **10**: 1213-1222 [PMID: 36381104 DOI: 10.14218/JCTH.2022.00239]
24 **Fordtran JS**, Rector FC Jr, Carter NW. The mechanisms of sodium absorption in the human small intestine. *J Clin Invest* 1968; **47**: 884-900 [PMID: 5641624 DOI: 10.1172/JCI105781]
25 **Kwong EJL**, Whiting S, Bunge AC, Leven Y, Breda J, Rakovac I, Cappuccio FP, Wickramasinghe K. Population-level salt intake in the WHO European Region in 2022: a systematic review. *Public Health Nutr* 2022; 1-14 [PMID: 36263661 DOI: 10.1017/S136898002200218X]
26 **Johnson C**, Praveen D, Pope A, Raj TS, Pillai RN, Land MA, Neal B. Mean population salt consumption in India: a systematic review. *J Hypertens* 2017; **35**: 3-9 [PMID: 27755388 DOI: 10.1097/HJH.0000000000001141]
27 **Tan M**, He FJ, Wang C, MacGregor GA. Twenty-Four-Hour Urinary Sodium and Potassium Excretion in China: A Systematic Review and

Meta-Analysis. *J Am Heart Assoc* 2019; **8**: e012923 [PMID: 31295409 DOI: 10.1161/JAHA.119.012923]

28 **Morando F**, Rosi S, Gola E, Nardi M, Piano S, Fasolato S, Stanco M, Cavallin M, Romano A, Sticca A, Caregaro L, Gatta A, Angeli P. Adherence to a moderate sodium restriction diet in outpatients with cirrhosis and ascites: a real-life cross-sectional study. *Liver Int* 2015; **35**: 1508-1515 [PMID: 24811138 DOI: 10.1111/liv.12583]

29 **Sumarsono A**, Wang J, Xie L, Chiang GC, Tielleman T, Messiah SE, Singal AG, Mufti A, Chen C, Leveno M. Prognostic Value of Hypochloremia in Critically Ill Patients With Decompensated Cirrhosis. *Crit Care Med* 2020; **48**: e1054-e1061 [PMID: 32947468 DOI: 10.1097/CCM.0000000000004620]

30 **Ji Y**, Li L. Lower serum chloride concentrations are associated with increased risk of mortality in critically ill cirrhotic patients: an analysis of the MIMIC-III database. *BMC Gastroenterol* 2021; **21**: 200 [PMID: 33933032 DOI: 10.1186/s12876-021-01797-3]

31 **Hanberg JS**, Rao V, Ter Maaten JM, Laur O, Brisco MA, Perry Wilson F, Grodin JL, Assefa M, Samuel Broughton J, Planavsky NJ, Ahmad T, Bellumkonda L, Tang WH, Parikh CR, Testani JM. Hypochloremia and Diuretic Resistance in Heart Failure: Mechanistic Insights. *Circ Heart Fail* 2016; **9** [PMID: 27507113 DOI: 10.1161/CIRCHEARTFAILURE.116.003180]

32 **Kumar R**, Kumar S, Prakash SS. Compensated liver cirrhosis: Natural course and disease modifying strategies. World J Methodol 2023; 13: 179-193 [DOI: 10.5662/wjm.v13.i4.179]

33 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS, Bosch J; Portal Hypertension Collaborative Group. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [PMID: 17681169 DOI: 10.1053/j.gastro.2007.05.024]

34 **Jindal A**, Bhardwaj A, Kumar G, Sarin SK. Clinical Decompensation and Outcomes in Patients With Compensated Cirrhosis and a Hepatic Venous Pressure Gradient ≥20 mm Hg. *Am J Gastroenterol* 2020; **115**: 1624-1633 [PMID: 32453061 DOI: 10.14309/ajg.0000000000000653]

35 **Meneton P**, Jeunemaitre X, de Wardener HE, MacGregor GA. Links between dietary salt intake, renal salt handling, blood pressure, and cardiovascular diseases. *Physiol Rev* 2005; **85**: 679-715 [PMID: 15788708 DOI: 10.1152/physrev.00056.2003]

36 **Rosengren A**. Salt: the sweet spot? *Eur Heart J* 2022; **43**: 2889-2891 [PMID: 35808993 DOI: 10.1093/eurheartj/ehac336]

37 **Sun M**, Cui H, Liang M, Wang W, Wang Y, Liu X, Liu S, Cao L. Perceived dietary salt intake and the risk of primary liver cancer: a population-based prospective study. *J Hum Nutr Diet* 2020; **33**: 833-840 [PMID: 32548912 DOI: 10.1111/jhn.12761]

38 **Pashayee-Khamene F**, Hajimohammadebrahim-Ketabforoush M, Saber-Firoozi M, Hatami B, Naseri K, Karimi S, Ahmadzadeh S, Kord H, Saadati S, Hekmatdoost A. Salt consumption and mortality risk in cirrhotic patients: results from a cohort study. *J Nutr Sci* 2022; **11**: e99 [PMID: 36405096 DOI: 10.1017/jns.2022.69]

39 **Weinberger MH**, Miller JZ, Luft FC, Grim CE, Fineberg NS. Definitions and characteristics of sodium sensitivity and blood pressure resistance. *Hypertension* 1986; **8**: II127-II134 [PMID: 3522418 DOI: 10.1161/01.hyp.8.6_pt_2.ii127]

40 **Mishra S**, Ingole S, Jain R. Salt sensitivity and its implication in clinical practice. *Indian Heart J* 2018; **70**: 556-564 [PMID: 30170653 DOI: 10.1016/j.ihj.2017.10.006]

41 **Olde Engberink RHG**, Selvarajah V, Vogt L. Clinical impact of tissue sodium storage. *Pediatr Nephrol* 2020; **35**: 1373-1380 [PMID: 31363839 DOI: 10.1007/s00467-019-04305-8]

42 **Titze J**, Shakibaei M, Schafflhuber M, Schulze-Tanzil G, Porst M, Schwind KH, Dietsch P, Hilgers KF. Glycosaminoglycan polymerization may enable osmotically inactive Na+ storage in the skin. *Am J Physiol Heart Circ Physiol* 2004; **287**: H203-H208 [PMID: 14975935 DOI: 10.1152/ajpheart.01237.2003]

43 **Machnik A**, Neuhofer W, Jantsch J, Dahlmann A, Tammela T, Machura K, Park JK, Beck FX, Müller DN, Derer W, Goss J, Ziomber A, Dietsch P, Wagner H, van Rooijen N, Kurtz A, Hilgers KF, Alitalo K, Eckardt KU, Luft FC, Kerjaschki D, Titze J. Macrophages regulate salt-dependent volume and blood pressure by a vascular endothelial growth factor-C-dependent buffering mechanism. *Nat Med* 2009; **15**: 545-552 [PMID: 19412173 DOI: 10.1038/nm.1960]

44 **Kumar R**, Anand U, Priyadarshi RN. Lymphatic dysfunction in advanced cirrhosis: Contextual perspective and clinical implications. *World J Hepatol* 2021; **13**: 300-314 [PMID: 33815674 DOI: 10.4254/wjh.v13.i3.300]

45 **McLean R**, Cameron C, Butcher E, Cook NR, Woodward M, Campbell NRC. Comparison of 24-hour urine and 24-hour diet recall for estimating dietary sodium intake in populations: A systematic review and meta-analysis. *J Clin Hypertens (Greenwich)* 2019; **21**: 1753-1762 [PMID: 31769168 DOI: 10.1111/jch.13729]

46 **Lucko AM**, Doktorchik C, Woodward M, Cogswell M, Neal B, Rabi D, Anderson C, He FJ, MacGregor GA, L'Abbe M, Arcand J, Whelton PK, McLean R, Campbell NRC; TRUE Consortium. Percentage of ingested sodium excreted in 24-hour urine collections: A systematic review and meta-analysis. *J Clin Hypertens (Greenwich)* 2018; **20**: 1220-1229 [PMID: 30101426 DOI: 10.1111/jch.13353]

47 **Brand A**, Visser ME, Schoonees A, Naude CE. Replacing salt with low-sodium salt substitutes (LSSS) for cardiovascular health in adults, children and pregnant women. *Cochrane Database Syst Rev* 2022; **8**: CD015207 [PMID: 35944931 DOI: 10.1002/14651858.CD015207]

48 **Nurmilah S**, Cahyana Y, Utama GL, Aït-Kaddour A. Strategies to Reduce Salt Content and Its Effect on Food Characteristics and Acceptance: A Review. *Foods* 2022; **11** [PMID: 36230196 DOI: 10.3390/foods11193120]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1091-1108

DOI: 10.4254/wjh.v15.i10.1091

ISSN 1948-5182 (online)

REVIEW

Progress on traditional Chinese medicine in improving hepatic fibrosis through inhibiting oxidative stress

Zhen Li, Jun-Feng Zhu, Hao Ouyang

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Cheng TH, Taiwan; Radhakrishnan K, South Korea

Received: June 26, 2023
Peer-review started: June 26, 2023
First decision: August 16, 2023
Revised: August 26, 2023
Accepted: September 19, 2023
Article in press: September 19, 2023
Published online: October 27, 2023

Zhen Li, Jun-Feng Zhu, Hao Ouyang, Department of Liver, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

Jun-Feng Zhu, Department of Liver, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China

Corresponding author: Jun-Feng Zhu, Doctor, PhD, Chief Physician, Full Professor, Department of Liver, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, No. 110 Ganhe Road, Hongkou District, Shanghai 201203, China. zhujftongling@163.com

Abstract

Hepatic fibrosis is a common pathological process that occurs in the development of various chronic liver diseases into cirrhosis and liver cancer, characterized by excessive deposition of the extracellular matrix. In the past, hepatic fibrosis was thought to be a static and irreversible pathological process. In recent years, with the rapid development of molecular biology and the continuous in-depth study of the liver at the microscopic level, more and more evidence has shown that hepatic fibrosis is a dynamic and reversible process. Therefore, it is particularly important to find an effective, simple, and inexpensive method for its prevention and treatment. Traditional Chinese medicine (TCM) occupies an important position in the treatment of hepatic fibrosis due to its advantages of low adverse reactions, low cost, and multi-target effectiveness. A large number of research results have shown that TCM monomers, single herbal extracts, and TCM formulas play important roles in the prevention and treatment of hepatic fibrosis. Oxidative stress (OS) is one of the key factors in the occurrence and development of hepatic fibrosis. Therefore, this article reviews the progress in the understanding of the mechanisms of TCM monomers, single herbal extracts, and TCM formulas in preventing and treating hepatic fibrosis by inhibiting OS in recent years, in order to provide a reference and basis for drug therapy of hepatic fibrosis.

Key Words: Hepatic fibrosis; Oxidative stress; Traditional Chinese medicine monomer; Single herbal extract; Traditional Chinese medicine formula

Core Tip: Hepatic fibrosis is a common pathological process that occurs in the development of various chronic liver diseases into cirrhosis and liver cancer, characterized by excessive deposition of the extracellular matrix. This article reviews the progress in the understanding of the mechanisms of traditional Chinese medicine (TCM) monomers, single herbal extracts, and TCM formulas in preventing and treating hepatic fibrosis by inhibiting oxidative stress in recent years, in order to provide a reference and basis for drug therapy of hepatic fibrosis.

Citation: Li Z, Zhu JF, Ouyang H. Progress on traditional Chinese medicine in improving hepatic fibrosis through inhibiting oxidative stress. *World J Hepatol* 2023; 15(10): 1091-1108
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1091.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1091

INTRODUCTION

The prevalence of hepatic fibrosis ranges from 2% to 19%, and it remains a major cause of morbidity and mortality worldwide[1,2]. It leads to notorious complications such as ascites, portal hypertension, hepatic encephalopathy, and liver failure, and increases the risk of hepatocellular carcinoma, posing a heavy burden on individuals, society, and healthcare systems[3,4]. Currently, Western medical treatments for hepatic fibrosis include antiviral drugs, corticosteroids, hepatoprotective drugs, and liver support, but they have low efficacy rates, significant resistance, and side effects [5]. Traditional Chinese medicine (TCM), as a unique medical approach in China, has better safety and effectiveness and is widely used to treat hepatic fibrosis[6]. Continuous oxidative stress (OS) in the liver can induce biological changes in hepatocytes, leading to fibrotic changes in the liver[7]. Based on this, experts and scholars have conducted relevant experimental studies to demonstrate that TCM monomers, single herbal extracts, and TCM formulas can significantly improve the condition of hepatic fibrosis by regulating OS. Therefore, this article reviews the progress in the the understanding of the mechanisms of TCM in preventing and treating hepatic fibrosis by using OS as a starting point to provide references for the clinical treatment of hepatic fibrosis with TCM.

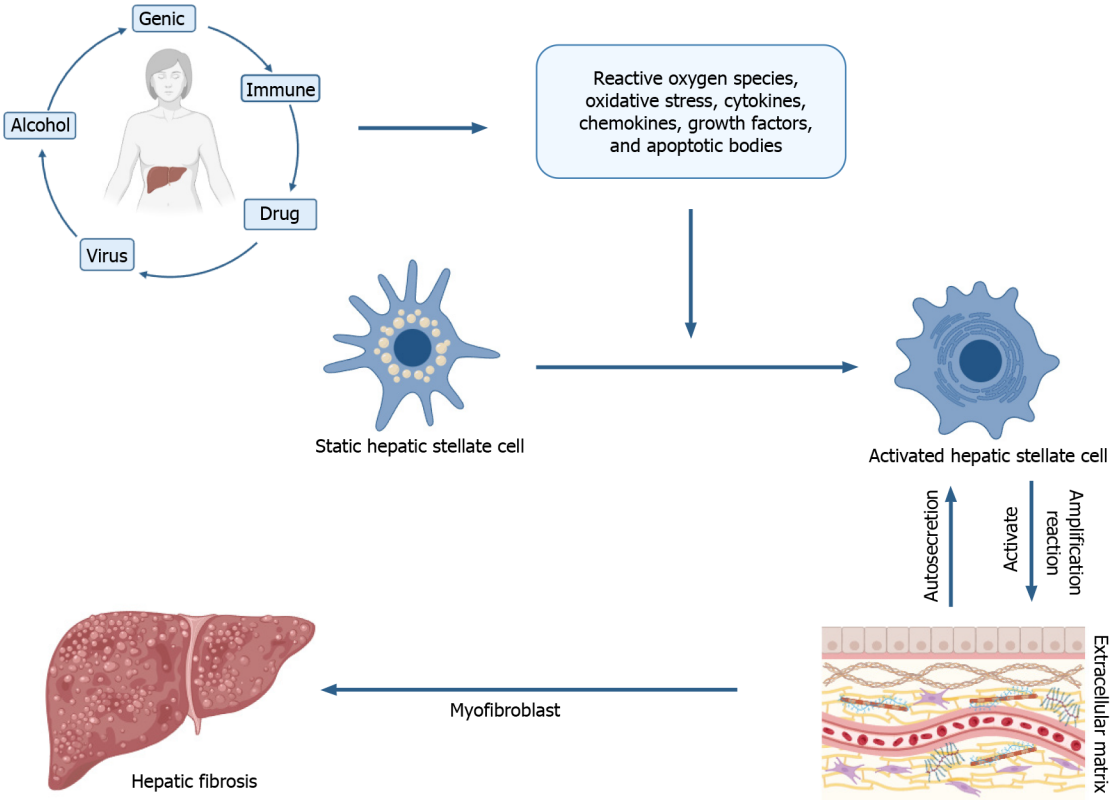
MECHANISMS OF HEPATIC FIBROSIS

Hepatic fibrosis is a chronic wound healing response to cellular damage and inflammation caused by increased synthesis and deposition of extracellular matrix (ECM) components and decreased or unbalanced ECM degradation (Figure 1)[8,9]. Various etiologies such as alcohol abuse, viral hepatitis infection, genetic abnormalities, non-alcoholic fatty liver disease, autoimmune disorders, and other non-infectious diseases can cause continuous wound healing response and liver injury, leading to hepatic fibrosis[10]. The main mechanism of hepatic fibrosis is believed to be the activation of myofibroblast precursor cells, which leads to an increase in ECM deposition surrounding the sinusoidal cell layer in the Disse space[11, 12]. ECM increase is the main feature of hepatic fibrosis, and the ECM is composed of five types of substances, collagen, non-collagenous proteins, elastic fibers, proteoglycans, and glycosaminoglycans. It is mainly divided into basement membrane and interstitial matrix according to its distribution site[13]. In human patients and rodent models of liver disease, fibrotic livers contain multiple types of collagen (types I, III, and V), non-fibrillar collagens (IV and VI), and glycosaminoglycans and proteoglycans (such as fibronectin, tenascin, laminin, basement membrane proteoglycans, decorin, biglycan, and fibrillin)[11].

In addition, hepatic stellate cells (HSCs) (Ito cells and lipocytes), portal-resident fibroblasts (portal or central veins), epithelial cells undergoing epithelial-to-mesenchymal transition, bone marrow-derived fibroblasts, vascular smooth muscle cells, and sinusoidal peri-hematopoietic stem cells are the main cell types that produce the ECM during hepatic fibrosis[14]. In human patients, HSCs constitute 5%-8% of the total liver cells involved in growth, differentiation, and regeneration. They are not only the main source of myofibroblasts but also the main cell type leading to hepatic fibrosis [15]. Portal fibroblasts are thought to play an important role in fibrosis in cholestatic liver disease[16,17]. The static HSC to myofibroblast differentiation is a multi-step process involving reactive oxygen species (ROS), cytokines, chemokines, growth factors, and apoptotic bodies from hepatocytes[18]. Chronic liver injury involves HSCs undergoing phenotypic activation towards myofibroblasts, which is characterized by increased expression of cell markers such as alpha-smooth muscle actin (α-SMA) and collagen. In addition, transforming growth factor-beta 1 (TGF-β1) inhibits liver regeneration during HSC to myofibroblast differentiation, ultimately leading to hepatic fibrosis[19].

OS-MEDIATED HEPATIC FIBROSIS

OS refers to the imbalance between the normal oxidant scavenging enzyme system [such as superoxide dismutase (SOD), catalase, and glutathione (GSH)] and the production of ROS in the cells, which is considered a key driving factor in hepatic fibrosis[20]. Oxidants, also known as ROS, include superoxide anion radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen[21]. In addition, they also contain some nitrogen oxides, lipid peroxide radicals, and



DOI: 10.4254/wjh.v15.i10.1091

Figure 1 The mechanism of hepatic fibrosis. Due to various factors such as alcohol abuse, viral hepatitis infection, genetic abnormalities, alcoholic fatty liver disease, autoimmune diseases, and medications, the body may produce an excessive amount of reactive oxygen species, leading to oxidative stress. At the same time, these factors may also induce cells to release cytokines, chemokines, growth factors, and apoptotic bodies, as well as activate hepatic stellate cells (HSC) and transform them into myofibroblasts. Consequently, large amounts of extracellular matrix (ECM) substances, such as collagen, non-fibrillar collagens, glycosaminoglycans, and proteoglycans, are autosecreted. The presence of these ECM substances can further stimulate the autosecretion of HSCs, ultimately resulting in fibrosis and impaired liver function.

hypochlorous acid. The process of oxidant formation begins with oxygen being reduced to water, resulting in the production of free radicals such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals[22].

Under physiological conditions, ROS are the result of normal cellular metabolism and are maintained in dynamic equilibrium with antioxidants[23]. Under pathological conditions, excess ROS can stimulate pathological oxidative-reductive signal transduction, leading to OS. Various organic compounds such as DNA, lipids, carbohydrates, and proteins are structurally damaged, resulting in cell damage and various diseases[24]. Lee *et al*[25] first demonstrated a possible molecular link between enhanced lipid peroxidation and induced collagen gene expression in cultured fibroblasts, suggesting that OS plays a direct pathogenic role in hepatic fibrogenesis. Sustained OS in the liver directly or indirectly affects hepatocytes and alters the structure of cell membranes and organelles, causing damage, necrosis, and apoptosis[26]. These processes lead to cell damage and the release of various cytokines and growth factors, inducing quiescent HSC activation into myofibroblasts expressing α-SMA as a characteristic marker[27]. Activated HSCs lose lipid droplets (vitamin A), rapidly proliferate, and upregulate many genes, especially collagen, fibronectin, laminin, and hyaluronic acid, beginning to increase the synthesis of connective tissue proteins, especially collagen, leading to fibrosis formation and further development into liver cirrhosis and even liver cancer[19,28]. In addition, excess ROS also enhance the secretion of the fibrogenic factor TGF-β1, which is highly involved in HSC activation, exacerbating ECM deposition in the liver and progressing to hepatic fibrosis[29]. The mechanism of OS-mediated hepatic fibrosis is shown in Figure 2.

UNDERSTANDING HEPATIC FIBROSIS FROM TCM PERSPECTIVE

Ancient literature did not have a clear concept of "hepatic fibrosis" as a disease name. Based on its main clinical manifestations of hypochondriac pain, palpable masses in the hypochondrium, and jaundice, modern physicians categorize it under disease categories such as distension and swelling, hypochondriac pain, accumulation, and jaundice[6]. The Ling Shu section of the Huangdi Neijing states: "If the evil is in the liver, then there is pain in both flank regions, the patient feels cold, and stagnant blood circulates within, causing restricted joint movements, and occasional foot swelling. Acupuncture at Jia Jian was used to activate the meridian around the liver area and warm up the stomach, extract blood through veins to eliminate stagnant blood, and take out the green vein by the ear to alleviate cramps[30]." The Huangdi Neijing says: "Wind, cold, and dampness combined cause obstruction of channels and collaterals, known as arthralgia.

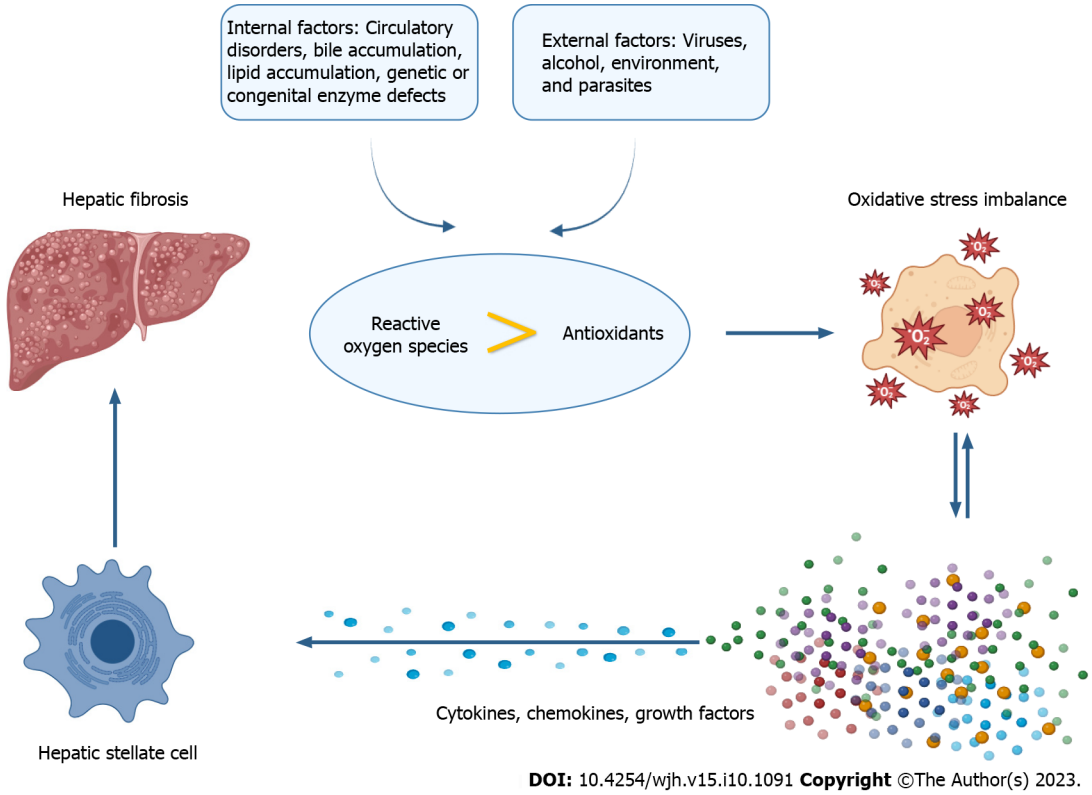
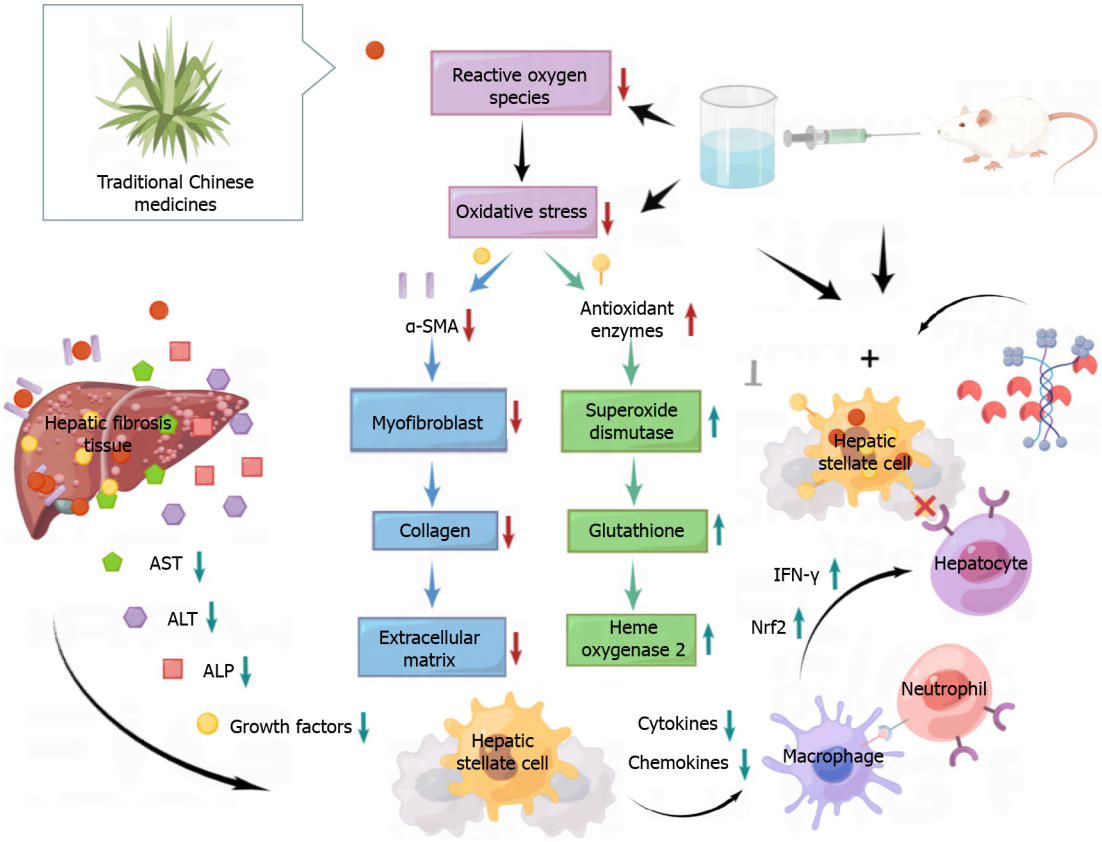


Figure 2 The mechanism of oxidative stress-mediated hepatic fibrosis. Circulatory disorders, bile accumulation, lipid accumulation, genetic or congenital enzyme defects, as well as factors such as viruses, alcohol, environment, and parasites, can all contribute to an imbalance between the production of reactive oxygen species and the body's ability to remove them, resulting in oxidative stress. This imbalance can further stimulate the secretion of cytokines, chemokines, and growth factors, which in turn activate hepatic stellate cells and contribute to the development of hepatic fibrosis.

Furthermore, if this occurs in spring, it is called tendon arthralgia. Tendon arthralgia continues stubbornly and is further affected by evil forces, which reside in the liver interior. All organs have junctions, so if an illness remains lingering, it will settle in the junction of that organ. Since the liver's junction is with the tendons, the arthralgia continues, and when affected by evil forces, it settles in the liver[31]." Therefore, the TCM understanding of the pathogenesis of hepatic fibrosis can be summarized as weakened vital qi, allowing external pathogenic factors such as excessive exposure to the "Six Pathogens" or inappropriate emotional responses from the "Seven Emotions" to invade, resulting in Qi stagnation and blood stasis[32]. This progression is often slow and persistent, ultimately leading to hepatic fibrosis. Based on classic single herbal extracts and TCM formulas, many effective preventive and therapeutic TCM formulas have been developed and applied clinically. Currently, TCM monomers, single herbal extracts, and TCM formulas can regulate HSCs and ECM expression levels by affecting OS, achieving a state of balance between yin and yang in the body, and thereby preventing and treating hepatic fibrosis[33]. Therefore, this article analyzes and discusses the research progress in preventing and treating hepatic fibrosis through the inhibition of OS by using TCM monomers, single herbal extracts, and TCM formulas, based on recent domestic and international studies on hepatic fibrosis, in order to provide a theoretical basis for future research.

MECHANISMS OF TCM IN ANTI-FIBROSIS

As TCM has shown good therapeutic effects in various chronic diseases, its role and mechanism in preventing and treating hepatic fibrosis have attracted widespread attention from scientists. Scholars from various countries have begun using modern pharmacological research methods to explore the mechanisms of TCM in anti-fibrosis. It has been confirmed that TCM monomers (such as flavonoids, glycosides, alkaloids, and polysaccharides), single herbal extracts (such as *Salvia miltiorrhiza*, *Ginkgo biloba* leaf, clove basil, *Ceratonia siliqua* pod extract, grape seed, pomegranate extract, *Taraxacum officinale* root extract, and *Myrtus communis*), and TCM formulas [such as Yin-Chen-Hao-Tang (YCHT), Xiaochaihu Tang (XCHT), Fu Zheng Hua Yu Fang, Chunggan extract, and Huangjia Ruangan Granule] can be used to inhibit OS and prevent and treat hepatic fibrosis (Figure 3).



DOI: 10.4254/wjh.v15.i10.1091

Figure 3 Treatment of hepatic fibrosis by oxidative stress mediated by traditional Chinese medicine. Traditional Chinese medicine employs various mechanisms to reduce the production of reactive oxygen species in the body, thereby suppressing oxidative stress reactions. They can enhance the secretion of antioxidant enzymes such as superoxide dismutase, glutathione, and heme oxygenase 2, as well as increase the activity of interferon-γ and nuclear factor erythroid 2-related factor. Moreover, traditional Chinese medicine can inhibit the secretion of growth factors, cytokines, chemokines, macrophages, and neutrophils. They can also hinder the activation of quiescent hepatic stellate cells into myofibroblasts and facilitate the degradation of extracellular matrix components like collagen. These actions contribute to the protection of liver function and hepatocytes, ultimately mitigating and reversing hepatic fibrosis. ALP: Alkaline phosphatase; ALT: Alanine transaminase; AST: Aspartate aminotransferase; α-SMA: Alpha-smooth-muscle actin; IFN-γ: Interferon-γ; Nrf2: Nuclear factor erythroid 2-related factor.

TCM MONOMERS

In recent years, numerous experimental studies have demonstrated that TCM monomers, such as flavonoids, glycosides, alkaloids, and polysaccharides, can reduce the activation rate of HSCs by inhibiting OS and reducing excessive ECM deposition. This in turn can suppress hepatic fibrosis and connective tissue proliferation or improve liver function and delay the progression of hepatic fibrosis.

FLAVONOIDS

Flavonoids are a class of compounds that belong to the family of polyphenols. They are widely found in plant-based foods such as vegetables, fruits, and grains[34]. Flavonoids are known for their beneficial effects on human health, such as antioxidant and anti-inflammatory properties[35]. The most common flavonoids include quercetin, kaempferol, and myricetin, which are found in many fruits and vegetables[34]. Flavonoids have been linked to a reduced risk of chronic diseases such as cardiovascular disease, cancer, and diabetes[36]. Briefly, the consumption of flavonoid-rich foods is an important part of a healthy diet. Extensive pharmacological research has shown that flavonoids possess the ability to inhibit the pathological production of ROS, suppress OS, boost the antioxidant capacity of the body, and offer protection against hepatic fibrosis[37].

Quercetin is an important plant chemical substance belonging to the polyphenolic flavonoid group[38]. The chemical formula of quercetin is $C_{15}H_{10}O_7$, and its structure shares a common flavonoid nucleus composed of two benzene rings linked by a heterocyclic pyran ring[39]. Quercetin is commonly found in various fruits and vegetables, including apples, berries, cherries, red leaf lettuce, onions, and asparagus, with small amounts present in pepper, broccoli, peas, and tomatoes[40]. It is well known that onions contain the highest levels of quercetin[41]. Quercetin is one of the most extensively researched flavonoids and has been found to exhibit exceptional antioxidant activity. Its effects on GSH and ROS activity, as well as its regulation of various signaling pathways including heme oxygenase 1/nuclear factor erythroid 2-related factor (Nrf2), mitogen-activated protein kinase (MAPK), Toll-like receptor 4 (TLR4)/phosphatidylin-

ositol-3-kinase (PI3K), and 5'adenosine monophosphate-activated protein kinase have been demonstrated in numerous studies[42]. Studies have also shown that quercetin can enhance the activities of antioxidant enzymes like SOD and increase GSH levels. Furthermore, it can inhibit OS, down-regulate inflammatory cytokine expression, and reduce tissue histopathological changes induced by thioacetamide, thus mitigating hepatic fibrosis[43]. In addition, Khodarahmi *et al* [44] proposed that quercetin can improve hepatic fibrosis by inhibiting ROS-related OS-mediated inflammatory cascades. Concisely, these findings suggest that quercetin has potential therapeutic benefits for hepatic fibrosis through its ability to enhance antioxidant enzyme activities, reduce OS and inflammation, and inhibit ROS-mediated cascades. However, more research is needed to fully understand the mechanism of action and optimal dosage of quercetin for treating hepatic fibrosis.

Isorhamnetin, with the chemical formula $C_{16}H_{12}O_7$, is a 3'-O-methylated gut metabolite of quercetin[45]. Isorhamnetin belongs to the flavonoid family, more specifically the flavonol group[46]. It can be found in several plants, such as sorbus, ginkgo leaves, or cactus, which have traditionally been used as medicinal plants in various cultures[47]. *In vitro*, isorhamnetin can scavenge diphenylpicrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radicals, inhibit liver mitochondrial lipid peroxidation, and exhibit antioxidant activity[48]. Furthermore, isorhamnetin can block TGF-β1-induced ROS production and GSH depletion, reduce phosphorylated Smad3, TGF-β1, α-SMA, and plasminogen activator inhibitor-1 expression, and collagen expression in primary mouse HSCs and LX-2 cells, alleviate OS, and inhibit HSC activation, thereby preventing hepatic fibrosis[49]. These findings suggest that isorhamnetin may have potential therapeutic applications for hepatic fibrosis.

Naringin is a natural organic compound with the molecular formula $C_{15}H_{12}O_5$[50]. Naringin and its glycosides are present in various herbs and fruits, including grapefruit, Buddha's hand citron, lime, tart cherry, tomato, cocoa, Greek hay, water mint, and legumes[51]. Due to its hydroxyl substituents, naringin exhibits high reactivity towards ROS and reactive nitrogen species and has strong inhibitory effects on lipid peroxidation in mouse liver, brain, and heart tissues [52]. In 2017, Hernández-Aquino *et al*[53] reported that administration of naringin could potentially prevent an increase in liver enzymes such as alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase, and GSH peroxidase (GSH-Px). Additionally, naringin was shown to enhance the body's antioxidant capacity and effectively prevent liver inflammation, hepatocyte necrosis, and hepatic fibrosis induced by carbon tetrachloride (CCl_4)[53]. Building on this work, Hernández-Aquino *et al*[54] conducted further research in 2019 and found that naringin has the potential to inhibit OS and exert its anti-fibrotic effect by blocking the nuclear transcription factor-κB (NF-κB), TGF-β-Smad3, and c-Jun N-terminal kinase-Smad3 pathways. These findings suggest that naringin could be a promising candidate for treating human fibrosis.

Additionally, *Mallotus apelta* (Lour.) Muell.Arg. leaf and *Bidens bipinnata* L. contain total flavonoids along with other beneficial compounds such as puerarin, hesperidin, alpinetin, fisetin, glabridin, morin, and astilbin. These compounds have been shown to inhibit OS and promote a stable internal environment within the body, thereby improving hepatic fibrosis (Table 1).

GLYCOSIDES

Glycosides are a diverse group of biologically active compounds that are widely distributed in the plant kingdom[55]. They consist of a sugar molecule linked to a non-sugar compound, such as a flavonoid or terpenoid. Glycosides have been extensively studied for their pharmacological properties, which include anti-inflammatory, antibacterial, antifungal, and antioxidant effects[56]. In particular, glycosides have been found to possess potent antioxidant properties that can prevent or reduce OS in various tissues and organs, including the liver[57].

Saikosaponin-D is a type of glycoside monomer component extracted from the dried roots of *Bupleurum chinense* DC. and *Bupleurum scorzonerifolium* Willd, both plants belonging to the Umbelliferae family[58]. Its molecular formula is $C_{42}H_{68}O_{13}$[58]. Saikosaponin-D possesses various pharmacological effects such as antioxidant, sedative, antiviral, anti-inflammatory, immune-regulatory, hepatoprotective, and anticancer activities[59]. Saikosaponin-D can delay the development of hepatic fibrosis by alleviating liver cell damage caused by OS[57]. Researchers such as Que *et al*[60] have proposed that saikosaponin-D may downregulate the ROS/MAPK signaling pathway. This not only significantly inhibits the proliferation and activation of HSC-T6 cells induced by OS but also reduces the deposition of the ECM, such as tissue inhibitors of TGF-β1, hydroxyproline, collagen-1, and matrix metalloproteinase (MMP)-1, which indicates its potential as a therapeutic agent for hepatic fibrosis[60]. However, despite its promising pharmacological properties, the clinical use of saikosaponin-D is limited due to its low bioavailability and poor water solubility. Further research is needed to develop effective delivery systems and optimize its pharmacokinetic properties to allow for its use in clinical settings.

Resveratrol glucoside is a polyphenol and monocrystalline natural compound belonging to the stilbene class[61]. Vitaceae, Liliaceae, and Leguminosae families are the important sources of resveratrol glucoside extraction[62]. It is mainly isolated from the rhizome and roots of *Polygonum cuspidatum*, and also found in daily foods such as grapes and red wine[62]. Various studies have shown that resveratrol glucoside has a variety of pharmacological activities, such as anti-inflammatory, anti-apoptotic, anti-tumor, lipid-lowering, and cardiovascular protective effects, particularly strong antioxidant pharmacological activity[63]. Resveratrol glucoside has been shown to have antioxidant biological activity and therapeutic action on many liver diseases, including hepatic fibrosis. For example, resveratrol glucoside has been found to inhibit the production of 4-hydroxydecenoic acid in the liver and the expression of nicotinamide adenine dinucleotide phosphate oxidase 4, thereby reducing OS and inflammation and improving chronic liver injury and fibrosis [64,65]. Moreover, research has demonstrated that resveratrol glucoside can also downregulate the nicotinamide adenine dinucleotide phosphate oxidase 4 enzyme, thus decreasing TLR4/NF-κB p65 signaling pathway-related inflammatory

Table 1 Flavonoids improve hepatic fibrosis by inhibiting oxidative stress

No.	Flavonoids	Model	Upregulated molecules	Downregulated molecules	Mechanism	Ref.
1	*Mallotus apelta* (Lour.) Muell.Arg. leaf	Rat	IkBa, SOD, GSH-Px	HYP, PC-III, Col-I, HA, LN, α-SMA, Col-III, MDA, TNF-α, IL-1β, IL-6, MMP-2/9, TIMP-1, TGF-β1/Smad, IKKβ, NF-κB p65, pNF-κBp65, IκBα, p-IκBα	Improving hepatic fibrosis and reducing ECM accumulation are related to inhibiting OS and regulating the TGF-β1/Smad signaling pathway and NF-κB-dependent inflammatory response	Zhang *et al* [117]
2	*Bidens bipinnata* L.	Rat	SOD, GSH-Px	NF-κB, α-SMA, TGF-β1	Inhibiting OS to improve liver injury and protect rats from CCl4-induced hepatic fibrosis	Yuan *et al* [118]
3	Puerarin	Rat	SOD, CAT	TG, TCHO, LDL, α-SMA, Col-I/III, MDA, IL-1β, IL-6, TNF-α, MCP-1, NF-κB/p65, TGF-β, TGF-β/Smad2/3	Inhibiting OS and inflammation associated with NF-κB signal inactivation, thereby blocking the upregulation of pro-inflammatory cytokines (IL-1β and TNF-α) and chemokines (MCP-1), ultimately improving hepatic fibrosis	Hou *et al* [119]
4	Hesperidin	Mice		ROS	Regulating liver OS and alleviating mitochondrial dysfunction to reduce fibrosis	Li *et al*[120]
5	Alpinetin	Mice	IL-10, CAT, GSH-Px, SOD, GSH, Nrf2	HYP, α-SMA, fibronectin, α1(I) procollagen, IL-1β, IL-6, TNF-α, Cox-2, iNOS, MDA, VEGF, VEGFR2, PDGF-βR, HIF-1α	Preventing and treating hepatic fibrosis by inhibiting the anti-inflammatory activity mediated by NLRP3 and the anti-OS activity mediated by Nrf2	Zhu *et al* [121]
6	Fisetin	Rat	GSH, GSK-3β	MDA, TNF-α, IL-6, TGF-β1, Col-I, TIMP-1, Wnt3a, β-catenin, α-SMA, Cyclin D1	Inhibiting liver OS can suppress Wnt/β-catenin pathway and inhibit HSC activation and proliferation, regulate MMP-9 and TIMP-1, and suppress multiple pro-fibrotic factors, to prevent and treat hepatic fibrosis	El-Fadaly *et al*[122]
7	Glabridin	Mice	PPAR-γ, GSH, T-AOC	α-SMA, fibronectin, Col-α1(I), MDA	Activation of PPARγ can inhibit inflammation and OS, thereby inhibiting HSC activation and hepatic fibrosis	Zhang *et al* [123]
8	Morin	Rat	GSH	NO, HYP, TNF-α, iNOS, NF-κB (p65)	Receiving MDA and nitric oxide levels elevation, and restoring GSH to normal levels can inhibit OS and improve hepatic fibrosis	Heeba and Mahmoud [124]
9	Astilbin	Rat	SOD, GSH, GCLC, GCLM, HO-1, NQO1, Nrf2	TGF-β, Col-I/III, HYP, TNF-α, IL-1β, IL-6, MDA	Significant reduction in collagen production, inflammation and OS *in vivo* can prevent CCL4-induced hepatic fibrosis in rats	Sun *et al* [125]

α-SMA: Alpha-smooth-muscle actin; ECM: Extracellular matrix; OS: Oxidative stress; TGF-β1: Transforming growth factor-beta 1; NF-κB: Nuclear transcription factor-κB; SOD: Superoxide dismutase; GSH: Glutathione; GSH-Px: Glutathione peroxidase; IL: Interleukin; TNF: Tumor necrosis factor; ROS: Reactive oxygen species; Nrf2: Nuclear factor erythroid 2-related factor; HSC: Hepatic stellate cell.

reactions and macrophage expression, which suggests that it could be an effective therapeutic agent for preventing and treating hepatic fibrosis[66]. To sum up, resveratrol glucoside shows promise as a natural compound for preventing and treating chronic liver injury and fibrosis. Further research is needed to fully understand its mechanisms of action and optimal dosage for therapeutic use.

Geniposide is an organic compound with the molecular formula $C_{17}H_{24}O_{10}$[67]. It is derived from the dried mature fruit of *Gardenia jasminoides Ellis*, a plant belonging to the Rubiaceae family[68]. Geniposide is mainly found in *Gardenia jasminoides*, but has also been detected in other commonly used Chinese herbal medicines such as *Eucommia ulmoides*, *Rehmannia glutinosa*, and *Scutellaria baicalensis*[68]. Geniposide not only upregulates endogenous antioxidant enzymes to slow down cell damage, but also increases the activity of antioxidant enzymes and pathways such as liver lipid peroxidation, GSH S-transferases, GSH, GSH-Px, and copper- and zinc-containing SOD, which can prevent OS damage, protect hepatocytes, and improve hepatic fibrosis[69]. The study by Yang *et al*[70] investigated the protective effects of geniposide on hepatic fibrosis in a rat model induced by CCl_4 administration. The researchers found that geniposide treatment significantly reduced hepatic fibrosis and improved liver function, as evidenced by decreased levels of serum ALT, aspartate aminotransferase (AST), and ALP. Further analysis revealed that geniposide exerted its anti-fibrotic effects through multiple mechanisms[70]. First, geniposide increased the activities of two important antioxidant enzymes, SOD and GSH-Px, which scavenge free radicals and protect cells from oxidative damage. This was accompanied by a reduction in the levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, in the liver tissue[70]. In brief, the study suggested that geniposide has potential as a therapeutic agent for hepatic fibrosis by targeting OS. However, further studies are needed to confirm these findings in human subjects and to explore the optimal dosage and duration of geniposide treatment.

In addition, glycoside compounds such as baicalin, vitexin, and forsythoside A (Table 2) can also improve hepatic fibrosis by inhibiting the body's OS, regulating intestinal flora bile acid metabolism, increasing antioxidant and phase II

Table 2 Glycosides improve hepatic fibrosis by inhibiting oxidative stress

No.	Glycosides	Model	Upregulated molecules	Downregulated molecules	Mechanism	Ref.
1	Wogonoside	Rat	SOD, GSH, IL-10	HA, LN, HYP, MDA, TNF-α, IL-1β, IL-6, TGF-β, MMP-2/9, Smad-3, PI3K/Akt/mTOR, NF-κB p65, PI3K/Akt/mTOR/p70S6K, α-SMA, Col-I	Inhibition of OS can improve hepatic fibrosis	Wang *et al* [126]
2	Hyperoside	Mice	SOD, GSH-Px, CAT, GSH, Nrf2	GOT, GPT, MDA, TGF-β, Col-I/III	Activating Nrf2 nuclear translocation, inhibiting OS to increase antioxidant and phase II detoxifying enzyme activity can improve hepatic fibrosis	Zou *et al*[127]
3	Forsythiaside A	Mice	SOD, GSH, CAT, GSH-Px, p450 7a1, FXR, SHP, LRH-1, BSEP, NTCP, OATP-1	HA, LN, PC-III, Col-I/IV, HYP, α-SMA, TNF-α, IL-1β, IL-6, LCA, DCA, HDCA, β-MCA, alloLCA, NorDCA, NorCA, CA, FGF-R4, MRP-2	Inhibiting OS and inflammation, regulating gut microbiota and BA metabolism can improve hepatic fibrosis	Fu *et al* [128]

α-SMA: Alpha-smooth-muscle actin; OS: Oxidative stress; SOD: Superoxide dismutase; GSH: Glutathione; GSH-Px: Glutathione peroxidase; IL: Interleukin; Nrf2: Nuclear factor erythroid 2-related factor.

detoxification enzyme activity. To recap, the evidence suggests that glycosides can prevent or reduce hepatic fibrosis by inhibiting ROS production, reducing OS, and modulating HSC activation. These findings highlight the potential of glycosides as therapeutic agents for hepatic fibrosis and other OS-related diseases. However, further studies are needed to elucidate the molecular mechanisms underlying the protective effects of glycosides and identify optimal doses and treatment regimens.

ALKALOIDS

Alkaloids are a class of naturally occurring organic compounds that are characterized by their bitter taste and basic properties[71]. They are found in many plants and have a wide range of biological activities, including analgesic, anti-inflammatory, and anti-cancer properties. Alkaloids have complex interactions with ROS and hepatic fibrosis[72].

Some alkaloids have protective effects against hepatic fibrosis by reducing ROS production and promoting antioxidant activity. Further research is needed to better understand the mechanisms underlying these effects and to identify new alkaloids with potential therapeutic applications in hepatic fibrosis.

Berberine is a naturally occurring compound found in various plants such as goldenseal, barberry, and oregon grape [73]. It has been widely used in traditional medicine for its anti-inflammatory, anti-microbial, and anti-diabetic properties [74]. In recent years, there has been growing interest in the potential of berberine as a treatment for hepatic fibrosis. Studies have shown that berberine has potent antioxidant properties that help reduce OS and ROS levels in the liver[75]. It achieves this by activating various cellular defense mechanisms that protect liver cells from oxidative damage[75]. Furthermore, studies suggest that berberine can prevent the activation of HSCs, which are responsible for producing the scar tissue that leads to hepatic fibrosis[76]. Domitrović *et al*[77] demonstrated that the administration of high-dose berberine (9 mg/kg) is effective in reducing OS, decreasing the expression of tumor necrosis factor-alpha (TNF-α) and TGF-β1, increasing MMP-2 levels, and promoting the removal of fibrous deposits to ameliorate hepatic fibrosis. In essence, these findings suggest that berberine has great potential as a therapeutic agent in the treatment of hepatic fibrosis. By reducing OS and preventing the activation of HSCs, berberine may help slow or even reverse the progression of hepatic fibrosis. However, further research is necessary to fully understand the mechanisms through which berberine exerts its beneficial effects on hepatic fibrosis.

Betaine, also known as trimethylglycine, is a naturally occurring compound found in many foods, including spinach, beets, and whole grains[78]. It is used as a dietary supplement to improve athletic performance, promote liver health, and reduce the risk of liver disease[79]. Research suggests that betaine may help to alleviate hepatic fibrosis by reducing OS. In a study conducted by Bingül *et al*[80], betaine supplementation was found to significantly reduce ROS levels, decrease lipid peroxidation, and increase antioxidant enzyme activity in rats with hepatic fibrosis induced by CCl_4 exposure. Additionally, betaine treatment reduced collagen deposition and improved liver function in these rats, indicating that it may have therapeutic potential for hepatic fibrosis[81]. Another study conducted by Kim *et al*[82] investigated the effects of betaine on OS and fibrosis in liver cells. The researchers found that betaine treatment reduced ROS levels and lipid peroxidation, increased GSH levels (an important antioxidant in the body), and inhibited the expression of fibrotic markers in HSCs, the primary cells responsible for hepatic fibrosis. These results suggest that betaine may exert its anti-fibrotic effects by modulating OS and reducing fibrogenic signaling pathways in liver cells[82]. In conclusion, the relationship between betaine and hepatic fibrosis is complex, but emerging evidence suggests that betaine may help to alleviate hepatic fibrosis by reducing OS, inhibiting fibrogenic signaling pathways, and promoting liver function. Further research is needed to fully elucidate the mechanisms underlying betaine's therapeutic effects on hepatic fibrosis and determine optimal dosages and treatment durations for clinical use.

Lycorine is a natural alkaloid that is found in various plant species such as the Amaryllidaceae family[83]. It has been known to possess multiple pharmacological properties, including anti-cancer, anti-inflammatory, and antiviral activities [84]. Recently, lycorine has also been studied for its effect on hepatic fibrosis, a chronic condition that occurs due to the accumulation of ECM proteins in the liver tissue. Lycorine has been shown to inhibit ROS production and reduce OS, thereby reducing HSC activation and regulating the fibrotic process[83]. Furthermore, lycorine may also have a protective effect against liver injury caused by OS. In a study conducted on animal models of acute liver injury induced by CCl_4, lycorine was found to prevent liver damage by reducing OS and inflammation in liver tissue[85]. To conclude, the relationship between lycorine, ROS, OS, and hepatic fibrosis is complex and multifaceted. More research is needed to explore the potential therapeutic effects of lycorine in hepatic fibrosis and other related liver diseases. However, the current evidence suggests that lycorine may hold promise as a natural therapeutic agent for hepatic fibrosis.

To put it briefly, alkaloids have shown potential as therapeutic agents for the treatment of hepatic fibrosis. However, more research is needed to determine their efficacy and safety, especially at higher doses. It is important to work with a healthcare professional to determine the best course of action for managing hepatic fibrosis and to monitor liver function regularly.

POLYSACCHARIDES

Polysaccharides are complex carbohydrates that play an important role in the body's physiological processes[86]. They are found in a variety of sources, including plants, fungi, and animals, and are known for their ability to confer a range of health benefits[86]. One area where polysaccharides have shown particular promise is in the treatment of hepatic fibrosis. Recent research has suggested that polysaccharides may offer a promising alternative for managing hepatic fibrosis. In particular, studies have shown that certain polysaccharides have antioxidant properties, which can help reduce OS in the liver. OS is known to play a key role in the development and progression of hepatic fibrosis.

Cordyceps is a type of fungus that has been used for centuries in traditional medicine to treat a variety of ailments[87]. In recent years, researchers have been investigating the therapeutic potential of Cordyceps polysaccharides, one of the main bioactive compounds found in the fungus[88]. Cordyceps polysaccharides have been shown to have antioxidant and anti-inflammatory properties, making them a promising candidate for treating hepatic fibrosis, a condition characterized by scarring and damage to the liver[89]. Cordyceps polysaccharides protect hepatocytes from hydrogen peroxide-induced mitochondrial dysfunction by reducing ROS production and regulating mitochondrial apoptotic signaling *via* cytochrome C, apoptosis regulator Bax, and mitochondrial-related apoptotic proteins in HepG2 cells[90]. In addition, studies have shown that Cordyceps polysaccharides can reduce the release of pro-inflammatory cytokines and cell apoptosis by regulating TLR4/myeloid differentiation factor 88/NF-κB, Bcl-2/Bax, and caspase family signaling pathways, thereby reducing OS, serum enzymes, α-SMA, Col-III, TGF-β1, p-Smad3, and collagen volume fraction, inhibiting OS, enhancing the body's antioxidant defense system, and improving hepatic fibrosis[91]. Recapitulating, Cordyceps polysaccharides have demonstrated hepatoprotective effects against liver injury caused by OS. They can regulate various signaling pathways to promote liver cell survival, reduce fibrosis, and improve liver function. These findings suggest that polysaccharides may be a promising therapeutic agent for liver diseases.

The relationship between polysaccharides, OS, and hepatic fibrosis is complex, and additional research is needed to fully understand the mechanisms underlying this relationship. However, these findings suggest that polysaccharides may offer a promising new avenue for the treatment of hepatic fibrosis.

SINGLE HERBAL EXTRACTS

Single herbal extracts such as *Salvia miltiorrhiza*, *Ginkgo biloba* leaf, clove basil, *Ceratonia siliqua* pod extract, grape seed, pomegranate extract, *Taraxacum officinale* root, and *Myrtus communis* have demonstrated significant potential in combating hepatic fibrosis. These extracts target OS pathways, regulate HSC activation and apoptosis, and inhibit collagen deposition in liver tissue, as outlined in Table 3.

Salvia miltiorrhiza, also known as Danshen, is a TCM that has been used for centuries to treat various ailments, including liver disease[92]. Recent studies have shown that one of the mechanisms by which *Salvia miltiorrhiza* exerts its hepatoprotective effects is through the modulation of OS pathways in the liver[93]. Research has demonstrated that *Salvia miltiorrhiza* can alleviate hepatic fibrosis by reducing the generation of ROS and the consequent activation of HSCs[94]. In a recent study, Zhang *et al*[94] investigated the effect of *Salvia miltiorrhiza* on ROS-induced hepatic fibrosis in rats. The researchers found that treatment with *Salvia miltiorrhiza* significantly reduced the levels of ROS and lipid peroxidation products in the liver tissue, and also inhibited the activation of HSCs[94]. Furthermore, they observed that *Salvia miltiorrhiza* treatment increased the expression of antioxidant enzymes, such as SOD and catalase, which help protect against OS in the liver[95]. Another study by Wang *et al*[96] explored the potential molecular mechanisms underlying the anti-fibrotic effects of *Salvia miltiorrhiza*. The researchers found that *Salvia miltiorrhiza* could inhibit the expression of fibrogenic genes in HSCs, such as *Col-I* and *α-SMA*, by downregulating the activity of the TGF-β signaling pathway, which is known to be a key regulator of fibrogenesis in the liver[96]. Moreover, they observed that *Salvia miltiorrhiza* treatment reduced the production of ROS and enhanced the activity of antioxidant enzymes in HSCs, which may contribute to its anti-fibrotic effects[97]. Summarily, these studies provide evidence that *Salvia miltiorrhiza* can protect against hepatic fibrosis by modulating OS pathways in the liver. Further research is needed to fully understand the molecular mechanisms underlying this effect and to explore the potential of *Salvia miltiorrhiza* as a therapeutic agent for

Table 3 Single herbal extracts improve hepatic fibrosis by inhibiting oxidative stress

No.	Single herbal extracts	Model	Upregulated molecules	Downregulated molecules	Mechanism	Ref.
1	*Salvia miltiorrhiza*	Mice	SOD, GSH	Col-1/III, TGF-β, MDA, IL-1α, TNF-α	Reducing MDA content, increasing SOD activity and GSH content, and suppressing OS while also reducing TNF-α and IL-1α can improve hepatic fibrosis	Zhang *et al* [94]
2	*Ginkgo biloba* leaf	Rat		MDA, TGF-β1, Col-1	Effectively regulating the OS thioacetamide-induced hepatic fibrosis	Al-Attar *et al* [100]
3	Clove basil	Rat	CAT	α-SMA, Col-α	Inhibiting OS can significantly suppress HSC activation, as well as the expressions of α-SMA and Col-α, exhibiting its anti-hepatic fibrosis properties	Chiu *et al* [103]
4	*Ceratonia siliqua* pod	Mice	GSH, SOD, CAT, GST, GPx, GR	TIMP-2, NO	Inhibiting the production of LPO and NO, increasing the content of GSH, and restoring the activity of antioxidant enzymes can suppress OS, granuloma formation, and hepatic fibrosis	Al-Olayan *et al*[129]
5	Grape seed	Rat	AOC, GSH	TNF-α, MDA, MPO	Inhibiting neutrophil infiltration and lipid peroxidation, suppressing OS, restoring the oxidative and antioxidant status in tissues can help protect against hepatic fibrosis	Dulundu *et al*[130]
6	Pomegranate	Rat	SOD, GST, CAT, Nrf2	NF-κB, α-SMA, Cox-2	Reducing OS by regulating Nrf2 and NF-κB can eliminate hepatic fibrosis	Husain *et al* [131]
7	*Taraxacum officinale* root	Mice	SOD, mt I/II	α-SMA	Inhibiting OS, inducing HSC inactivation and enhancing liver regeneration capacity are effective in the treatment of hepatic fibrosis	Domitrović *et al*[132]
8	*Myrtus communis*	Rat	GSH, SOD	TNF-α, IL-1β, MDA, MPO, TGF-β	Its antioxidant and free radical scavenging activities protect liver tissue from OS damage after bile duct ligation , thereby exerting its anti-fibrotic effects	Sen *et al*[133]

α-SMA: Alpha-smooth-muscle actin; OS: Oxidative stress; SOD: Superoxide dismutase; GSH: Glutathione; IL: Interleukin; Nrf2: Nuclear factor erythroid 2-related factor; HSC: Hepatic stellate cell.

hepatic fibrosis.

Ginkgo biloba leaf is a popular herb that has been used for centuries for various health benefits[98]. Ginkgo contains flavonoids and terpenoids, which are known to have antioxidant and anti-inflammatory properties[45]. These compounds make ginkgo a potential treatment option for hepatic fibrosis[99]. *Ginkgo biloba* leaf has the potential therapeutic effect of effectively regulating OS induced by thioacetamide and exerting an effect on thioacetamide-induced hepatic fibrosis[100]. Studies have demonstrated that *Ginkgo biloba* leaf can inhibit HSC activation and reduce collagen deposition in liver tissue by regulating OS pathways[100]. In addition, *Ginkgo biloba* leaf has been found to upregulate the expression of Nrf2, a transcription factor that plays a key role in the cellular response to OS[101]. This suggests that *Ginkgo biloba* leaf may be a promising therapeutic agent for hepatic fibrosis.

Clove basil, also known as *Ocimum gratissimum*, is a species of basil that is native to parts of Africa and Asia[102]. It has been used in traditional medicine to treat a variety of conditions, including liver diseases. Recent studies have demonstrated the potential of clove basil extract in preventing the development of hepatic fibrosis through its antioxidant properties. Clove basil contains high levels of phenolic compounds, such as rosmarinic acid, which have potent antioxidant activity. Studies have shown that the clove basil extract possesses anti-hepatic fibrosis properties by inhibiting serum-induced HSC activation and reducing the expression of α-SMA and Col-a[103]. This effect is attributed to the antioxidant components in the extract, which suppress OS and prevent hepatic fibrosis[103]. Additionally, studies have shown that clove basil extract can maintain levels of ALT and AST, as well as MDA, catalase, and α-SMA levels induced by CCl_4, indicating its ability to protect the liver from oxidative damage and promote healing of hepatic fibrosis [104]. Synthetically, clove basil extract has the potential to be used as a therapeutic agent in the treatment of hepatic fibrosis due to its antioxidant and anti-HSC activation properties. However, further studies are needed to explore its mechanism of action and its potential use in clinical settings.

These findings suggest that single herbal extracts may be a potential treatment option for hepatic fibrosis. However, more research is needed to fully understand their effectiveness and potential side effects. It is important to consult with a healthcare professional before taking any herbal supplements, including clove basil.

TCM FORMULAS

The TCM formula stands out for its multi-component, multi-target, and low adverse reaction properties. It employs syndrome differentiation treatment and holistic therapy to regulate the body functions and status, improves prognosis, and eliminates pathogenic factors without harming healthy ones. As a result, TCM formulas show remarkable potential in preventing and treating hepatic fibrosis. This article presents a summary of TCM formulas (Table 4) which have been

Table 4 Traditional Chinese medicine formulas improve hepatic fibrosis by inhibiting oxidative stress

No.	Traditional Chinese medicine formulas	Composition	Efficacy	Model	Upregulated molecules	Downregulated molecules	Mechanism	Ref.
1	Yin-Chen-Hao-Tang	*Artemisia annua* L. 18 g, *Gardenia jasminoides Ellis* 12 g, *Rheum palmatum* 6 g	Clearing heat and promoting diuresis to relieve dampness and jaundice	Rat		Col-I, α-SMA, TGF-β, HYP, ROS	Alleviating hepatic fibrosis by enhancing antioxidant capacity, reducing ROS levels, suppressing OS, and inhibiting inflammatory response	Lee *et al* [106], Mountford *et al*[108], Chen *et al* [109]
2	Xiaochaihu Tang (XCHT)	Bupleurum root 12 g, *Scutellaria baicalensis* 9 g, Ginseng 6g, *Pinellia ternata* 9 g, Roasted Licorice 5 g, Fresh Ginger 9 g, 4 Red Dates	Soothing liver and relieving depression, regulating stomach and lowering qi counterflow, tonifying qi and strengthening spleen, clearing liver and purging fire	Rat	Nrf2, Nqo1, HO-2, GCLC, GCLM	α-SMA, HA, PCIII, LN	Activating the Nrf2 pathway can attenuate OS and further suppress activated HSCs, thereby improving hepatic fibrosis	Li *et al*[111], Hu *et al*[112]
3	Fu Zheng Hua Yu Fang	*Salvia miltiorrhiza*, Fermented *Cordyceps sinensis* powder, Peach kernel, Pine pollen, *Lycopodium clavatum*, *Schisandra chinensis* (processed)	Activating blood circulation and removing blood stasis, nourishing essence and nurturing liver	Mice		α-SMA, Nqo1, HO-2, GCLC, GCLM, TIMP1, TGF-β1, TGF-RI, PDGF-Rβ, MDA, Col-1aI, TGF-βRI, PDGF-β, MCP-1, MIP-1 mRNA	Effectively suppressing the production of liver ROS, inhibiting OS, and exerting anti-fibrotic effects	Jiang *et al* [116]
4	CGX	*Artemisia annua* 5g, *Trionyx sinensis* shell 5g, Radish seed 5g; White Atractylodes 3g, Pogostemon cablin 3g, Alisma orientale 3g, White Atractylodes 3g, *Salvia miltiorrhiza* 3g; *Poria cocos* 2g, Aurantium fruit 2g, Amomum cardamomum 2g, Licorice 1g, *Saussurea costus* 1g	Clearing liver and activating blood circulation, strengthening spleen and transforming dampness	Rat	GSH-Px, GSH-Rd, GST, SOD, IFN-γ	MDA, PDGF-BB, TGF-β1, CTGF	It inhibits the levels of liver tissue HYP and MDA, while increasing the total GSH content and the activity of GSH-oxidation reduction system enzymes, which suppresses OS and exerts its anti-hepatic fibrosis effects	Kim *et al* [134]
5	Huangjia Ruangan Granule	*Astragalus membranaceus*, *Trionyx sinensis* shell, *Pueraria lobata* root, Bupleurum root, *Ganoderma lucidum*, *Paeonia lactiflora*, *Salvia miltiorrhiza*, *Panax notoginseng*, *Lycopodium* clavatum, *Phyllanthus urinaria*	Regulating qi and soothing liver, activating blood circulation and relieving hardness	Rat	SOD, GSH	PC-III, Col-IV, LN, HA, α-SMA, MDA, MPO, TNF-α, IL-1β, IL-6, Cox-2, iNOS, TNFR1, p-IκBα, p-P65/P65, p-ERK/ERK, p-JNK/JNK, MAPK p-P38/P38	Antagonizing OS effectively inhibits hepatic fibrosis by regulating TNF/MAPK and NF-κB signaling pathways to suppress liver inflammation	Cai *et al* [135]

α-SMA: Alpha-smooth-muscle actin; OS: Oxidative stress; SOD: Superoxide dismutase; GSH: Glutathione; IL: Interleukin; IFN: Interferon; Nrf2: Nuclear factor erythroid 2-related factor; HSC: Hepatic stellate cell.

reported to prevent and improve hepatic fibrosis by inhibiting OS.

YCHT is a TCM formula that has been used for centuries to treat liver diseases[105]. It is composed of three herbs, *Artemisia annua L.* (Qing Hao), *Gardenia jasminoides Ellis* (Zhi Zi), and *Rheum palmatum L.* (Da Huang), and is commonly used in clinics in China and other Asian countries[105]. Studies have suggested that YCHT can decrease ROS production and alleviate hepatic fibrosis. One of the mechanisms by which YCHT works is by regulating the balance between the pro- and anti-inflammatory cytokines in the liver[106]. Inflammatory cytokines such as TNF-α, interleukin (IL)-6, and IL-1 β are known to promote ROS production and hepatic fibrosis[107]. YCHT inhibits the expression of these cytokines and promotes the secretion of anti-inflammatory cytokines such as TGF-β and IL-10, which can reduce ROS production and hepatic fibrosis[108]. Another mechanism by which YCHT inhibits ROS production is by increasing the expression of antioxidant enzymes, such as SOD[109]. SOD converts superoxide radicals, one of the primary ROS, into hydrogen peroxide, which is less toxic. This conversion reduces OS in the liver and improves liver function[109]. In short, the

beneficial effects of YCHT on hepatic fibrosis may be attributed to its antioxidant properties. Further studies are needed to fully elucidate the underlying mechanisms and to establish its clinical efficacy and safety for the treatment of hepatic fibrosis.

XCHT is a TCM formula that has been used for centuries to alleviate various ailments, including hepatic fibrosis[110]. Recent studies have shown that XCHT may effectively reduce hepatic fibrosis by inhibiting ROS and OS pathways. Recent research has suggested that XCHT can suppress the production of ROS and reduce OS, leading to improvement in antioxidant capacity[111]. XCHT contains various active compounds such as baicalin, baicalein, and saikosaponin[111]. These compounds have all been shown to possess potent antioxidant properties, which may help to explain XCHT's ability to reduce oxidative liver damage and improve hepatic fibrosis[111]. Furthermore, XCHT has shown promising results in treating hepatic fibrosis by increasing levels of Nqo1, HO-2, GCLC, and GCLM - key components of the Nrf2 pathway in the liver[111]. This mechanism of action is likely responsible for its effectiveness in improving hepatic fibrosis. XCHT has been found to upregulate OS through the Nrf2 pathway, while also inhibiting the proliferation and activation of HSCT6 cells, which contributes to its ability to improve hepatic fibrosis[112]. To put it briefly, XCHT has shown promise in reducing hepatic fibrosis by inhibiting ROS and OS pathways, enhancing antioxidant capacity, and modulating the immune system and ECM remodeling processes. Further research is needed to fully understand the molecular mechanisms underlying XCHT's therapeutic effects in hepatic fibrosis and to optimize its clinical application in treating hepatic fibrosis.

Fuzheng Huayu Fang is a formula used in TCM for treating liver diseases and related complications[113]. This formula aims to strengthen the body's immune system, promote blood circulation, and reduce inflammation, hence helping to alleviate the symptoms associated with liver disorders[114]. Fuzheng Huayu Fang contains several herbs with antioxidant and anti-inflammatory properties that help to regulate ROS levels and protect liver cells from damage[115]. For instance, the herb Danshen (*Salvia miltiorrhiza*) has been shown to reduce ROS levels and inhibit the production of pro-inflammatory cytokines in liver cells, thereby improving liver function and reducing hepatic fibrosis[96]. Moreover, some studies have found that Fuzheng Huayu Fang can also help to lower OS and inflammation by regulating the expression of genes involved in these processes[116]. To cut a long story short, Fuzheng Huayu Fang is a useful formula for treating hepatic fibrosis due to its antioxidant and anti-inflammatory properties. By reducing ROS levels and promoting liver regeneration, this formula can help to alleviate the symptoms associated with liver disorders and improve the overall health of the liver.

In essence, TCM formulas have shown great potential in preventing and treating hepatic fibrosis. These formulas can regulate the body's functions and status and improve prognosis without causing adverse reactions. The mechanisms underlying their anti-fibrotic effects involve the suppression of ROS production, inhibition of HSC activation, and regulation of cytokine expression. Further studies are needed to validate their efficacy and safety in clinical practice.

DISCUSSION

Hepatic fibrosis is a compensatory response to liver injury and is also a risk factor for liver cirrhosis, liver cancer, and liver failure. Although the pathogenesis of hepatic fibrosis is complex and not fully understood, OS has been shown to play an important role in the development of hepatic fibrosis diseases. Therefore, the inhibition of OS can prevent hepatic fibrosis and related liver diseases. TCM is a treasure of Chinese traditional medicine, with unique advantages and profound connotations. TCM monomers, single herb extracts, and TCM formulas are increasingly being studied by researchers for their low adverse reactions, cost-effectiveness, and broad targeting of multiple pathways in the prevention and treatment of hepatic fibrosis. Based on the development of modern scientific technologies such as pharmacology and genomics, TCM monomers, single herb extracts, and TCM formulas have been extensively studied for their molecular mechanisms of anti-hepatic fibrosis by targeting OS and intracellular signal transduction processes, achieving good results and having broad application prospects.

However, there are also some issues that need to be addressed at present: Clinical trials are still lacking, and current studies on the role of TCM monomers, single herb extracts, and TCM formulas in inhibiting OS to treat hepatic fibrosis are mainly focused on animal experiments, with limited large-scale clinical trials, which limits the further research and transformation of these TCM monomers, single herb extracts, and TCM formulas. Therefore, how to apply the results of basic experiments to clinical practice and verify the anti-fibrosis effects of TCM monomers, single herb extracts, and TCM formulas in large-scale clinical trials has become another challenge in the treatment of hepatic fibrosis.

In addition, the structure-activity relationship study is not sufficient. Since most polysaccharides, single herb extracts, and TCM formulas are mixtures, their pharmacological mechanisms are difficult to fully elucidate, and they may be limited to the accumulation of effective components of single herb extracts or may be the result of the formation of a systemic pharmacological mechanism of mixtures. This limits the practical application of these TCM in clinical practice. Therefore, TCM theory should be combined with modern medical theory. Based on the summary of previous research, individual differences in pathogenesis and disease progression should be analyzed to further explore how TCM inhibits OS to address hepatic fibrosis.

As well as, some people may be allergic to certain TCMs. If symptoms such as rash, itching, difficulty breathing, or throat swelling occur, medication should be immediately discontinued and medical help should be sought. Additionally, individuals with pre-existing health conditions or those taking other medications should consult healthcare professionals before starting any TCM treatment. This is crucial to ensure the absence of potential drug interactions or worsening of current health issues. Besides, it is important to purchase Chinese medicine products from reputable suppliers to ensure their quality and safety. Counterfeit or adulterated products can pose serious health risks and should be avoided. In

summary, while TCM can provide therapeutic benefits, understanding and addressing potential adverse reactions and preventive measures associated with its use are crucial. By following proper guidelines, consulting healthcare professionals, and using legitimate products, risks can be minimized and benefits can be maximized. This will help find better inducing effects, with fewer adverse reactions and lower prices for TCM, providing a more reliable theoretical basis for achieving true and effective reversal of hepatic fibrosis.

CONCLUSION

Hepatic fibrosis is a condition characterized by inflammation and excessive growth of fibrous tissue in the liver, resulting from long-term exposure to harmful factors. OS is considered a key mechanism in the development and progression of hepatic fibrosis. TCM monomers, single herb extracts, and TCM formulas play important roles in treating hepatic fibrosis by inhibiting OS. This review summarizes the role and effectiveness of TCM monomers, single herb extracts, and TCM formulas in inhibiting OS for the treatment of hepatic fibrosis, based on relevant research. The findings demonstrate that TCM monomers, single herb extracts, and TCM formulas possess significant antioxidant properties, effectively reducing OS levels in the liver and alleviating the occurrence and progression of hepatic fibrosis.

FOOTNOTES

Author contributions: Zhu JF and Li Z designed the study; Li Z searched, analyzed, and, and summarized the literature results; Li Z and Ouyang H collected the data and wrote the manuscript; Zhu JF and Li Z checked and revised the article; all authors contributed to the article and approved the submitted version.

Supported by the Construction Project of Traditional Chinese Medicine Specialty in Yueyang Hospital of Integrated Traditional Chinese and Western Medicine; Preclinical Study of a New Chinese Herbal Medicine for the Treatment of Ascites of Liver Cirrhosis (Spleen and Kidney Yang Deficiency Type) with the Clinical Formula of Qigui Xiaogu Cataplasm, No. 23S21900100; National Natural Science Foundation of China, No. 82074386; Clinical Research Plan of SHDC, No. SHDC2020CR3095B; and Construction of Special Disease Alliance of Traditional Chinese Medicine in East China Area and Municipal Level, Shanghai Special Disease Alliance of Traditional Chinese Medicine for Liver Cirrhosis Ascites (Water Sickness).

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Country/Territory of origin: China

ORCID number: Zhen Li 0000-0002-2940-6464; Jun-Feng Zhu 0000-0003-0245-4092; Hao Ouyang 0000-0001-6930-2363.

S-Editor: Lin C
L-Editor: Wang TQ
P-Editor: Yuan YY

REFERENCES

1 **He Z**, Yang DY, Fan XL, Zhang MWW, Li Y, Gu XB, Yang MY. The Roles and Mechanisms of lncRNAs in Liver Fibrosis. *Int J Mol Sci* 2020; **21**: 1482 [DOI: 10.3390/ijms21041482]
2 **Huang DQ**, Mathurin P, Cortez-Pinto H, Loomba R. Global epidemiology of alcohol-associated cirrhosis and HCC: trends, projections and risk factors. *Nat Rev Gastroenterol Hepatol* 2023; **20**: 37-49 [PMID: 36258033 DOI: 10.1038/s41575-022-00688-6]
3 **Xie R**, Xiao M, Li L, Ma N, Liu M, Huang X, Liu Q, Zhang Y. Association between SII and hepatic steatosis and liver fibrosis: A population-based study. *Front Immunol* 2022; **13**: 925690 [PMID: 36189280 DOI: 10.3389/fimmu.2022.925690]
4 **Atta HM**. Reversibility and heritability of liver fibrosis: Implications for research and therapy. *World J Gastroenterol* 2015; **21**: 5138-5148 [PMID: 25954087 DOI: 10.3748/wjg.v21.i17.5138]
5 **Zhang D**, Zhang Y, Sun B. The Molecular Mechanisms of Liver Fibrosis and Its Potential Therapy in Application. *Int J Mol Sci* 2022; **23** [PMID: 36293428 DOI: 10.3390/ijms232012572]
6 **Wei C**, Qiu J, Wu Y, Chen Z, Yu Z, Huang Z, Yang K, Hu H, Liu F. Promising traditional Chinese medicine for the treatment of cholestatic liver disease process (cholestasis, hepatitis, liver fibrosis, liver cirrhosis). *J Ethnopharmacol* 2022; **297**: 115550 [PMID: 35863612 DOI: 10.1016/j.jep.2022.115550]
7 **Sonoda S**, Murata S, Yamaza H, Yuniartha R, Fujiyoshi J, Yoshimaru K, Matsuura T, Oda Y, Ohga S, Tajiri T, Taguchi T, Yamaza T. Targeting hepatic oxidative stress rescues bone loss in liver fibrosis. *Mol Metab* 2022; **66**: 101599 [PMID: 36113772 DOI: 10.1016/j.molmet.2022.101599]

8 **Karsdal MA**, Hjuler ST, Luo Y, Rasmussen DGK, Nielsen MJ, Holm Nielsen S, Leeming DJ, Goodman Z, Arch RH, Patel K, Schuppan D. Assessment of liver fibrosis progression and regression by a serological collagen turnover profile. *Am J Physiol Gastrointest Liver Physiol* 2019; **316**: G25-G31 [PMID: 30160980 DOI: 10.1152/ajpgi.00158.2018]

9 **Chen L**, Brenner DA, Kisseleva T. Combatting Fibrosis: Exosome-Based Therapies in the Regression of Liver Fibrosis. *Hepatol Commun* 2019; **3**: 180-192 [PMID: 30766956 DOI: 10.1002/hep4.1290]

10 **Poilil Surendran S**, George Thomas R, Moon MJ, Jeong YY. Nanoparticles for the treatment of liver fibrosis. *Int J Nanomedicine* 2017; **12**: 6997-7006 [PMID: 29033567 DOI: 10.2147/IJN.S145951]

11 **Eulenberg VM**, Lidbury JA. Hepatic Fibrosis in Dogs. *J Vet Intern Med* 2018; **32**: 26-41 [PMID: 29194760 DOI: 10.1111/jvim.14891]

12 **Xu F**, Liu C, Zhou D, Zhang L. TGF-β/SMAD Pathway and Its Regulation in Hepatic Fibrosis. *J Histochem Cytochem* 2016; **64**: 157-167 [PMID: 26747705 DOI: 10.1369/0022155415627681]

13 **Ambrosini YM**, Piedra-Mora C, Jennings S, Webster CRL. Serum 25-hydroxyvitamin D and C-reactive protein and plasma von Willebrand concentrations in 23 dogs with chronic hepatopathies. *J Vet Intern Med* 2022; **36**: 966-975 [PMID: 35420222 DOI: 10.1111/jvim.16424]

14 **Crosas-Molist E**, Fabregat I. Role of NADPH oxidases in the redox biology of liver fibrosis. *Redox Biol* 2015; **6**: 106-111 [PMID: 26204504 DOI: 10.1016/j.redox.2015.07.005]

15 **Dong Z**, Li S, Wang X, Si L, Ma R, Bao L, Bo A. lncRNA GAS5 restrains CCl(4)-induced hepatic fibrosis by targeting miR-23a through the PTEN/PI3K/Akt signaling pathway. *Am J Physiol Gastrointest Liver Physiol* 2019; **316**: G539-G550 [PMID: 30735452 DOI: 10.1152/ajpgi.00249.2018]

16 **Heo MJ**, Yun J, Kim SG. Role of non-coding RNAs in liver disease progression to hepatocellular carcinoma. *Arch Pharm Res* 2019; **42**: 48-62 [PMID: 30610616 DOI: 10.1007/s12272-018-01104-x]

17 **Wei L**, Wang X, Lv L, Liu J, Xing H, Song Y, Xie M, Lei T, Zhang N, Yang M. The emerging role of microRNAs and long noncoding RNAs in drug resistance of hepatocellular carcinoma. *Mol Cancer* 2019; **18**: 147 [PMID: 31651347 DOI: 10.1186/s12943-019-1086-z]

18 **George J**, Tsuchishima M, Tsutsumi M. Molecular mechanisms in the pathogenesis of N-nitrosodimethylamine induced hepatic fibrosis. *Cell Death Dis* 2019; **10**: 18 [PMID: 30622238 DOI: 10.1038/s41419-018-1272-8]

19 **Hong SW**, Jung KH, Lee HS, Zheng HM, Choi MJ, Lee C, Hong SS. Suppression by fucoidan of liver fibrogenesis *via* the TGF-β/Smad pathway in protecting against oxidative stress. *Biosci Biotechnol Biochem* 2011; **75**: 833-840 [PMID: 21597183 DOI: 10.1271/bbb.100599]

20 **Xiang M**, Lu Y, Xin L, Gao J, Shang C, Jiang Z, Lin H, Fang X, Qu Y, Wang Y, Shen Z, Zhao M, Cui X. Role of Oxidative Stress in Reperfusion following Myocardial Ischemia and Its Treatments. *Oxid Med Cell Longev* 2021; **2021**: 6614009 [PMID: 34055195 DOI: 10.1155/2021/6614009]

21 **Ping Z**, Peng Y, Lang H, Xinyong C, Zhiyi Z, Xiaocheng W, Hong Z, Liang S. Oxidative Stress in Radiation-Induced Cardiotoxicity. *Oxid Med Cell Longev* 2020; **2020**: 3579143 [PMID: 32190171 DOI: 10.1155/2020/3579143]

22 **Taleb A**, Ahmad KA, Ihsan AU, Qu J, Lin N, Hezam K, Koju N, Hui L, Qilong D. Antioxidant effects and mechanism of silymarin in oxidative stress induced cardiovascular diseases. *Biomed Pharmacother* 2018; **102**: 689-698 [PMID: 29604588 DOI: 10.1016/j.biopha.2018.03.140]

23 **Kong D**, Zhang Z, Chen L, Huang W, Zhang F, Wang L, Wang Y, Cao P, Zheng S. Curcumin blunts epithelial-mesenchymal transition of hepatocytes to alleviate hepatic fibrosis through regulating oxidative stress and autophagy. *Redox Biol* 2020; **36**: 101600 [PMID: 32526690 DOI: 10.1016/j.redox.2020.101600]

24 **Sadasivam N**, Kim YJ, Radhakrishnan K, Kim DK. Oxidative Stress, Genomic Integrity, and Liver Diseases. *Molecules* 2022; **27** [PMID: 35630636 DOI: 10.3390/molecules27103159]

25 **Lee KS**, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. *J Clin Invest* 1995; **96**: 2461-8 [PMID: 7593635 DOI: 10.1172/JCI118304]

26 **Arroyave-Ospina JC**, Wu Z, Geng Y, Moshage H. Role of Oxidative Stress in the Pathogenesis of Non-Alcoholic Fatty Liver Disease: Implications for Prevention and Therapy. *Antioxidants (Basel)* 2021; **10** [PMID: 33530432 DOI: 10.3390/antiox10020174]

27 **Gu J**, Chen C, Wang J, Chen T, Yao W, Yan T, Liu Z. Withaferin A Exerts Preventive Effect on Liver Fibrosis through Oxidative Stress Inhibition in a Sirtuin 3-Dependent Manner. *Oxid Med Cell Longev* 2020; **2020**: 2452848 [PMID: 33029279 DOI: 10.1155/2020/2452848]

28 **Huang W**, Zheng Y, Feng H, Ni L, Ruan YF, Zou XX, Ye M, Zou SQ. Total phenolic extract of Euscaphis konishii hayata Pericarp attenuates carbon tetrachloride (CCl(4))-induced liver fibrosis in mice. *Biomed Pharmacother* 2020; **125**: 109932 [PMID: 32036214 DOI: 10.1016/j.biopha.2020.109932]

29 **Di Paola R**, Modafferi S, Siracusa R, Cordaro M, D'Amico R, Ontario ML, Interdonato L, Salinaro AT, Fusco R, Impellizzeri D, Calabrese V, Cuzzocrea S. S-Acetyl-Glutathione Attenuates Carbon Tetrachloride-Induced Liver Injury by Modulating Oxidative Imbalance and Inflammation. *Int J Mol Sci* 2022; **23** [PMID: 35457246 DOI: 10.3390/ijms23084429]

30 **Gao YT**, Pan HW, Wu SB. Gross conception of anatomical structure of zang-fu viscera in Huangdi Neijing. *Zhong Xi Yi Jie He Xue Bao* 2006; **4**: 339-342 [PMID: 16834967 DOI: 10.3736/jcim20060404]

31 **Jaimes PN**, Reyes AM, Lara DG, Coyuca ACP. Correlation Between the Sinew Channels with the Myofascial System, Pathology, and Treatment. *J Acupunct Meridian Stud* 2022; **15**: 201-213 [PMID: 36521769 DOI: 10.51507/j.jams.2022.15.4.201]

32 **Byoung-Hee L**, Min-Yong K, Young-Bae P, Young-Jae P. Development of a valid and reliable seven emotions impairment questionnaire and assessment of its predictability for phlegm and blood stasis. *J Tradit Chin Med* 2016; **36**: 547-554 [PMID: 28459523 DOI: 10.1016/s0254-6272(16)30073-5]

33 **Ge C**, Tan J, Lou D, Zhu L, Zhong Z, Dai X, Sun Y, Kuang Q, Zhao J, Wang L, Liu J, Wang B, Xu M. Mulberrin confers protection against hepatic fibrosis by $Trim^3$1/Nrf2 signaling. *Redox Biol* 2022; **51**: 102274 [PMID: 35240537 DOI: 10.1016/j.redox.2022.102274]

34 **Xiao J**, Muzashvili TS, Georgiev MI. Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnol Adv* 2014; **32**: 1145-1156 [PMID: 24780153 DOI: 10.1016/j.biotechadv.2014.04.006]

35 **Parhiz H**, Roohbakhsh A, Soltani F, Rezaee R, Iranshahi M. Antioxidant and anti-inflammatory properties of the citrus flavonoids hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. *Phytother Res* 2015; **29**: 323-331 [PMID: 25394264 DOI: 10.1002/ptr.5256]

36 **Maleki SJ**, Crespo JF, Cabanillas B. Anti-inflammatory effects of flavonoids. *Food Chem* 2019; **299**: 125124 [PMID: 31288163 DOI: 10.1016/j.foodchem.2019.125124]

37 **Ma M**, Duan R, Zhong H, Liang T, Guo L. The Crosstalk between Fat Homeostasis and Liver Regional Immunity in NAFLD. *J Immunol Res* 2019; **2019**: 3954890 [PMID: 30719457 DOI: 10.1155/2019/3954890]

38 **Hosseini A**, Razavi BM, Banach M, Hosseinzadeh H. Quercetin and metabolic syndrome: A review. *Phytother Res* 2021; **35**: 5352-5364 [PMID: 34101925 DOI: 10.1002/ptr.7144]

39 **Patel RV**, Mistry BM, Shinde SK, Syed R, Singh V, Shin HS. Therapeutic potential of quercetin as a cardiovascular agent. *Eur J Med Chem* 2018; **155**: 889-904 [PMID: 29966915 DOI: 10.1016/j.ejmech.2018.06.053]

40 **Alizadeh SR**, Ebrahimzadeh MA. Quercetin derivatives: Drug design, development, and biological activities, a review. *Eur J Med Chem* 2022; **229**: 114068 [PMID: 34971873 DOI: 10.1016/j.ejmech.2021.114068]

41 **Dabeek WM**, Marra MV. Dietary Quercetin and Kaempferol: Bioavailability and Potential Cardiovascular-Related Bioactivity in Humans. *Nutrients* 2019; **11** [PMID: 31557798 DOI: 10.3390/nu11102288]

42 **Shen P**, Lin W, Deng X, Ba X, Han L, Chen Z, Qin K, Huang Y, Tu S. Potential Implications of Quercetin in Autoimmune Diseases. *Front Immunol* 2021; **12**: 689044 [PMID: 34248976 DOI: 10.3389/fimmu.2021.689044]

43 **Aslam A**, Sheikh N, Shahzad M, Saeed G, Fatima N, Akhtar T. Quercetin ameliorates thioacetamide-induced hepatic fibrosis and oxidative stress by antagonizing the Hedgehog signaling pathway. *J Cell Biochem* 2022; **123**: 1356-1365 [PMID: 35696520 DOI: 10.1002/jcb.30296]

44 **Khodarahmi A**, Eshaghian A, Safari F, Moradi A. Quercetin Mitigates Hepatic Insulin Resistance in Rats with Bile Duct Ligation Through Modulation of the STAT3/SOCS3/IRS1 Signaling Pathway. *J Food Sci* 2019; **84**: 3045-3053 [PMID: 31529802 DOI: 10.1111/1750-3841.14793]

45 **Gong G**, Guan YY, Zhang ZL, Rahman K, Wang SJ, Zhou S, Luan X, Zhang H. Isorhamnetin: A review of pharmacological effects. *Biomed Pharmacother* 2020; **128**: 110301 [PMID: 32502837 DOI: 10.1016/j.biopha.2020.110301]

46 **Hu J**, Zhang Y, Jiang X, Zhang H, Gao Z, Li Y, Fu R, Li L, Li J, Cui H, Gao N. ROS-mediated activation and mitochondrial translocation of CaMKII contributes to Drp1-dependent mitochondrial fission and apoptosis in triple-negative breast cancer cells by isorhamnetin and chloroquine. *J Exp Clin Cancer Res* 2019; **38**: 225 [PMID: 31138329 DOI: 10.1186/s13046-019-1201-4]

47 **Li X**, Chen H, Zhang Z, Xu D, Duan J, Li X, Yang L, Hua R, Cheng J, Li Q. Isorhamnetin Promotes Estrogen Biosynthesis and Proliferation in Porcine Granulosa Cells *via* the PI3K/Akt Signaling Pathway. *J Agric Food Chem* 2021; **69**: 6535-6542 [PMID: 34096286 DOI: 10.1021/acs.jafc.1c01543]

48 **Kalai FZ**, Boulaaba M, Ferdousi F, Isoda H. Effects of Isorhamnetin on Diabetes and Its Associated Complications: A Review of In Vitro and In Vivo Studies and a Post Hoc Transcriptome Analysis of Involved Molecular Pathways. *Int J Mol Sci* 2022; **23** [PMID: 35054888 DOI: 10.3390/ijms23020704]

49 **Yang JH**, Kim SC, Kim KM, Jang CH, Cho SS, Kim SJ, Ku SK, Cho IJ, Ki SH. Isorhamnetin attenuates liver fibrosis by inhibiting TGF-β/Smad signaling and relieving oxidative stress. *Eur J Pharmacol* 2016; **783**: 92-102 [PMID: 27151496 DOI: 10.1016/j.ejphar.2016.04.042]

50 **Chen R**, Qi QL, Wang MT, Li QY. Therapeutic potential of naringin: an overview. *Pharm Biol* 2016; **54**: 3203-3210 [PMID: 27564838 DOI: 10.1080/13880209.2016.1216131]

51 **Zeng W**, Jin L, Zhang F, Zhang C, Liang W. Naringenin as a potential immunomodulator in therapeutics. *Pharmacol Res* 2018; **135**: 122-126 [PMID: 30081177 DOI: 10.1016/j.phrs.2018.08.002]

52 **Hernández-Aquino E**, Muriel P. Beneficial effects of naringenin in liver diseases: Molecular mechanisms. *World J Gastroenterol* 2018; **24**: 1679-1707 [PMID: 29713125 DOI: 10.3748/wjg.v24.i16.1679]

53 **Hernández-Aquino E**, Zarco N, Casas-Grajales S, Ramos-Tovar E, Flores-Beltrán RE, Arauz J, Shibayama M, Favari L, Tsutsumi V, Segovia J, Muriel P. Naringenin prevents experimental liver fibrosis by blocking TGFβ-Smad3 and JNK-Smad3 pathways. *World J Gastroenterol* 2017; **23**: 4354-4368 [PMID: 28706418 DOI: 10.3748/wjg.v23.i24.4354]

54 **Hernández-Aquino E**, Quezada-Ramírez MA, Silva-Olivares A, Casas-Grajales S, Ramos-Tovar E, Flores-Beltrán RE, Segovia J, Shibayama M, Muriel P. Naringenin attenuates the progression of liver fibrosis *via* inactivation of hepatic stellate cells and profibrogenic pathways. *Eur J Pharmacol* 2019; **865**: 172730 [PMID: 31618621 DOI: 10.1016/j.ejphar.2019.172730]

55 **Kren V**, Martínková L. Glycosides in medicine: "The role of glycosidic residue in biological activity". *Curr Med Chem* 2001; **8**: 1303-1328 [PMID: 11562268 DOI: 10.2174/0929867013372193]

56 **Zou Y**, Fang W. Naturally Derived Glycosides with Potential Activity against Staphylococcus Aureus. *Curr Top Med Chem* 2021; **21**: 2500-2512 [PMID: 34649487 DOI: 10.2174/1568026621666211015091539]

57 **Fan J**, Li X, Li P, Li N, Wang T, Shen H, Siow Y, Choy P, Gong Y. Saikosaponin-d attenuates the development of liver fibrosis by preventing hepatocyte injury. *Biochem Cell Biol* 2007; **85**: 189-195 [PMID: 17534399 DOI: 10.1139/O07-010]

58 **Manoharan S**, Deivendran B, Perumal E. Chemotherapeutic Potential of Saikosaponin D: Experimental Evidence. *J Xenobiot* 2022; **12**: 378-405 [PMID: 36547471 DOI: 10.3390/jox12040027]

59 **Li X**, Li X, Huang N, Liu R, Sun R. A comprehensive review and perspectives on pharmacology and toxicology of saikosaponins. *Phytomedicine* 2018; **50**: 73-87 [PMID: 30466994 DOI: 10.1016/j.phymed.2018.09.174]

60 **Que R**, Shen Y, Ren J, Tao Z, Zhu X, Li Y. Estrogen receptorβdependent effects of saikosaponind on the suppression of oxidative stressinduced rat hepatic stellate cell activation. *Int J Mol Med* 2018; **41**: 1357-1364 [PMID: 29286085 DOI: 10.3892/ijmm.2017.3349]

61 **Weiskirchen S**, Weiskirchen R. Resveratrol: How Much Wine Do You Have to Drink to Stay Healthy? *Adv Nutr* 2016; **7**: 706-718 [PMID: 27422505 DOI: 10.3945/an.115.011627]

62 **Pandey RP**, Parajuli P, Shin JY, Lee J, Lee S, Hong YS, Park YI, Kim JS, Sohng JK. Enzymatic Biosynthesis of Novel Resveratrol Glucoside and Glycoside Derivatives. *Appl Environ Microbiol* 2014; **80**: 7235-7243 [PMID: 25239890 DOI: 10.1128/AEM.02076-14]

63 **Tong J**, Gao J, Liu Q, He C, Zhao X, Qi Y, Yuan T, Li P, Niu M, Wang D, Zhang L, Li W, Wang J, Zhang Z, Peng S. Resveratrol derivative excited postsynaptic potentiation specifically *via* PKCβ-NMDA receptor mediation. *Pharmacol Res* 2020; **152**: 104618 [PMID: 31891789 DOI: 10.1016/j.phrs.2019.104618]

64 **Jang IA**, Kim EN, Lim JH, Kim MY, Ban TH, Yoon HE, Park CW, Chang YS, Choi BS. Effects of Resveratrol on the Renin-Angiotensin System in the Aging Kidney. *Nutrients* 2018; **10** [PMID: 30424556 DOI: 10.3390/nu10111741]

65 **Ding KN**, Lu MH, Guo YN, Liang SS, Mou RW, He YM, Tang LP. Resveratrol relieves chronic heat stress-induced liver oxidative damage in broilers by activating the Nrf2-Keap1 signaling pathway. *Ecotoxicol Environ Saf* 2023; **249**: 114411 [PMID: 36525949 DOI: 10.1016/j.ecoenv.2022.114411]

66 **Yu B**, Qin SY, Hu BL, Qin QY, Jiang HX, Luo W. Resveratrol improves CCL4-induced liver fibrosis in mouse by upregulating endogenous IL-10 to reprogramme macrophages phenotype from M(LPS) to M(IL-4). *Biomed Pharmacother* 2019; **117**: 109110 [PMID: 31252263 DOI: 10.1016/j.biopha.2019.109110]

67 **Wang Y**, Wu H, Gui BJ, Liu J, Rong GX, Deng R, Bu YH, Zhang H. Geniposide alleviates VEGF-induced angiogenesis by inhibiting VEGFR2/PKC/ERK1/2-mediated SphK1 translocation. *Phytomedicine* 2022; **100**: 154068 [PMID: 35358930 DOI:

10.1016/j.phymed.2022.154068]
68 **Jin Z**, Li J, Pi J, Chu Q, Wei W, Du Z, Qing L, Zhao X, Wu W. Geniposide alleviates atherosclerosis by regulating macrophage polarization *via* the FOS/MAPK signaling pathway. *Biomed Pharmacother* 2020; **125**: 110015 [PMID: 32187958 DOI: 10.1016/j.biopha.2020.110015]
69 **Wen M**, Liu Y, Chen R, He P, Wu F, Li R, Lin Y. Geniposide suppresses liver injury in a mouse model of DDC-induced sclerosing cholangitis. *Phytother Res* 2021; **35**: 3799-3811 [PMID: 33763888 DOI: 10.1002/ptr.7086]
70 **Yang L**, Bi L, Jin L, Wang Y, Li Y, Li Z, He W, Cui H, Miao J, Wang L. Geniposide Ameliorates Liver Fibrosis Through Reducing Oxidative Stress and Inflammatory Respose, Inhibiting Apoptosis and Modulating Overall Metabolism. *Front Pharmacol* 2021; **12**: 772635 [PMID: 34899328 DOI: 10.3389/fphar.2021.772635]
71 **Ziegler J**, Facchini PJ. Alkaloid biosynthesis: metabolism and trafficking. *Annu Rev Plant Biol* 2008; **59**: 735-769 [PMID: 18251710 DOI: 10.1146/annurev.arplant.59.032607.092730]
72 **Bhambhani S**, Kondhare KR, Giri AP. Diversity in Chemical Structures and Biological Properties of Plant Alkaloids. *Molecules* 2021; **26** [PMID: 34204857 DOI: 10.3390/molecules26113374]
73 **Feng X**, Sureda A, Jafari S, Memariani Z, Tewari D, Annunziata G, Barrea L, Hassan STS, Šmejkal K, Malaník M, Sychrová A, Barreca D, Ziberna L, Mahomoodally MF, Zengin G, Xu S, Nabavi SM, Shen AZ. Berberine in Cardiovascular and Metabolic Diseases: From Mechanisms to Therapeutics. *Theranostics* 2019; **9**: 1923-1951 [PMID: 31037148 DOI: 10.7150/thno.30787]
74 **Habtemariam S**. Berberine pharmacology and the gut microbiota: A hidden therapeutic link. *Pharmacol Res* 2020; **155**: 104722 [PMID: 32105754 DOI: 10.1016/j.phrs.2020.104722]
75 **Eissa LA**, Kenawy HI, El-Karef A, Elsherbiny NM, El-Mihi KA. Antioxidant and anti-inflammatory activities of berberine attenuate hepatic fibrosis induced by thioacetamide injection in rats. *Chem Biol Interact* 2018; **294**: 91-100 [PMID: 30138605 DOI: 10.1016/j.cbi.2018.08.016]
76 **Yi J**, Wu S, Tan S, Qin Y, Wang X, Jiang J, Liu H, Wu B. Berberine alleviates liver fibrosis through inducing ferrous redox to activate ROS-mediated hepatic stellate cells ferroptosis. *Cell Death Discov* 2021; **7**: 374 [PMID: 34864819 DOI: 10.1038/s41420-021-00768-7]
77 **Domitrović R**, Jakovac H, Marchesi VV, Blažeković B. Resolution of liver fibrosis by isoquinoline alkaloid berberine in CCl_4-intoxicated mice is mediated by suppression of oxidative stress and upregulation of MMP-2 expression. *J Med Food* 2013; **16**: 518-528 [PMID: 23734997 DOI: 10.1089/jmf.2012.0175]
78 **Zhao G**, He F, Wu C, Li P, Li N, Deng J, Zhu G, Ren W, Peng Y. Betaine in Inflammation: Mechanistic Aspects and Applications. *Front Immunol* 2018; **9**: 1070 [PMID: 29881379 DOI: 10.3389/fimmu.2018.01070]
79 **Arumugam MK**, Chava S, Perumal SK, Paal MC, Rasineni K, Ganesan M, Donohue TM Jr, Osna NA, Kharbanda KK. Acute ethanol-induced liver injury is prevented by betaine administration. *Front Physiol* 2022; **13**: 940148 [PMID: 36267591 DOI: 10.3389/fphys.2022.940148]
80 **Bingül İ**, Başaran-Küçükgergin C, Aydın AF, Çoban J, Doğan-Ekici I, Doğru-Abbasoğlu S, Uysal M. Betaine treatment decreased oxidative stress, inflammation, and stellate cell activation in rats with alcoholic liver fibrosis. *Environ Toxicol Pharmacol* 2016; **45**: 170-178 [PMID: 27314760 DOI: 10.1016/j.etap.2016.05.033]
81 **Bingül İ**, Aydın AF, Başaran-Küçükgergin C, Doğan-Ekici I, Çoban J, Doğru-Abbasoğlu S, Uysal M. High-fat diet plus carbon tetrachloride-induced liver fibrosis is alleviated by betaine treatment in rats. *Int Immunopharmacol* 2016; **39**: 199-207 [PMID: 27494683 DOI: 10.1016/j.intimp.2016.07.028]
82 **Kim SK**, Seo JM, Chae YR, Jung YS, Park JH, Kim YC. Alleviation of dimethylnitrosamine-induced liver injury and fibrosis by betaine supplementation in rats. *Chem Biol Interact* 2009; **177**: 204-211 [PMID: 18930038 DOI: 10.1016/j.cbi.2008.09.021]
83 **Liang Q**, Cai W, Zhao Y, Xu H, Tang H, Chen D, Qian F, Sun L. Lycorine ameliorates bleomycin-induced pulmonary fibrosis *via* inhibiting NLRP3 inflammasome activation and pyroptosis. *Pharmacol Res* 2020; **158**: 104884 [PMID: 32428667 DOI: 10.1016/j.phrs.2020.104884]
84 **Roy M**, Liang L, Xiao X, Feng P, Ye M, Liu J. Lycorine: A prospective natural lead for anticancer drug discovery. *Biomed Pharmacother* 2018; **107**: 615-624 [PMID: 30114645 DOI: 10.1016/j.biopha.2018.07.147]
85 **Ilavenil S**, Kaleeswaran B, Ravikumar S. Protective effects of lycorine against carbon tetrachloride induced hepatotoxicity in Swiss albino mice. *Fundam Clin Pharmacol* 2012; **26**: 393-401 [PMID: 21480982 DOI: 10.1111/j.1472-8206.2011.00942.x]
86 **Jin M**, Shi J, Zhu W, Yao H, Wang DA. Polysaccharide-Based Biomaterials in Tissue Engineering: A Review. *Tissue Eng Part B Rev* 2021; **27**: 604-626 [PMID: 33267648 DOI: 10.1089/ten.TEB.2020.0208]
87 **Yang S**, Yang X, Zhang H. Extracellular polysaccharide biosynthesis in Cordyceps. *Crit Rev Microbiol* 2020; **46**: 359-380 [PMID: 32720528 DOI: 10.1080/1040841X.2020.1794788]
88 **Liu Y**, Li Y, Zhang H, Li C, Zhang Z, Liu A, Chen H, Hu B, Luo Q, Lin B, Wu W. Polysaccharides from Cordyceps miltaris cultured at different pH: Sugar composition and antioxidant activity. *Int J Biol Macromol* 2020; **162**: 349-358 [PMID: 32574745 DOI: 10.1016/j.ijbiomac.2020.06.182]
89 **Peng J**, Li X, Feng Q, Chen L, Xu L, Hu Y. Anti-fibrotic effect of Cordyceps sinensis polysaccharide: Inhibiting HSC activation, TGF-β1/Smad signalling, MMPs and TIMPs. *Exp Biol Med (Maywood)* 2013; **238**: 668-677 [PMID: 23918878 DOI: 10.1177/1535370213480741]
90 **Liu Y**, E Q, Zuo J, Tao Y, Liu W. Protective effect of Cordyceps polysaccharide on hydrogen peroxide-induced mitochondrial dysfunction in HL-7702 cells. *Mol Med Rep* 2013; **7**: 747-754 [PMID: 23258306 DOI: 10.3892/mmr.2012.1248]
91 **Zhao H**, Li D, Li M, Liu L, Deng B, Jia L, Yang F. Coprinus comatus polysaccharides ameliorated carbon tetrachloride-induced liver fibrosis through modulating inflammation and apoptosis. *Food Funct* 2022; **13**: 11125-11141 [PMID: 36205351 DOI: 10.1039/d2fo01349e]
92 **Jia Q**, Zhu R, Tian Y, Chen B, Li R, Li L, Wang L, Che Y, Zhao D, Mo F, Gao S, Zhang D. Salvia miltiorrhiza in diabetes: A review of its pharmacology, phytochemistry, and safety. *Phytomedicine* 2019; **58**: 152871 [PMID: 30851580 DOI: 10.1016/j.phymed.2019.152871]
93 **Wang X**, Liu W, Jin G, Wu Z, Zhang D, Bao Y, Shi W. Salvia miltiorrhiza polysaccharides alleviates florfenicol induced kidney injury in chicks *via* inhibiting oxidative stress and apoptosis. *Ecotoxicol Environ Saf* 2022; **233**: 113339 [PMID: 35219255 DOI: 10.1016/j.ecoenv.2022.113339]
94 **Zhang Y**, Zhang Y, Xie Y, Gao Y, Ma J, Yuan J, Li J, Wang J, Li L, Zhang J, Chu L. Multitargeted inhibition of hepatic fibrosis in chronic iron-overloaded mice by Salvia miltiorrhiza. *J Ethnopharmacol* 2013; **148**: 671-681 [PMID: 23707206 DOI: 10.1016/j.jep.2013.05.028]
95 **Gao Y**, Wang N, Zhang Y, Ma Z, Guan P, Ma J, Zhang X, Wang J, Zhang J, Chu L. Mechanism of protective effects of Danshen against iron overload-induced injury in mice. *J Ethnopharmacol* 2013; **145**: 254-260 [PMID: 23147497 DOI: 10.1016/j.jep.2012.10.060]
96 **Wang R**, Wang J, Song F, Li S, Yuan Y. Tanshinol ameliorates CCl(4)-induced liver fibrosis in rats through the regulation of Nrf2/HO-1 and NF-κB/IκBα signaling pathway. *Drug Des Devel Ther* 2018; **12**: 1281-1292 [PMID: 29844659 DOI: 10.2147/DDDT.S159546]
97 **Li S**, Wang R, Song F, Chen P, Gu Y, Chen C, Yuan Y. Salvianolic acid A suppresses CCl(4)-induced liver fibrosis through regulating the Nrf2/HO-1, NF-κB/IκBα, p38 MAPK, and JAK1/STAT3 signaling pathways. *Drug Chem Toxicol* 2023; **46**: 304-313 [PMID: 35057680 DOI: 10.1080/01480545.2022.2028822]

98 **Yoshikawa T**, Naito Y, Kondo M. Ginkgo biloba leaf extract: review of biological actions and clinical applications. *Antioxid Redox Signal* 1999; **1**: 469-480 [PMID: 11233145 DOI: 10.1089/ars.1999.1.4-469]

99 **Ding J**, Yu J, Wang C, Hu W, Li D, Luo Y, Luo H, Yu H. Ginkgo biloba extract alleviates liver fibrosis induced by CCl in rats. *Liver Int* 2005; **25**: 1224-1232 [PMID: 16343076 DOI: 10.1111/j.1478-3231.2005.01169.x]

100 **Al-Attar AM**. Attenuating effect of Ginkgo biloba leaves extract on liver fibrosis induced by thioacetamide in mice. *J Biomed Biotechnol* 2012; **2012**: 761450 [PMID: 23091357 DOI: 10.1155/2012/761450]

101 **Jeong HS**, Kim KH, Lee IS, Park JY, Kim Y, Kim KS, Jang HJ. Ginkgolide A ameliorates non-alcoholic fatty liver diseases on high fat diet mice. *Biomed Pharmacother* 2017; **88**: 625-634 [PMID: 28142119 DOI: 10.1016/j.biopha.2017.01.114]

102 **Brum A**, Pereira SA, Cardoso L, Chagas EC, Chaves FCM, Mouriño JLP, Martins ML. Blood biochemical parameters and melanomacrophage centers in Nile tilapia fed essential oils of clove basil and ginger. *Fish Shellfish Immunol* 2018; **74**: 444-449 [PMID: 29353078 DOI: 10.1016/j.fsi.2018.01.021]

103 **Chiu YW**, Chao PY, Tsai CC, Chiou HL, Liu YC, Hung CC, Shih HC, Lai TJ, Liu JY. Ocimum gratissimum is effective in prevention against liver fibrosis *in vivo* and in vitro. *Am J Chin Med* 2014; **42**: 833-852 [PMID: 25004878 DOI: 10.1142/S0192415X14500530]

104 **Chen YH**, Chiu YW, Shyu JC, Tsai CC, Lee HH, Hung CC, Hwang JM, Liu JY, Wang WH. Protective effects of Ocimum gratissimum polyphenol extract on carbon tetrachloride-induced liver fibrosis in rats. *Chin J Physiol* 2015; **58**: 55-63 [PMID: 25687492 DOI: 10.4077/CJP.2015.BAD285]

105 **Xiang H**, Wang G, Qu J, Xia S, Tao X, Qi B, Zhang Q, Shang D. Yin-Chen-Hao Tang Attenuates Severe Acute Pancreatitis in Rat: An Experimental Verification of In silico Network Target Prediction. *Front Pharmacol* 2016; **7**: 378 [PMID: 27790147 DOI: 10.3389/fphar.2016.00378]

106 **Lee TY**, Chang HH, Lo WC, Lin HC. Alleviation of hepatic oxidative stress by Chinese herbal medicine Yin-Chen-Hao-Tang in obese mice with steatosis. *Int J Mol Med* 2010; **25**: 837-844 [PMID: 20428786 DOI: 10.3892/ijmm_00000412]

107 **Seki E**, Brenner DA. Recent advancement of molecular mechanisms of liver fibrosis. *J Hepatobiliary Pancreat Sci* 2015; **22**: 512-518 [PMID: 25869468 DOI: 10.1002/jhbp.245]

108 **Mountford S**, Effenberger M, Noll-Puchta H, Griessmair L, Ringleb A, Haas S, Denk G, Reiter FP, Mayr D, Dinarello CA, Tilg H, Bufler P. Modulation of Liver Inflammation and Fibrosis by Interleukin-37. *Front Immunol* 2021; **12**: 603649 [PMID: 33746950 DOI: 10.3389/fimmu.2021.603649]

109 **Chen Z**, Wang X, Li Y, Wang Y, Tang K, Wu D, Zhao W, Ma Y, Liu P, Cao Z. Comparative Network Pharmacology Analysis of Classical TCM Prescriptions for Chronic Liver Disease. *Front Pharmacol* 2019; **10**: 1353 [PMID: 31824313 DOI: 10.3389/fphar.2019.01353]

110 **Wang SJ**, Ye W, Li WY, Tian W, Zhang M, Sun Y, Feng YD, Liu CX, Liu SY, Cao W, Meng JR, Li XQ. Effects and mechanisms of Xiaochaihu Tang against liver fibrosis: An integration of network pharmacology, molecular docking and experimental validation. *J Ethnopharmacol* 2023; **303**: 116053 [PMID: 36529247 DOI: 10.1016/j.jep.2022.116053]

111 **Li J**, Hu R, Xu S, Li Y, Qin Y, Wu Q, Xiao Z. Xiaochaihutang attenuates liver fibrosis by activation of Nrf2 pathway in rats. *Biomed Pharmacother* 2017; **96**: 847-853 [PMID: 29078262 DOI: 10.1016/j.biopha.2017.10.065]

112 **Hu R**, Jia WY, Xu SF, Zhu ZW, Xiao Z, Yu SY, Li J. Xiaochaihutang Inhibits the Activation of Hepatic Stellate Cell Line T6 Through the Nrf2 Pathway. *Front Pharmacol* 2018; **9**: 1516 [PMID: 30666206 DOI: 10.3389/fphar.2018.01516]

113 **Sun S**, Dai J, Wang W, Cao H, Fang J, Hu YY, Su S, Zhang Y. Metabonomic Evaluation of ZHENG Differentiation and Treatment by Fuzhenghuayu Tablet in Hepatitis-B-Caused Cirrhosis. *Evid Based Complement Alternat Med* 2012; **2012**: 453503 [PMID: 22690245 DOI: 10.1155/2012/453503]

114 **Chen J**, Gao W, Zhou P, Ma X, Tschudy-Seney B, Liu C, Zern MA, Liu P, Duan Y. Enhancement of hepatocyte differentiation from human embryonic stem cells by Chinese medicine Fuzhenghuayu. *Sci Rep* 2016; **6**: 18841 [PMID: 26733102 DOI: 10.1038/srep18841]

115 **Zhang ZG**, Lu XB, Xiao L, Tang L, Zhang LJ, Zhang T, Zhan XY, Ma XM, Zhang YX. Antioxidant effects of the Uygur herb, Foeniculum Vulgare Mill, in a rat model of hepatic fibrosis. *Zhonghua Gan Zang Bing Za Zhi* 2012; **20**: 221-226 [PMID: 22475144 DOI: 10.3760/cma.j.issn.1007-3418.2012.03.017]

116 **Jiang C**, Iwaisako K, Cong M, Diggle K, Hassanein T, Brenner DA, Kisseleva T. Traditional Chinese Medicine Fuzheng Huayu Prevents Development of Liver Fibrosis in Mice. *Arch Clin Biomed Res* 2020; **4**: 561-580 [PMID: 33210080 DOI: 10.26502/acbr.50170125]

117 **Zhang B**, Lai L, Tan Y, Liang Q, Bai F, Mai W, Huang Q, Ye Y. Hepatoprotective effect of total flavonoids of Mallotus apelta (Lour.) Muell.Arg. leaf against carbon tetrachloride-induced liver fibrosis in rats *via* modulation of TGF-β1/Smad and NF-κB signaling pathways. *J Ethnopharmacol* 2020; **254**: 112714 [PMID: 32105750 DOI: 10.1016/j.jep.2020.112714]

118 **Yuan LP**, Chen FH, Ling L, Bo H, Chen ZW, Li F, Zhong MM, Xia LJ. Protective effects of total flavonoids of Bidens bipinnata L. against carbon tetrachloride-induced liver fibrosis in rats. *J Pharm Pharmacol* 2008; **60**: 1393-1402 [PMID: 18812033 DOI: 10.1211/jpp/60.10.0016]

119 **Hou B**, Zhao Y, Qiang G, Yang X, Xu C, Chen X, Liu C, Wang X, Zhang L, Du G. Puerarin Mitigates Diabetic Hepatic Steatosis and Fibrosis by Inhibiting TGF-β Signaling Pathway Activation in Type 2 Diabetic Rats. *Oxid Med Cell Longev* 2018; **2018**: 4545321 [PMID: 30057680 DOI: 10.1155/2018/4545321]

120 **Li S**, Li X, Chen F, Liu M, Ning L, Yan Y, Zhang S, Huang S, Tu C. Nobiletin mitigates hepatocytes death, liver inflammation, and fibrosis in a murine model of NASH through modulating hepatic oxidative stress and mitochondrial dysfunction. *J Nutr Biochem* 2022; **100**: 108888 [PMID: 34695558 DOI: 10.1016/j.jnutbio.2021.108888]

121 **Zhu Z**, Hu R, Li J, Xing X, Chen J, Zhou Q, Sun J. Alpinetin exerts anti-inflammatory, anti-oxidative and anti-angiogenic effects through activating the Nrf2 pathway and inhibiting NLRP3 pathway in carbon tetrachloride-induced liver fibrosis. *Int Immunopharmacol* 2021; **96**: 107660 [PMID: 33862553 DOI: 10.1016/j.intimp.2021.107660]

122 **El-Fadaly AA**, Afifi NA, El-Eraky W, Salama A, Abdelhameed MF, El-Rahman SSA, Ramadan A. Fisetin alleviates thioacetamide-induced hepatic fibrosis in rats by inhibiting Wnt/β-catenin signaling pathway. *Immunopharmacol Immunotoxicol* 2022; **44**: 355-366 [PMID: 35255766 DOI: 10.1080/08923973.2022.2047198]

123 **Zhang L**, Zhang H, Gu J, Xu W, Yuan N, Sun J, Li H. Glabridin inhibits liver fibrosis and hepatic stellate cells activation through suppression of inflammation and oxidative stress by activating PPARγ in carbon tetrachloride-treated mice. *Int Immunopharmacol* 2022; **113**: 109433 [PMID: 36371863 DOI: 10.1016/j.intimp.2022.109433]

124 **Heeba GH**, Mahmoud ME. Therapeutic potential of morin against liver fibrosis in rats: modulation of oxidative stress, cytokine production and nuclear factor kappa B. *Environ Toxicol Pharmacol* 2014; **37**: 662-671 [PMID: 24583409 DOI: 10.1016/j.etap.2014.01.026]

125 **Sun XH**, Zhang H, Fan XP, Wang ZH. Astilbin Protects Against Carbon Tetrachloride-Induced Liver Fibrosis in Rats. *Pharmacology* 2021; **106**: 323-331 [PMID: 33780953 DOI: 10.1159/000514594]

126 **Wang Q**, Wen R, Lin Q, Wang N, Lu P, Zhu X. Wogonoside Shows Antifibrotic Effects in an Experimental Regression Model of Hepatic Fibrosis. *Dig Dis Sci* 2015; **60**: 3329-3339 [PMID: 26130019 DOI: 10.1007/s10620-015-3751-4]

127 **Zou L**, Chen S, Li L, Wu T. The protective effect of hyperoside on carbon tetrachloride-induced chronic liver fibrosis in mice *via* upregulation of Nrf2. *Exp Toxicol Pathol* 2017; **69**: 451-460 [PMID: 28434817 DOI: 10.1016/j.etp.2017.04.001]

128 **Fu K**, Ma C, Wang C, Zhou H, Gong L, Zhang Y, Li Y. Forsythiaside A alleviated carbon tetrachloride-induced liver fibrosis by modulating gut microbiota composition to increase short-chain fatty acids and restoring bile acids metabolism disorder. *Biomed Pharmacother* 2022; **151**: 113185 [PMID: 35623173 DOI: 10.1016/j.biopha.2022.113185]

129 **Al-Olayan EM**, El-Khadragy MF, Alajmi RA, Othman MS, Bauomy AA, Ibrahim SR, Abdel Moneim AE. Ceratonia siliqua pod extract ameliorates Schistosoma mansoni-induced liver fibrosis and oxidative stress. *BMC Complement Altern Med* 2016; **16**: 434 [PMID: 27821159 DOI: 10.1186/s12906-016-1389-1]

130 **Dulundu E**, Ozel Y, Topaloglu U, Toklu H, Ercan F, Gedik N, Sener G. Grape seed extract reduces oxidative stress and fibrosis in experimental biliary obstruction. *J Gastroenterol Hepatol* 2007; **22**: 885-892 [PMID: 17565645 DOI: 10.1111/j.1440-1746.2007.04875.x]

131 **Husain H**, Latief U, Ahmad R. Pomegranate action in curbing the incidence of liver injury triggered by Diethylnitrosamine by declining oxidative stress *via* Nrf2 and NFκB regulation. *Sci Rep* 2018; **8**: 8606 [PMID: 29872102 DOI: 10.1038/s41598-018-26611-1]

132 **Domitrović R**, Jakovac H, Romić Z, Rahelić D, Tadić Z. Antifibrotic activity of Taraxacum officinale root in carbon tetrachloride-induced liver damage in mice. *J Ethnopharmacol* 2010; **130**: 569-577 [PMID: 20561925 DOI: 10.1016/j.jep.2010.05.046]

133 **Sen A**, Ozkan S, Recebova K, Cevik O, Ercan F, Kervancıoglu Demirci E, Bitis L, Sener G. Effects of Myrtus communis extract treatment in bile duct ligated rats. *J Surg Res* 2016; **205**: 359-367 [PMID: 27664884 DOI: 10.1016/j.jss.2016.06.094]

134 **Kim HG**, Han JM, Lee HW, Lee JS, Son SW, Choi MK, Lee DS, Wang JH, Son CG. CGX, a multiple herbal drug, improves cholestatic liver fibrosis in a bile duct ligation-induced rat model. *J Ethnopharmacol* 2013; **145**: 653-662 [PMID: 23228913 DOI: 10.1016/j.jep.2012.12.005]

135 **Cai Q**, Wang Z, Zhang R, Zhang L, Cui S, Lin H, Tang X, Yang D, Lin X, Bai S, Gao J, Yang L. Huangjia Ruangan Granule Inhibits Inflammation in a Rat Model with Liver Fibrosis by Regulating TNF/MAPK and NF-κB Signaling Pathways. *Evid Based Complement Alternat Med* 2022; **2022**: 8105306 [PMID: 35942372 DOI: 10.1155/2022/8105306]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1109-1126

DOI: 10.4254/wjh.v15.i10.1109

ISSN 1948-5182 (online)

REVIEW

Challenges and dilemmas in pediatric hepatic Wilson's disease

Upasana Ghosh, Moinak Sen Sarma, Arghya Samanta

Specialty type: Gastroenterology & hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: El-Koofy NM, Egypt; Wang YG, China

Received: July 31, 2023
Peer-review started: July 31, 2023
First decision: August 22, 2023
Revised: September 23, 2023
Accepted: October 8, 2023
Article in press: October 8, 2023
Published online: October 27, 2023

Upasana Ghosh, Department of Pediatric Gastroenterology, Sanjay Gandhi Post graduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India

Moinak Sen Sarma, Arghya Samanta, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

Corresponding author: Moinak Sen Sarma, MBBS, MD, Adjunct Associate Professor, Doctor, Department of Pediatric Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India. moinaksen@yahoo.com

Abstract

Wilson disease is an autosomal recessive disorder affecting the ATP7B gene located on chromosome 13q. This leads to copper deposition in various organs, most importantly in the liver and brain. The genetic mutations are vast, well reported in the West but poorly documented in developing countries. Hence the diagnosis is made with a constellation of clinico-laboratory parameters which have significant overlap with other liver diseases and often pose a significant dilemma for clinicians. Diagnostic scoring systems are not fool-proof. The availability and affordability of chelators in developing countries impact the drug compliance of patients. While D-penicillamine is a potent drug, its side effects lead to drug discontinuation. Trientine is cost-prohibitive in developing countries. There is no single test to assess the adequacy of chelation. Exchangeable urinary copper is an essential upcoming diagnostic and prognostic tool. In the presence of cirrhosis, hypersplenism clouds the assessment of myelosuppression of drugs. Similarly, it may be difficult to distinguish disease tubulopathy from drug-induced glomerulonephritis. Neurological worsening due to chelators may appear similar to disease progression. Presentation as fulminant hepatic failure requires rapid workup. There is a limited window of opportunity to salvage these patients with the help of plasmapheresis and other liver-assisted devices. This review addresses the challenges and clinical dilemmas faced at beside in developing countries.

Key Words: Wilson's disease; Children; Hepatic Wilson disease; D-penicillamine; Trientine; Exchangeable copper

Core Tip: Wilson's disease is an important and common cause of chronic liver disease among children with multi-organ affection. There are various biochemical parameters that aid in making the diagnosis but are nonetheless fraught with limitations. Treatment is lifelong and it's important to maintain adequate chelation while monitoring for its adverse effects. It is important to distinguish the adverse effects of chelators from the disease manifestations per se, as the management is diagonally opposite, for example, differentiating D-penicillamine-induced paradoxical neurological worsening from neurological manifestations of Wilson disease. This review discusses these challenges in making a diagnosis and treating pediatric hepatic Wilson's disease.

Citation: Ghosh U, Sen Sarma M, Samanta A. Challenges and dilemmas in pediatric hepatic Wilson's disease. *World J Hepatol* 2023; 15(10): 1109-1126
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1109.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1109

INTRODUCTION

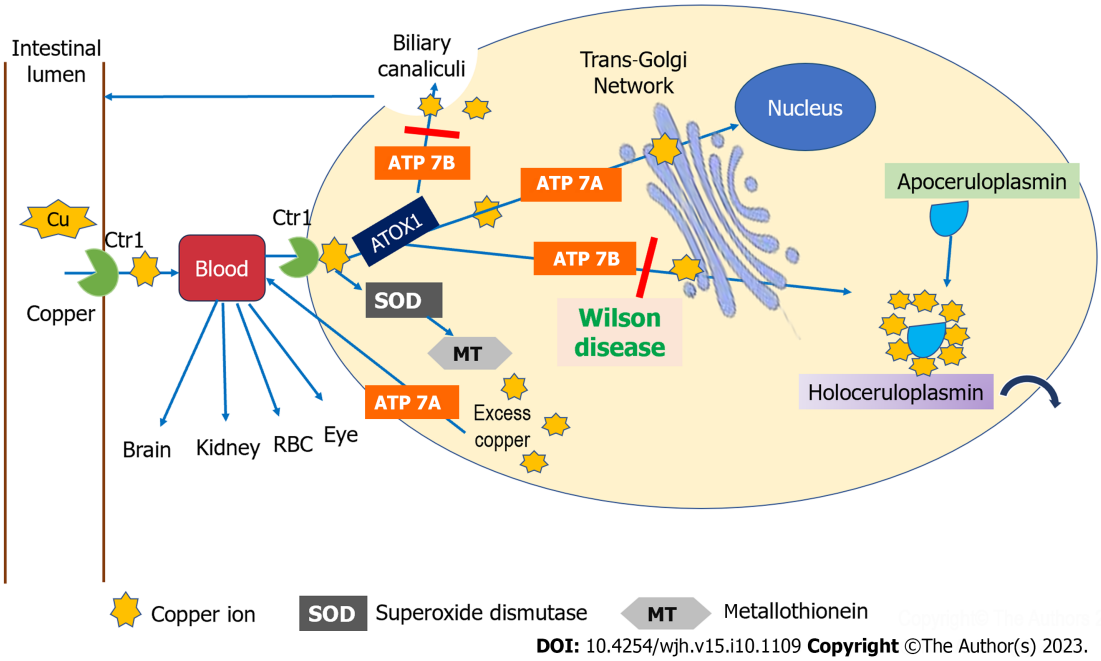
Wilson disease (WD) is a genetic disorder of copper metabolism, with an autosomal recessive mode of inheritance. The estimated prevalence of WD is approximately 30 per million population[1]. Among all causes of hepatobiliary disorders in children, in a 3.5-year study period, in a tertiary care centre in India, WD accounted for 7.6% of total cases[2]. It is important to make an early diagnosis for the prevention of disease progression and the identification of pre-symptomatic cases. This paper addresses the management difficulties in Wilson disease in terms of the identification of varied atypical clinical presentations, diagnostic dilemmas, and pharmacotherapy-related problems.

Pathogenesis

Figure 1 depicts the etiopathogenesis of WD. WD is caused by a mutation in the ATP7B gene, located on chromosome no. 13 Long arm, (13q14.3). This gene codes for an ATPase (a metal-transporting P-type adenosine triphosphatase), which is membrane-bound and mediates trans-Golgi migration of copper within the hepatocyte and its subsequent excretion into the biliary canaliculi[3]. Copper (Cu^{+}) from the diet enters the hepatocyte *via* the copper transport receptor 1 (CTR1) located in the apical membrane. Then it binds to Atox1 (antioxidant protein1) and is then handed over to ATPase (gene product of ATP7B) for its transport into the trans-Golgi network (TGN)[4]. Ceruloplasmin has two functions- i. it transports copper to the TGN where apoceruloplasmin binds to 6 atoms of copper to form holo-ceruloplasmin ii. in copper loaded condition, ATP7B goes to the endocytic vesicles to mediate copper excretion into the bile canaliculi[5]. Thus, in normal conditions, ATP7B is present in the trans-Golgi network and helps in the synthesis of holo-ceruloplasmin. But, in the copper-excess state, the protein moves towards the canalicular membrane to promote copper excretion[5]. Apo-ceruloplasmin is the inactive form of ceruloplasmin and is less stable. In the absence of this transporter, apoceruloplasmin degrades, and non-TGN-transported copper accumulates in the liver and gets bound to metallothioneins (endogenous chelators). When the copper binding capacity of metallothioneins is overwhelmed, the free copper spills into the lysosomes and causes free radical-mediated cell damage. Ultimately, this free copper comes to the peripheral circulation, where it is weakly bound to albumin and gets deposited in other tissues (brain, kidneys, cornea) (Figure 1). Apoceruloplasmin is a plasma protein and carrier of copper, mainly produced by the liver. In normal physiology, after copper reaches the liver *via* the portal circulation, 20% of copper is re-excreted into the GI tract *via* bile and the remaining 80% is transported to the periphery, bound to ceruloplasmin (Apoceruloplasmin + copper = holo-ceruloplasmin). However, in WD, holoceruloplasmin is reduced, and free copper (non-ceruloplasmin bound copper) is elevated, which leads to high urinary excretion of copper[6,7]. Hepatic involvement is the most common presentation in children with WD, as liver is the major organ responsible for copper homeostasis. Initially, copper binds with metallothionein and accumulates in lysosomes and progressively causes mitochondrial damage leading to hepatic steatosis, fibrosis, and cirrhosis. Subsequently, non-ceruloplasmin-bound excess and toxic copper leaks into the blood and accumulates in the other tissues like brain, leading to neurological manifestations, which is the commonest presentation in older children and adults.

DIAGNOSTIC DIFFICULTIES

Hepatic Wilson disease may present as acute hepatitis, persistent or intermittent elevations of transaminases, organomegaly, fluctuating or worsening jaundice, resembling autoimmune hepatitis, fatty liver, acute liver failure or as decompensated CLD[6,8,9]. Other major presentations of WD include neurological presentation with extrapyramidal symptoms and psychiatric manifestations in the form of tremors, dystonia, dysarthria, gait disturbances, and psychiatric manifestations, like behavioral abnormalities and psychiatric manifestations, like bipolar affective disorder and psychosis with cognitive decline. It is hypothesized that it is because of copper which gets deposited in the basal ganglia by penetrating through the choroid plexus (fenestrated endothelium)[4]. Minor presentations include renal tubular acidosis presenting with microscopic hematuria, osseo-muscular involvement in the form of pathological fractures, arthralgia,



DOI: 10.4254/wjh.v15.i10.1109 **Copyright** ©The Author(s) 2023.

Figure 1 Dietary copper (Cu) is taken up in the small intestine by intestinal enterocytes by copper transport receptor 1 (Ctr1) on the apical site. Most of the newly absorbed copper is taken up by the liver, mediated primarily by Ctr1. Accumulated copper is sequestered by superoxide dismutase and stored in metallothioneins. Specialized chaperons shuttle copper to its specific cellular targets: Antioxidant protein 1 (Atox1) to the copper-transporting ATPases ATP7A and ATP7B. Both ATP7A and ATP7B transport copper into the trans-Golgi network for subsequent incorporation into copper-dependent enzymes. ATP7B provides copper for incorporation into ceruloplasmin as well as for biliary excretion of copper, whereas ATP7A has role in copper mobilization from hepatic stores into the bloodstream during peripheral copper deficiency. Mutation in ATP7B gene leads to impaired biliary excretion of copper, causing copper overload in liver and other tissues as well as failure of loading of ceruloplasmin with copper. MT: Metallothioneins.

proximal muscle weakness.), and importantly haematological presentation in the form of acute fulminant liver failure with Coomb's negative haemolytic anemia.

To make the diagnosis of Wilson disease, we use the presence of a Kayser-Fleischer (K-F) ring on slit lamp exam, 24-h urine copper of > 100 micrograms (mcg) per day, and ceruloplasmin < 20 milligrams/decilitre (mg/dL) commonly. These biochemical parameters are not to be interpreted in isolation; it is prudent to remember various factors that affect them. Table 1 summarizes all the diagnostic tests and their interpretation. Table 2 enlists various diagnostic tests used in suspected WD patients and for family screening.

Ceruloplasmin

Apo-ceruloplasmin combines with copper and is secreted from the liver in the form of holo-ceruloplasmin. Serum ceruloplasmin is measured by two methods; either enzymatically by measuring its copper-dependent ferroxidase activity (holo-ceruloplasmin) or by antibody-based tests such as radioimmunoassay, radial immunodiffusion, or nephelometry[10]. The enzymatic method measures holo-ceruloplasmin, whereas immunologic assays measure both apo ceruloplasmin and holo-ceruloplasmin and thus can overestimate ceruloplasmin levels[10,11]. It has been suggested that the enzymatic method is better than the immunologic method to diagnose Wilson's disease[12]. In those patients with impaired synthetic functions due to causes other than WD, the specificity of both these tests dropped, but the enzymatic test had better specificity as compared to immunologic assays (84.5% *vs* 68.9%)[12]. However, in routine practice, immunological assays are more readily available, rapid and commonly used. The normal value of ceruloplasmin ranges between 20-40 mg/dL. Ceruloplasmin level < 20 mg/dL with the presence of Kayser Fleischer (KF) ring is considered consistent with WD[8]. Ceruloplasmin < 5 mg/dL is strongly suggestive of WD[6]. Also, ceruloplasmin level < 14 mg/dL has a 100% positive predictive value for the diagnosis of WD and a value < 5 mg/dL is strongly suggestive of WD[8,13]. Serum ceruloplasmin is also an acute phase reactant and may be falsely elevated in the presence of sepsis and it may be falsely low in conditions like fulminant hepatic failure of any etiology, advanced liver disease, celiac disease and aceruloplasminemia, nephrotic syndrome, and 20% of heterozygotes[10,11,14].

24-h urine copper excretion

It is another indirect marker of non-ceruloplasmin-bound copper, which is elevated in WD. 24-h urine copper excretion of more than 100 mcg per day is diagnostic of Wilson's disease[15]. However, certain other conditions like autoimmune hepatitis, chronic cholestatic liver disease and acute fulminant liver failure of any aetiology may give rise to high 24-h urine copper excretion[10]. Basal copper excretion may be low in children and asymptomatic siblings, thus a normal value doesn't rule out WD. The test may give rise to false results in case of improper urine collection and copper contamination if kept in certain types of containers. 24-h urine copper of more than 40 mcg per day may indicate WD in asymptomatic individuals. In addition to making a diagnosis, this is also a valuable test in monitoring the adequacy of

Table 1 Various laboratory investigations used in the diagnosis and monitoring of Wilson disease

Diagnostic tests	Cut-off values in diagnosis	Disease monitoring	Problems in interpretation
Serum cerulo-plasmin	< 10 mg/dL (strong evidence), 10-20 mg/dL (needs further evaluation), 20-40 mg/dL (normal value, but does not exclude diagnosis)	Not helpful	Can be normal in fulminant presentation and acute inflammation as it is an acute phase reactant
24-h urine copper	> 100 mcg/d (virtually diagnostic in symptomatic patients), > 40 mcg/d (may indicate disease, needs further evaluation)	> 500 mcg/d during initial phase, 200-500 mcg/d in the maintenance phase	Difficult to perform, to be done in reliable laboratories
Hepatic copper	> 250 mcg/g dry weight (diagnostic), 50-250 mcg/g dry weight (needs further evaluation), < 50 mcg/g dry weight (normal)	Not recommended	Inhomogenous distribution of copper (sampling error), elevated in long-standing cholestasis
Serum total copper	> 25 micromol/L (needs further evaluation), 14-24 micromol/L (90-150 mg/dL) normal	N/A[a]	
Non-ceruloplasmin copper	10-15 mcg/dL (normal person), > 25 μg/dL (untreated patients), Not recommended for diagnosis	> 15 mg/dL (poor compliance)[a] < 5 mg/dL (over-chelation)	
Exchangeable copper	> 2.08 micromol/L (more likelihood of extrahepatic organ involvement), 0.62 and 1.15 micromol/L (normal)	Experimental	Requires equipped laboratories and expertise
Relative exchangeable copper	> 15% (100% sensitivity and specificity for diagnosis)	Experimental	Requires equipped laboratories and expertise

[a]The serum free copper or NCC has a role in monitoring: Initial phase: > 25 mcg/dL, maintenance phase: 10-15 mcg/dL, under-chelation: > 15 mcg/dL, over-chelation: < 5 mcg/dL.

Table 2 Diagnostic tests for suspected Wilson disease patients and asymptomatic siblings

Parameters	Tests for suspected patients	Tests for asymptomatic siblings
Clinical examination	Yes	Yes
Liver function test	Yes	Yes
Slit lamp examination for Kayser–Fleischer ring	Yes	Yes
Serum ceruloplasmin	Yes	Yes
24 h urine copper	Yes	Yes
Genetic analysis for ATP7B gene mutation analysis	Yes, if feasible	Yes (if proband sample is available)
Liver biopsy	Yes, ancillary test	No
Hepatic copper	Yes, to be done in cases of ambiguity	No

chelation, a 24-h urine copper of 200-500 mcg per day is considered the target[8].

Liver biopsy and hepatic copper

On liver biopsy, early changes include steatosis, and fibrosis and late changes include changes of chronic active hepatitis with bridging fibrosis, occasionally resembling autoimmune hepatitis and, Mallory-Denk bodies[16]. Copper staining is demonstrated by orcein, which stains the copper-associated proteins, and rhodanine which stains the elemental copper. Copper-associated proteins may be elevated in various causes of advanced cholestatic liver disease (*e.g.*, Primary biliary cirrhosis or Chronic hepatitis B) other than Wilson's disease, thus histochemical staining for copper is not specific for Wilson's disease[17]. Also, negative histochemical staining for copper does not rule out the possibility of WD[18]. Copper deposition also depends on the stage of disease, early in the disease, copper staining is diffuse in the cytoplasm in the periportal region, and later in the disease, it is mainly seen within the lysosomes of hepatocytes in the periphery of the regenerating nodules. This pattern of distribution suggests that the cytoplasmic distribution leads to hepatocyte damage while copper in the lysosomes is likely to be less toxic. Initial cytoplasmic deposition of copper is due to the high affinity of the sulfhydryl-rich proteins (metallothionines) present in the cytoplasm as compared to lysosomes[19]. Hepatic copper of more than 250 mcg/gram (gm) dry weight of the liver is strongly suggestive of copper deposition in the liver but it may be also found in any advanced liver disease. Liver copper < 50 mcg/gm dry weight almost excludes the diagnosis of WD[8,20]. Also, the regional variation due to regenerative nodules and tissue fibrosis is to be kept in mind while interpreting hepatic copper[10].

Serum copper

It is important to measure the serum copper simultaneously while measuring ceruloplasmin, as it helps in the calculation of the "free copper" also known as "non-ceruloplasmin bound copper (NCC)". Total serum copper comprises of holo-ceruloplasmin (70%), albumin-bound (20%), peptide-bound, and free copper. Holo-ceruloplasmin level is low in WD. So, in most patients of WD, serum copper is low, unless there is a massive release of free copper from the liver due to liver necrosis[21]. This free copper, which is not bound to ceruloplasmin is known as "non-ceruloplasmin bound copper", is elevated in WD and is toxic. Serum copper is estimated by either atomic absorption or emission spectrometry or by inductively coupled plasma mass spectrometry (ICP-MS) methods[22]. Total serum copper (in mcg/L) = serum copper (in micromol/L) × 63.5[10]. The normal range of serum copper is 14- 24 micromol/L (90- 150 mg/dL)[11]. Holo-ceruloplasmin contains 3.15 mcg of copper per mg of ceruloplasmin, hence, ceruloplasmin-bound copper may be calculated as serum ceruloplasmin (mg/dL) x 3.15 (mcg copper per mg ceruloplasmin)[11]. Free copper or NCC = (Serum copper-ceruloplasmin bound copper). Non-ceruloplasmin-bound copper has been proposed as a diagnostic test for WD[23]. In normal individuals, the NCC (free copper) is 10-15 mcg/dL[3]. In untreated patients, it is > 25 mcg/dL[3]. The estimation of NCC depends on the methods used to estimate serum ceruloplasmin and copper levels. Falsely high ceruloplasmin calculated by immunological tests may lead to the calculation of a falsely low or negative value of free copper. In normal conditions in WD, serum copper is low. In acute fulminant WD, due to a massive release of copper from the liver, free copper may rise up to ten times and can cause intravascular hemolysis[23]. NCC is not validated for diagnosis of WD but is helpful in monitoring treatment response. With adequate chelation, the NCC decreases to < 5 mcg/dL. Among patients who are on chelation therapy, the target 24-hour urine copper excretion is 200-500 mcg/d. However, patients with poor compliance to chelators can also have low 24-h urine copper excretion as there is adequate chelator in the circulation to leach out copper. Such patients can be differentiated by estimating the NCC. Those with high NCC > 15 mg/dL have poor compliance while those with NCC < 5 mg/dL are over-chelated[10].

Exchangeable copper and relative exchangeable copper

Exchangeable copper is measured by adding ethylenediaminetetraacetic acid (EDTA) to the serum sample. As EDTA is a copper chelator, it binds with copper, and EDTA-bound copper is measured as exchangeable copper. It measures copper bound to albumin, and other amino acids, which easily gets exchanged with EDTA (copper-chelating agents)[21]. Unlike NCC, it doesn't depend on serum ceruloplasmin levels. Normal values for exchangeable copper (CuEx) are between 0.62 and 1.15 micromol/L[24]. Poujois *et al*[25] demonstrated that CuEx is normal to moderately increased in WD, but elevated in those with acute fulminant WD and WD with extrahepatic involvement. In patients with extra-hepatic manifestations, CuEx was the only biological marker to be positively correlated with the neurologic disease burden (assessed by the Unified Wilson Disease Rating Score). CuEx determination is, in consequence, useful when diagnosing WD with a value > 2.08 micromol/L, and is indicative of the severity of the extra-hepatic involvement. However, CuEx did not indicate the severity of liver damage. CuEx is an interesting experimental biomarker but needs to be interpreted with caution, especially in WD patients with hepatic manifestation. In this study, it was postulated that there may be a threshold concentration of CuEx above which organs like the brain and eyes (and possibly others such as kidneys and heart) may be affected by copper overload[25]. Relative exchangeable copper (REC) is the ratio of exchangeable copper to total serum copper. El Balkhi *et al*[26] showed that REC > 18.5% can be used to diagnose WD with a sensitivity and specificity of 100%. Trocello *et al*[27] showed that REC can also be used to diagnose WD in asymptomatic siblings, by taking REC cut-off of 15%, it can distinguish asymptomatic WD from heterozygotes accurately.

KF ring

It represents copper deposition of copper in Descemet's membrane of the cornea, visible on slit lamp examination, it is pathognomonic of WD. It is not specific to Wilson's disease and may be found in various cholestatic conditions. It is present in 44%-62% of hepatic WD and 95% of neurological WD[3]. KF-like rings have been described in primary biliary cirrhosis, autoimmune hepatitis and severe cholestasis where the total bilirubin is > 10 g/dL. They are called bilirubin rings and disappear with the resolution of jaundice[28]. It is important to note that the appearance of the KF ring is not related to disease severity and its resolution may take variable time, months to years[29,30]. Anterior segment-optical coherence tomography (AS-OCT) can also be used to diagnose the KF ring, where the KF ring is visualized as an intense reflectivity in Descemet's membrane and it may have better accuracy in diagnosing KF ring as compared to slit-lamp examination[31]. Broniek-Kowalik *et al*[32] showed that AS-OCT detected copper deposition in 15 additional patients of WD, in whom the KF ring was not seen in slit lamp examination.

Coomb's negative hemolytic anemia with a disproportionate elevation of aspartate transaminase (AST) as compared to alanine transaminase (ALT) is suggestive of acute fulminant WD. The ratio of AST/ALT > 2.2 and ALP/total bilirubin ratio < 4, when combined is suggestive of acute fulminant Wilson disease with a sensitivity and specificity of 100%[33]. Alkaline phosphatases are metalloenzymes and zinc is a co-factor. In fulminant WD, during the period of massive release of copper into circulation, copper may compete with zinc for incorporation into alkaline phosphatase apoenzymes. Copper-containing enzymes released into the circulation would have little or no enzymatic activity, and serum alkaline phosphatase values would be low[34].

Neurological imaging

The face of the giant panda sign, which is caused by the normal intensity of red nuclei, preservation of signal intensity in the lateral portion of pars reticulata of substantia nigra and hypointensity in the superior colliculus with hyperintense surrounding tegmentum, is considered pathognomonic of neurological WD[35]. This finding has been shown to reverse with chelation[36,37]. Other findings include lesions in the putamen, globus pallidus, caudate, thalamus, midbrain, pons,

and cerebellum as well as cortical atrophy and white matter changes. Usually, the lesions are hyperintense in T2 and hypointense in T1 weighted images. Magnetic resonance imaging (MRI) changes correlate with disease severity. Diffuse atrophy of the brain is one of the most common features[35]. MRI findings are universal in symptomatic patients and occasionally reported in pre-symptomatic patients[38].

Genetic analysis

Lastly, genetic analysis can be done in patients having ambiguity in diagnosis or for sibling screening. More than 600 pathogenic variants have been identified[39]. Most common mutations are single-nucleotide missense and nonsense mutations[39]. There is no genotype-phenotype correlation. Siblings may have varied presentations[40]. On screening the proband, the chances of a sibling being affected is 25% while either parent being affected is 0.5%[6].

Leipzig score

A diagnostic score named Leipzig score was proposed in 2001, which took into account various clinical, and biochemical, parameters to diagnose WD. A Leipzig score of more than 4 is highly suggestive of WD, while a score of 2-3 would merit further investigations and a score of less than 1 makes the diagnosis of WD unlikely[41]. There are practical difficulties in implementing the Leipzig score in developing countries. Hepatic copper is not universally available and inconsistent in reporting. Genetic testing is cumbersome and region-specific. Most regions do not report the mutations. The d-penicillamine challenge is not reliable. A revised Leipzig score was devised for the Asian setting, which gave importance to family history, greater points for the KF ring and an additional score for a ceruloplasmin level < 5 mg/dL[6].

Family screening

First-degree relatives of newly diagnosed patients should be screened by clinical examination, LFT, slit-lamp examination, serum ceruloplasmin, 24-h urine copper estimation and genetic testing, especially if the mutation is known in the proband. Screening by laboratory tests is usually deferred till 2 years of age though genetic screening is possible[6]. Preservation of the DNA sample of the proband is essential.

MANAGEMENT DIFFICULTIES

Differentiating manifestations of WD from chelation-related complications/Atypical symptoms of WD masquerading as chelation-related complications:

Kidney-related issues in WD

The renal manifestation of WD can be categorized into (1) renal involvement of underlying disease, or (2) treatment-related nephrotoxicity. The differentiating features in pathogenesis, laboratory investigations and management of these two conditions are summarized in Table 3. The renal involvement of WD can be in the form of renal calculi, hypercalciuria or tubulopathy. Renal manifestations of WD are due to copper deposition in the renal tubular cells. It has been shown that glomerular and tubular functions improve and normalize after starting D-penicillamine[42]. It is vital to differentiate the renal manifestations from the drug-induced glomerulonephritis as the management differs. Renal involvement in WD was first reported by Litin *et al*[43] in 1959 in the form of hypercalciuria. Acute renal failure, which is the most severe form, can be precipitated by massive intravascular hemolysis seen in WD[44]. Renal involvement can manifest with glomerular or non-glomerular (tubular) injury. Aminoaciduria was first detected in patients with Wilson disease by Uzman and Denny-Brown in 1948[45]. Tubular causes are tubulopathies [proximal or distal renal tubular acidosis (RTA)] presenting as renal rickets, polyuria, polydipsia, or macroscopic hematuria (nephrocalcinosis or renal stone), aminoaciduria, glucosuria, proteinuria, hyperphosphaturia, hypercalcemia and defective urinary acidification [46]. Sözeri *et al*[42] in their study of 10 patients of Wilson's disease, for whom excretion of tubular markers could be repeated between 3 mo to 12 years, N-acetyl-β-d-glucosaminidase (NAG), beta 2 microglobulin and low molecular weight proteins were higher in the first year, as compared to the subsequent period. This is in contrast to high molecular weight proteinuria, which increases after 1 year of treatment with D-penicillamine. Renal impairment may be mild to severe. It may present as sub-nephrotic or nephrotic range proteinuria, microscopic hematuria, hypercalciuria, renal stones, renal failure, and lastly D-penicillamine-associated glomerulonephritis[47].

Tubular damage/ RTA

The disease per se can cause glomerular as well as tubular dysfunction, however, tubulopathy is more common. Excess copper deposits in the epithelium of the proximal and distal convoluted tubules, and the thickening of the basement membrane interfere with reabsorptive function of the renal tubules, thereby leading to RTA[47]. Distal, as well as proximal RTA, has been reported in WD. In a cross-sectional study by Kapoor *et al*[48], done over 1 year duration, 14 out of 25 patients (56%) of WD, had renal tubular acidosis of which 24% (6/25) had distal RTA, 16% (4/25) had mixed RTA, and another 16% (4/25) had proximal RTA. Wolff *et al*[49] described post-mortem kidney biopsies of 5 patients, wherein, on histopathology, glomeruli were normal in all 5 patients, focal areas of degeneration and necrosis of tubular epithelial cells along with copper staining (rubeanic-acid staining) were seen in all patients. Elsas *et al*[50] showed progressive renal impairment in an adolescent with Wilson's disease for whom D-penicillamine was stopped for almost 18 mo (owing to D-penicillamine induced lupus nephritis), with simultaneous renal biopsy showing an increased number of conspicuous electron-dense bodies in the subapical areas of cytoplasm suggestive of metalloprotein complexes. Azizi *et al*[51]

Table 3 Differentiating features between Wilson disease-related renal tubular acidosis and D-penicillamine induced glomerulonephritis

	Wilson disease-related renal tubular acidosis	D-penicillamine induced glomerulonephritis
Mechanism	Copper induced tubular damage	Immune complex deposition
Presentation	Prior to starting chelation/during chelation	After starting chelation
Tests to differentiate	Normal anion gap metabolic acidosis, Urine pH, Urine for glucosuria, aminoaciduria, acidification test of urine	Urine for proteinuria, autoantibodies for glomerulonephritis, renal biopsy
Chelation	To be continued	To be stopped

described a 9-year-old boy, who first presented with renal colic due to hypercalciuria, only to be diagnosed as WD 1 year later. There are few case reports of children presenting with renal rickets who were finally diagnosed as WD[52]. In a retrospective study of 85 children with WD by Zhuang *et al*[47], renal impairment was found in 25 (29.4%) of the treatment-naïve patients. Seven of the twenty-five WD patients (28%) had symptoms of renal impairment (one each had acute nephritis, persistent glomerulonephritis, hemolytic uremic syndrome, and 2 had nephrotic syndrome). Five children had evidence of the glomerular cause of hematuria while the remaining two had non-glomerular hematuria. Twelve of the 25 (48%) had proteinuria, 14/25 (56%) had hematuria, and 5/25 (20%) had both proteinuria and hematuria, 4/25 (16%) had glucosuria[47]. Thus, by characterizing the type of hematuria (glomerular or non-glomerular) and proteinuria (low molecular weight or high molecular weight), the site of renal involvement can be ascertained. In the treatment -naïve patients, albuminuria with elevated serum creatinine, and low creatinine clearance suggest glomerular involvement. On the other hand, glycosuria, LMW proteinuria, non-glomerular hematuria, an increase in NAG and beta2 microglobulin, nephrocalcinosis, hypercalciuria, and non-anion gap metabolic acidosis indicate tubular involvement.

Glomerular involvement can be due to (1) copper deposition in the mesangium, leading to membranoproliferative glomerulonephritis, (2) IgA nephropathy caused by IgA deposits in the glomerulus, due to the loss of scavenging capacity of the liver, and (3) chelation (D-penicillamine) induced. IgA nephropathy: Gündüz *et al*[53] have reported a 13-year-old boy who presented with nephritic syndrome after 4 mo of diagnosis of Wilson disease, and was diagnosed as membranoproliferative glomerulonephritis on renal biopsy with positive immunofluorescence for IgA, suggestive of IgA nephropathy. Similar case of IgA vasculitis is described by Acharya *et al*[54] in an 11-year-old boy with Wilson disease with F2 fibrosis on liver biopsy (METAVIR staging), who presented with palpable purpura without any arthritis or gastrointestinal involvement. He was diagnosed with IgA nephropathy and mild tubular epithelial degeneration and atrophy on renal biopsy and had complete resolution of the rash and achievement of normal renal function within 3 to 6 mo of chelation. This is similar to primary IgA nephropathy, abnormally glycosylated IgA1 form large soluble IgA1 immune complexes by combining with IgG and IgA, which deposit in the mesangium and lead to mesangial injury. Clinical and histopathological changes may reverse after liver transplantation.

Treatment-related nephrotoxicity

D-penicillamine-induced nephrotic syndrome was first reported by Fellers and Shahidi in 1959[55]. D-penicillamine can cause inhibition of enzymes required for collagen synthesis, thus can damage the glomerular basement membrane and reduce GFR or it can act as a hapten and induce the formation of immune complexes leading to membranous glomerulonephritis[56]. D-penicillamine-induced proteinuria occurs in < 10% of patients of Wilson disease and usually begins after 1 year of treatment[57,58]. The spectrum of D-penicillamine-induced nephrotoxicity ranges from membranous glomerulonephritis, tubule-interstitial disease, crescentic glomerulonephritis, Goodpasture's syndrome and renal-limited vasculitis[59,60].

Proteinuria is the most common manifestation and is the first abnormality to be detected followed by progressive renal disease if D-penicillamine is not stopped[60]. In a case series reported by Sternlieb, all the patients were started on racemic penicillamine, the clinical features resembled nephrotic syndrome, 3 of whom improved following discontinuation of drugs, and another 5 required steroids and there was a recurrence of nephrotic syndrome in all 3 patients in whom penicillamine was re-started while 2 of them didn't have any recurrence after they were started on D isomer of penicillamine[61]. Initially, the racemic mixture of penicillamine was incriminated in the causation of nephrotic syndrome, however, later on, it was shown that even the D- isomer can cause proteinuria. Since 1960, the D-isomer of penicillamine has been available and was approved by the FDA in 1963[61]. Histological examination shows membranous nephropathy (most commonly) minimal change disease or membranoproliferative or rarely crescentic nephropathy[62-64]. D-penicillamine causes nephrotoxicity by immunological mechanism, ultrastructural changes include immune-complex deposits, subepithelial deposits and IgG deposits on immunofluorescence.

The incidence of nephrotoxicity due to D-penicillamine was more in conditions unrelated to copper metabolism, *e.g.* rheumatoid arthritis, cystinurias, and scleroderma[65,66]. In a case series of 33 patients, studied by Hall *et al*[59], who were followed up serially for a mean duration of 74 mo, it was found that the onset of proteinuria peaked after the first 6 mo, with 27/33 developing proteinuria within 1 year of starting D-penicillamine. There are reports to show that the time period from exposure of D-penicillamine to proteinuria may range from a few weeks to years[60]. Siafakas *et al*[67] report a 12-year-old boy who presented with nephrotic syndrome (nephrotic range proteinuria without hematuria, with renal biopsy showing minimal change disease and a negative immunofluorescence study) after 2 wk of D-penicillamine and showed resolution of proteinuria in 3 wk, after being started on steroids. There are studies to show that proteinuria increases even after stoppage of D-penicillamine (within 1-5 mo), and then gradually improves over a few months, which

can be explained by Wilson disease-related tubulopathy, as it improves over time with chelation[42,60,63]. In the study by Hall *et al*[59] the median first and last creatinine clearance almost remained the same, and none went into renal failure or required corticosteroids. The spontaneous resolution was seen within 2-32 mo of the stoppage of the drug[63]. According to Hall *et al*[59], among 33 patients of patients on D-penicillamine who had proteinuria, the number of patients in whom proteinuria resolved by 6, 12 and 18 mo were 12 (36%), 21(63%), 29 (88%) respectively. Renal biopsies done in all patients, 29/33 showed ultrastructural changes characteristic of membranous glomerulonephritis with IgG deposits and complement deposits on immunofluorescence, the remaining 4 patients' biopsies showed mesangial proliferation without any deposition of immunoglobulins or complement on immunofluorescence[59]. The severity of proteinuria is not related to the duration or dose of d-penicillamine or HLA typing[59]. In a retrospective study of 63 patients who developed nephrotic syndrome after being started on D-penicillamine, (75% had rheumatoid arthritis, 10% had Wilson disease), the mean duration of D-pen exposure to proteinuria was 7.6 (± 3.90) months and mean duration of drug exposure until the diagnosis of nephrotic syndrome was 11.9 (± 18.8) mo. Fifty-five percent of them had membranous glomerulonephritis and 27% had minimal change disease[63].

In a study of 8 patients of rheumatoid arthritis, who developed proteinuria while on D-penicillamine of variable duration, ranging from 3 to 48 mo and dosage ranging from 250 mg/d to 1.2 gm per day, 5 patients had scanty subepithelial deposits (spikes), 1 had plenty of sub-epithelial deposits, 2 had associated findings of glomerulosclerosis, interstitial scarring and, on immunofluorescence, 3 showed a positive granular pattern of fluorescence along basement membrane for IgG and C3[68]. After stopping D-penicillamine, proteinuria resolved in 3, progressive proteinuria was seen in one patient, mild proteinuria persisted in 4 others, with the persistence of proteinuria being seen up to 27 mo after stoppage of D-penicillamine[68]. Some studies have been done to see whether D-penicillamine can be resumed after the resolution of proteinuria[69]. In one of the studies in rheumatoid arthritis patients, D-penicillamine was re-introduced after 3 mo of resolution of proteinuria at a low dose of 50 mg/d, escalated to a maximum of 250 mg/d, showed that none of them had a relapse of proteinuria. However, this low dose is not enough to treat WD. In another study by Bacon *et al* [64] in 14 patients of rheumatoid arthritis, 3 developed proteinuria, and 11 developed nephrotic syndrome in a mean duration of 7.5 mo of starting D-penicillamine. Renal biopsies showed a picture similar to membranous nephropathy on light microscopy, granular deposits on immunofluorescence, positive for IgG and C3 and sub-epithelial dense deposits on electron microscopy, even after 3- 12 mo of stoppage of treatment. A renal biopsy done soon after stopping D-penicillamine showed effacement of podocytes with discrete electron-dense deposits in the subepithelial region and positive fine granular immunofluorescence with IgG and C3. When the biopsy was repeated after 6 mo of discontinuation, it showed normal foot processes with occasional dense deposits and focal weak immunofluorescence with IgG and C3. Among 3 patients in whom proteinuria persisted at a high level even after stoppage of the drug, biopsy done at 6, 8, 12 mo of discontinuing D-penicillamine, electron microscopy still showed discrete but much smaller electron-dense deposits, and brilliant fluorescence with IgG[64]. D-penicillamine-induced glomerulonephritis and Goodpasture syndrome have also been described[70].

To summarize, diagnosis of D-penicillamine-induced nephropathy can be made with routine urine microscopy, 24-h urine estimation for proteinuria, and type of proteinuria (tubular or glomerular), and if feasible, histopathological and electron microscopic examination (renal biopsy) especially in the first 18 mo. Membranous nephropathy is the most common nephropathy caused by D-penicillamine[59,63,68]. Serum MPLA2R antibodies (an immunofluorescence test) can be done as a supportive test. These antibodies are prevalent in primary membranous glomerulonephritis in up to 68.5% of cases and their absence on the face of renal biopsy picture of membranous nephropathy suggests the secondary cause of membranous glomerulonephritis[71]. Kumar *et al*[72] reported nephrotic syndrome in a 24-year-old lady, a case WD on D-penicillamine for 18 mo. Renal biopsy showed membranous nephropathy with positive immunofluorescence for IgG and C3, and with negative serum auto-antibodies to M-type phospholipase A2 receptor ($MPLA_2R$, which is usually seen in primary membranous nephropathy), thus supporting the diagnosis of secondary membranous glomerulonephritis.

Management of D-penicillamine nephrotoxicity includes stoppage of the drug and use of trientine as an alternative. In the study done by Neild *et al*[63], glucocorticoids had no effect on the natural history of nephropathy. In another case series by De Silva, among 35 patients who developed proteinuria while on D-penicillamine, 60% developed nephrotic syndrome (NS). Among NS patients, D-penicillamine was continued for 62%, stopped at a variable interval, and it was shown that within 4 mo of stopping D-penicillamine, proteinuria decreased to < 2 g/d and 12 mo after stopping D-penicillamine, proteinuria was 0-0.3 g/d[73]. AASLD recommends prompt withdrawal of D-penicillamine immediately.

Haematological manifestations

These include Coomb's negative hemolytic anaemia and acute renal failure, D-penicillamine-induced myelosuppression and lastly hypersplenism (splenomegaly).

Acute fulminant WD

Acute fulminant Wilson disease usually presents with acute intravascular hemolysis, which is due to free copper-mediated damage to the RBC membranes, mortality is 95%[74]. It needs to be tackled with plasmapheresis while awaiting liver transplantation[75].

Drug-induced cytopenia

The rate of myelotoxicity varies from D-penicillamine 0 to 7% and it is one of the most fatal adverse effects[76]. It has been hypothesized that marrow toxicity could be of two types: The first being an idiosyncratic reaction, leading to cytopenias occurring within the first year of treatment, and the other being a dose-dependent gradual fall[77]. Day *et al*[78] showed that of the 69 patients of rheumatoid arthritis, who were on D-penicillamine for more than 1 year, 15 (21.7%) had

developed dose-dependent hematological adverse effects; while those who were on < 500 mg per day, had no cytopenias. However in another case series of 10 patients of rheumatic arthritis with D-penicillamine induced myelosuppression, 7 had sudden onset myelosuppression, of these, 6 patients died, and the remaining showed gradual recovery of marrow over 1 year[79]. These studies are to be interpreted keeping in mind 2 points; first, both the studies were in rheumatoid arthritis patients, and myelosuppression was documented even with a low dose of D-penicillamine (in contrast to WD, where a higher dose of D-penicillamine is required), secondly, these patients didn't have concomitant hypersplenism (to confound the picture), thereby, cytopenia could easily be attributed to D-penicillamine, leading to faster decision making in terms of drug-discontinuation. Once myelosuppression is there, D-penicillamine is to be stopped. European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) has suggested monitoring blood counts initially for 1-3 monthly and later 3-6 monthly[80]. Table 4 enlists the risk factors that may complicate/mimic drug toxicity features.

Neurologic symptoms

Neurological symptoms include dystonia, dysarthria, gait abnormalities, and tremors, with dysarthria being the most common[8]. Other manifestations include abnormal gait, musculoskeletal symptoms, seizures, behavioral problems, drooling of saliva, and chorea[81]. Pediatric patients present with hepatic WD whereas neurological manifestations are more common among adults[82]. Neurological manifestations of WD are due to high concentrations of copper in brain tissue as well in CSF and anti-copper therapy leads to improvement in symptoms[83]. It is essential to characterize the disease extent in order to monitor the evolution of symptoms carefully. It is important to identify the subtle neurologic manifestations at the time of diagnosis as it has a bearing on choosing the medical therapy. Multiple studies have shown that neurological worsening on starting chelation is more commonly seen in those having neurological manifestations, within 1-3 mo of starting therapy. In one of the early studies, Brewer *et al*[84] showed that neurological worsening post-D-penicillamine occurred in 50% of neurologic WD, with more than half of the patients showing deterioration within 4 wk of chelation. Ranjan *et al*[85] performed an MRI brain at the time of neurological worsening after D-penicillamine and showed the appearance of new lesions in MRI in white matter, thalamus, pons, and midbrain which showed diffusion restriction with simultaneous blood investigations revealed increased free serum copper, malondialdehyde and reduced glutathione. Litwin *et al*[86] showed neurological worsening in 11% of neuro-hepatic WD in a mean time of 2.3 ± 1.9 mo from the time of treatment initiation independent of the type of chelation used. One hypothesis proposed is that there is a sudden release of free copper from the liver, which enters the blood-brain barrier and causes brain damage[84]. The second hypothesis is that the free copper is generated by the chelators within the brain tissue itself. This was substantiated by an increase in free copper concentration in serum and brain tissue, within 3 d of starting on D-penicillamine, measured by ultrafiltration in an animal model of WD. It was noted that at the time of the increase in brain tissue-free copper, there was a fall in protein-bound copper in the brain tissue. A simultaneous increase in immunofluorescent staining of ATP7A (copper transporting protein in neurons) and CTR1 (mediates copper uptake in mammalian tissue) in the cortex and basal ganglia, and not in the blood-brain barrier, suggests that the free copper is generated probably by mobilization of copper within the brain parenchymal cells and not *via* peripherally released free copper entry *via* the blood-brain barrier[87]. The expression of ATP7A present in the subcellular level to mediate biliary excretion of copper (in this animal model) correlated with the free copper in the cortex and basal ganglia. The third hypothesis proposed by Miki *et al*[88] is that the penicillamine-copper complexes that are generated are non-toxic, so, shouldn't cause tissue damage. However, these complexes can catalyze oxidation of the ghost membranes, due to changes in the redox potential of copper, Cu^{2+} to Cu^{1+}), thereby causing neurological symptoms.

TREATMENT

Once the diagnosis is established, the patient is to be started on lifelong pharmacotherapy, depending on the stage of the disease. WD patients can be broadly classified into three subtypes; symptomatic WD, asymptomatic WD with active disease (on biochemical, histological or imaging findings), and pre-symptomatic WD (mostly those detected on family screening). Those with symptomatic or asymptomatic active disease need to be started on chelation. Patients who are asymptomatic or without active disease can be treated with lower maintenance doses of chelators or zinc alone. It takes around 6-18 mo of consistent chelation therapy to improve the organ function and then patients can be shifted to a lower dose of chelator and zinc[8].

Table 5 summarizes all the drugs used in the treatment of WD, their mechanism of action, dose, storage and important side effects. There is a fundamental difference in the mechanism of action of chelators and zinc. Chelators decrease the copper load in the body by chelating the copper in the liver, enterocytes and extrahepatic circulation. Whereas zinc induces the synthesis of metallothioneins, which in turn bind to copper and sequester it in the enterocyte (copper is lost as the enterocytes shed off). Thus, the action of zinc is slow. In scenarios, which demand fast and immediate action, for example, advanced liver disease, chelators are a better choice than zinc.

D-penicillamine

D-penicillamine (D-3β,3β-dimethylcysteine; C5H11NO2S) has been the first line of treatment, since its discovery in 1956. It acts by chelating divalent metal ions using its thiol (-SH) group and forming a water-soluble complex which is excreted in urine. It chelates extracellular copper and mediates its excretion in urine. Also, it chelates the intracellular copper from tissue complexes[81]. In WD, the excess free copper in the cytoplasm of hepatocytes, after saturating the metallothioneins, deposits in the lysosomes and causes free radicle-mediated cell damage. D-penicillamine, trientine, as well as zinc, increase the expression of metallothioneins, which bind to copper ions. Metallothionein is a cysteine-rich protein that is

Table 4 Risk factors for side effects of drug therapy in Wilson's disease

Side effect of drugs	Risk factor	How to differentiate
Neurological worsening with D-penicillamine or trientine	Pre-existing neurological Wilson disease	Exchangeable copper and relative exchangeable copper
Cytopenia due to D-penicillamine	Co-existing hypersplenism due to portal hypertension	Bone marrow biopsy

Table 5 Drugs, their mechanism of action, dosage and side effects

Name of drug	Mechanism of action	Dose	When to start	Side effects
D-penicillamine	Induces cuprieuresis, induces hepatic metallothionine synthesis, reduces fibrosis (by preventing collagen formation)	20 mg/kg/d (maximum induction dose of 1500 mg/d and maintenance dose of 1000 mg/d), to be taken 1 h before or 2 h after meal, storage at room temperature	Chelator of choice in all hepatic phenotype	**Early (1-3 wk):** Fever, rash, arthralgia, cytopenia, proteinuria; **Late:** (1) Skin: degenerative dermatoses elastosis perforans serpingosa, cutis laxa, pseudoxanthoma elasticum, bullous dermatoses, psoriasiform dermatoses, lichen planus, seborrheic dermatitis alopecia, aphthous ulcerations, hair loss; (2) Connective tissue disorders: Lupus like syndrome, arthralgia, Rheumatoid arthritis, polymyositis; (3) Renal: proteinuria, hematuria, glomerulonephritis, nephrotic syndrome, renal vasculitis, Goodpasture's syndrome; (4) Nervous system: Paradoxical neurological worsening, neuropathies, myasthenia, hearing abnormalities, serous retinitis; (5) Gastrointestinal: Nausea, vomiting, diarrhea, elevated transaminases, cholestasis, hepatic siderosis; (6) Respiratory: Pneumonitis, pulmonary fibrosis, pleural effusion; (7) Hematological: Cytopenia, agranulocytosis, aplastic anemia, hemolytic anemia; and (8) Others: Immunoglobulin deficiency, breast enlargement, pyridoxine deficiency
Trientine	Induces cuprieuresis, induces hepatic metallothionine synthesis	20 mg/kg/d (maximum induction dose of 1500 mg/d and maintenance dose of 1000 mg/d), to be taken 1 h before or 2 h after meal, storage at 2^0-8^0 temperature	Indicated if intolerant to D-penicillamine	Paradoxical neurological worsening (10%-50%), sideroblastic anemia, bone marrow suppression, gastritis, skin rash, arthralgia, myalgia, hirsutism
Zinc	Induces intestinal synthesis of metallothioneins, prevents copper absorption	25 mg thrice daily (weight < 50 kg), 50 mg thrice daily (weight > 50 kg), taken in empty stomach	Maintenance phase of symptomatic hepatic WD; First-line induction treatment in selected patient subgroups (neurologic WD, intolerant to chelators, pre-symptomatic patients)	Gastric irritation (30%-40%)
Ammonium Tetra-thiomolybdate	Forms complexes with copper in blood, binds the copper present in food		Neurological WD	Neurological dysfunction (rare), hepatotoxicity, bone marrow suppression

WD: Wilson disease.

an endogenous chelator of copper[5,81]. D-penicillamine also solubilizes the copper deposited in the lysosomes, without much affecting the metallothionein-bound copper and reduces cell damage[89]. Thus, D-penicillamine causes the excretion of copper but can also lead to the sequestration of free intracellular copper with metallothioneins[5]. D-penicillamine also has an anti-inflammatory effect, which is why it is a second-line drug in rheumatic diseases[90]. The standard dose of D-penicillamine is 20 mg/kg/d in 2-3 divided doses, it is usually started at a dose of 1000-1500 mg/d, and after liver functions improve it can be reduced to a maintenance dose of 10-15 mg/kg/d or 750-1000 mg/d[8]. Around 40%-70% of D-penicillamine is absorbed in the proximal intestine, and peak plasma concentration is reached within 1-3 h. After long-standing treatment, it is eliminated within 4-6 d (slow pool of drug bound reversibly to the tissue). Eighty percent of the drug circulates in bound form with D-penicillamine and the rest in free form. The drug is eliminated *via* the kidneys[91]. In neurohepatic WD, the dictum is to start at a low dose and increase slowly. Indian National Association for the Study of the Liver (INASL) recommends starting D-penicillamine at 250 mg on alternate days and increasing by 250 mg every 2-3 wk, until the maximum dose of 1000-1500 mg per day, while keeping a watch on neurological symptoms

[6]. As D-penicillamine causes depletion of pyridoxine by direct effect, pyridoxine needs to be supplemented at a dose of 25-40 mg per day. D-penicillamine should be given either 1 hour before or 2 h after meals, as it may get bound to the copper in the diet before its absorption into the intestine[92]. Antacids and iron significantly decrease its absorption[8]. Brewer *et al*[93] showed that fecal excretion of copper is significantly less with penicillamine and zinc combination than with zinc alone.

Adverse effects of D-penicillamine are acute (early onset) and late-onset

Acute adverse effects include hypersensitivity reaction, in the form of fever, rash, urticaria, arthralgia, proteinuria and leukopenia, usually within the initial 3 wk of starting D-penicillamine, seen in up to 15% of patients[6,94]. Delayed hypersensitivity include pemphigus, lupus-like syndrome[6]. Other delayed adverse effects include various organ systems, such as hematological (both idiosyncratic and dose-dependent marrow suppression), renal (membranous glomerulonephritis, and neurological (worsening of neurological symptoms). Renal and hematological adverse effects have already been discussed in the article earlier. In case of early hypersensitivity reactions, the drug is to be stopped immediately. In case of isolated dermatological involvement, it may be re-started under steroid cover[6]. Other dermatological adverse effects include dermatopathies due to elastic fibre abnormalities *e.g.* elastosis perforans serpiginosa, and pseudo-pseudoxanthoma elasticum. About 15%-30% of patients develop these adverse effects due to high doses and a long duration of drug intake. These skin changes are caused due to inhibition of the aldol crosslinking of tropocollagen and take months to years to manifest (as it takes a long time for new weakened collagen to be synthesized)[95]. Penicillamine-induced autoimmune dermatoses include pemphigus, epidermolysis bullosa and lupus-erythematosus-like syndrome. Immune-mediated toxicities like Goodpasture syndrome, systemic lupus erythematosus, and nephrotic syndrome warrant immediate drug withdrawal[8,58] (Table 5).

In some cases, paradoxical neurological worsening is seen after starting d-penicillamine, likely due to peripheral mobilization of copper from the liver to the bloodstream and its subsequent deposition in CNS, seen in 11%-50%[84,86,96,97]. Large-scale comparative studies should be done to study the effect of chelators on neurological WD by correlating the dose of chelators with MRI changes and copper transporting molecules by *in vivo* studies. Reversal of neurologic deterioration was seen in only 50% of those affected as D-penicillamine was being continued[84]. In another retrospective study, partial/complete reversal was seen in 53% (8/15) and a further partial response in 13% (2/15) of cases during 9.2 ± 5.2 mo[86]. Thus, the solution to this problem is to start at a low dose and increase it gradually.

Trientine

It is also known as triethylenetetramine. It is an alternative to D-penicillamine for chelation, particularly for those who do not tolerate D-penicillamine. One molecule of trientine combines with copper in a 1:1 ratio to form a stable complex, which is excreted in the urine. Trientine dihydrochloride is the oral preparation, which requires cold storage at 2^0-8^0 Celsius to maintain its stability, failure to maintain cold chain is a common cause of drug discontinuation[98]. Since 2018, trientine is also available as trientine tetrahydrochloride. Woimant *et al*[99] showed that there was no difference in efficacy of the two drugs and in terms of adverse effects, there was a case of recurrence of lupus erythematosus-like syndrome with trientine dihydrochloride. Trientine tetrahydrochloride is stable at room temperature, has slightly more rapid absorption and higher bioavailability and greater systemic exposure. Also, it is a cheaper alternative. It is poorly absorbed with a bioavailability of 8-30%. It reaches maximum plasma concentration in 1-3 h[100,101]. Plasma concentration of trientine is significantly reduced when given after food[95]. Trientine has fewer side effects. Pancytopenia is rarely caused. It should not be given with oral iron because trientine-iron complexes are toxic. There are no hypersensitivity reactions reported. Other minor adverse effects include hemorrhagic gastritis, loss of taste and nausea, sideroblastic anemia and allergic rash[6,102,103]. In a multicenter retrospective study of 77 patients with WD, where patients were treated with trientine for an average duration of 8 years (range: 5 mo to 32.5 years), 49.4% had improved hepatic functions, 10.4% of patients remained unchanged, 5.2% showed worsening, and remaining were asymptomatic to begin with. Twenty-two percent of patients had trientine-associated minor adverse effects, with only one patient requiring treatment discontinuation due to anaemia[104]. Neurologic deterioration is reported with trientine, hence, it should be started at a low dose and increased gradually in patients having neurologic manifestations[8]. In a large retrospective cohort of 471 patients (326 receiving D-penicillamine vs. 141 receiving trientine), it was shown that hepatic and neurologic improvement was comparable in either group. Stable neurologic disease as first-line drug was comparable in either group (27.2% for D-penicillamine *vs* 20% for trientine). A higher rate of neurologic worsening was reported with trientine as the first-line agent (20% *vs* 5.3%)[105]. In another randomized control trial among neurologic WD patients, for comparison between tetrathiomolybdate, 6/23 (26%) in trientine had neurologic worsening as compared to 1/23 in the tetrathiomolybdate group[106]. The dose is 750-1000 mg per day or 20 mg/kg/d in 3 divided doses. It is to be given 1 h before or 2 h after food[100].

Zinc

It induces synthesis of metallothioneins, and thereby promotes copper-binding to metallothionein in the enterocyte and ultimately hinders its absorption (as copper is lost when the enterocyte is shed). It is a slow de-coppering agent, it decreases the copper absorption but doesn't lead to a sudden massive increase in free copper, hence, it is of choice in neurological WD. However, because of the slow reduction in copper, it is not suitable for treating florid symptoms[8]. Disease may show progression in the initial few months, because of its slow onset of action[106]. In the first pediatric study, in which trientine was used as the initial chelator, it was shown that once adequate chelation was achieved, zinc combination therapy and subsequently zinc monotherapy maintained normal ALT/AST levels[102]. Among those having weight < 50 kg, it is to be given in a dose of 25 mg thrice a day and in those weighing > 50 kg, 50 mg thrice a day[6].

Various zinc preparations are available; zinc acetate, zinc gluconate, and zinc sulfate. There is no statistically significant difference in various zinc preparations in terms of improvement of liver function[107]. There are few adverse effects, gastric irritation is the most common side effect, in 30%-40% of patients. It can also cause an asymptomatic elevation in amylase and lipase[8]. It is recommended to use zinc in pre-symptomatic patients or in the maintenance phase of treatment in symptomatic patients[8]. In a systematic review and meta-analysis, zinc showed better improvement as compared to D-penicillamine in neurologic WD, however, there was no difference in hepatic WD. Also, the incidence of adverse effects and neurologic deterioration was higher with D-penicillamine as compared to zinc (RR: 2.42, 95%CI: 1.20%–4.88%; P = 0.014) and RR: 1.96, 95%CI: 1.31%–2.93%; P = 0.001, respectively)[108].

Ammonium tetra-thiomolybdate

It acts by forming a tripartite complex with copper and protein, which is stable. It binds the copper present in food and prevents its absorption. When given without food, it is absorbed into the blood and forms complexes with copper bound to albumin, thus preventing its deposition in various organs[109]. Also, it enters the blood-brain barrier and enters neuronal cells[110]. In an open-label study, 55 neurologic WD were treated with tetrathiomolybdate (120-410 mg for 8 wk), followed by zinc maintenance therapy. Only 3.6% (2/55) showed neurologic deterioration. Among the other adverse effects, 5/22 (23%) of treatment-naïve patients had bone marrow suppression and 3/22 (14%) had an elevation in liver enzymes and both these adverse effects quickly responded to drug dose reduction[111]. Bone marrow suppression is caused by the depletion of copper and is reversed by decreasing the dose of tetrathiomolybdate. The rise in the liver enzymes could be due to the mobilization of hepatic copper (from hepatic pools including metallothioneins) in a heavily copper-loaded liver, which is reversible on dose reduction[111]. In a subsequent double-blind RCT between tetrathiomolybdate (120 mg per day) and trientine (1000 mg/d) among 48 patients of neurologic WD, as mentioned above, tetrathiomolybdate was better than trientine, only 4% worsened neurologically *vs* 26% in trientine arm[106]. Ammonium tetrathiomolybdate is unstable for routine use, bis-choline tetrathiomolybdate is a more stable complex and has a better availability. Recently, bis-choline tetrathiomolybdate underwent a phase II trial among 28 neurologic WD, where it was given for a span of 24 wk, with the target to achieve a primary end-point of normal value of non-ceruloplasmin bound $\text{copper}_{\text{corrected}}$ ($\text{NCC}_{\text{corrected}}$ stands for NCC corrected for copper contained in tetrathiomolybdate-copper-albumin complexes) or achievement of 25% reduction from baseline $\text{NCC}_{\text{corrected}}$. Twenty-two patients completed the study up to 24 wk and by week 24, 20 (71%) had achieved the primary end-point (treatment success) accompanied by improvement in neurologic status (without any paradoxical neurological worsening, being reported). There were 11 (25%) serious adverse effects which included 6 events of psychiatric disorders in 4 patients, gait disturbance in one patient and two events of raised aminotransferases, one agranulocytosis and a decline in neurologic functioning[112]. Also, Brewer *et al*[113] analysed free copper levels in patients treated with tetrathiomolybdate *vs* trientine and found that the mean free copper was significantly less in the tetrathiomolybdate group at week 4 and week 8 of treatment. Thus, tetrathiomolybdate is a fast copper-lowering agent with minimal adverse effects. The dose recommended is initially 120 mg per day for the first 2 wk, as, 20 mg thrice a day with meals and 20 mg daily between meals followed by 60 mg daily as 10 mg thrice a day with meals and 10 mg thrice daily between meals[113]. Currently, a phase 3 trial is going on and results are keenly awaited (NCT03403205).

COMPARISON BETWEEN VARIOUS CHELATORS

D-penicillamine vs trientine

In a large retrospective cohort of median follow-up of 13.3 years, both D-penicillamine and trientine were comparable in terms of improvement in hepatic WD (> 90% cases) as well as in neurologic WD (> 55% cases) while neurologic worsening was more common in trientine group as compared to D-penicillamine group (20% *vs* 5.3%, P = 0.042). Treatment discontinuation due to adverse effects was more common in the D-penicillamine group as compared to trientine (28.8% *vs* 7.1%, P = 0.039)[105]. In the most recent randomized open-label non-inferiority trial of D-penicillamine *vs* trientine, for maintenance therapy after 1 year of chelation with D-penicillamine, trientine was found non-inferior to D-penicillamine[114].

D-penicillamine vs zinc

There are studies to show that zinc was found to be more effective in ameliorating neurological symptoms in 90% of patients in the zinc arm as compared to 25% alone in the D-penicillamine arm[115]. In a head-to-head comparison between D-penicillamine and zinc, among 67 new patients, of whom the majority were asymptomatic or had neurological disease, 44% discontinued D-penicillamine owing to its adverse effects as compared to 12% in the zinc arm[116]. In Another retrospective study of 288 patients for a period of 17.1 years by Weiss *et al*[117] showed that zinc monotherapy led to 15.9% (14/88) hepatic treatment failure as compared to 1.2% (4/313) in the D-penicillamine group without any statistically significant difference in the adverse effects in either group. Further, these zinc non-responders responded to chelators (either D-penicillamine or trientine). This supports the use of chelators in asymptomatic patients with active disease.

Dhawan *et al*[118], Askari *et al*[119] and Santos *et al*[120] have shown favourable outcomes with combination therapy but earlier studies by Brewer *et al*[93] show no added advantage. A systematic review of 17 articles involving 1056 on combination therapy showed that combination therapies are significantly less effective than individual therapies (47.1 *vs* 78.6%)[121].

Adequacy of treatment

Treatment targets on chelators: Asymptomatic patients should remain asymptomatic and symptomatic patients should show improvement in liver functions in the initial 6-18 mo[8]. Patients with decompensation might take longer to improve. Patients are to be monitored clinically for symptom improvement/new symptom onset. Initially, LFTs are to be done, every 3 mo, and thereafter 3-6 mo depending on the disease severity[8]. Adequacy is monitored by monitoring liver function tests and by quantifying 24-h urine copper excretion or free copper estimation. Free copper estimation, is not a full-proof test if the ceruloplasmin is calculated by the immunologic method as it calculates both apoceruloplasmin and holoceruloplsmin[6]. Also, urinary copper excretion is to be interpreted carefully after taking proper treatment history. Urinary copper excretion may be high if chelation has been re-started after a period of non-adherence or it can be falsely low, in case of poor drug absorption or inadequate dosing itself. Another way to monitor adequacy is to measure REC.

For de-coppering agents, free copper is a marker of the adequacy of chelation, while for zinc, urine copper levels below a certain cut-off is recommended for good copper control (Table 1).

When to decrease chelation?

Transition to maintenance dose or zinc can be used once liver function improves. AASLD recommends a transition to maintenance after clinical and biochemical parameters improve (usually seen after 1 year of therapy). The drug of choice drug could be low-dose chelating agents or full-dose zinc. In a retrospective study of 31 symptomatic hepatic WD who were transitioned from D-penicillamine to zinc (28 due to financial constraints and 3 due to adverse effects), wherein the majority of patients belonged to Child's class C (54%) patients, the average duration of zinc therapy on follow-up was 363 (35–728) weeks. In the Child C cirrhosis group at presentation, who received D-penicillamine for 111 (2-230) weeks followed by zinc for 344 (41–652) weeks, 15 had significant improvement in liver function and disease severity scores [122]. In a prospective study of 44 hepatic WD patients, who received D-penicillamine plus zinc combination therapy for more than 2 years, and were in biochemical remission (defined by AST and ALT > 1.5 times upper limit of normal, serum albumin > 3.5 gm/dL and INR < 500 mcg/d and NCC < 15 mcg/dL, were shifted to zinc monotherapy and biochemical parameters were assessed on follow-up. They showed that 9/44 (20.4%) relapsed till the last follow-up[123]. More prospective studies are needed to establish the correct time of transition and establish guidelines for the same.

CONCLUSION

Wilson's disease is a treatable metabolic liver disease. Early diagnosis is imperative. Wilson's disease has varied manifestations. Certain disease manifestations need to be differentiated from drug-toxicities, *e.g.*, tubulopathies due to WD vs. D-penicillamine-induced nephrotoxicity and hypersplenism *vs* D-penicillamine-induced myelosuppression. Though there are difficulties in making the correct diagnosis, with the help of non-ceruloplasmin bound copper, relative exchangeable copper and newer methods to detect early Kayser-Fleischer ring (AS-OCT), diagnosis can be made in resource-limited conditions, where mutational analysis is cost-forbidding. Chelation remains the mainstay of treatment and is to be preferred in active disease whether symptomatic or asymptomatic. Regarding the transition to maintenance therapy, the exact timeline is not yet defined, but depends on the liver function and is to be decided on a case-to-case basis with close follow-up of the copper load in the body. Trientine has been shown to have a good clinical response in a recent clinical trial and its availability as tetrahydrochloride, which is cheaper and doesn't require cold storage gives hope in resource-limited conditions. Bis-choline tetrathiomolybdate is the new addition in the armamentarium, which rapidly decreases the free copper load and is undergoing phase III trials. In fulminant hepatic failure, plasmapheresis has shown (while awaiting liver transplantation) some hope.

FOOTNOTES

Author contributions: Ghosh U contributed to the literature review, analysis and drafting of the initial manuscript; Sen Sarma M contributed to the conception and design of the manuscript and critical revision of the initial manuscript; Samanta A contributed to the interpretation of the data and critical revision of the initial manuscript.

Conflict-of-interest statement: All authors declare no conflict of interest.

Country/Territory of origin: India

ORCID number: Upasana Ghosh 0009-0006-2060-6132; Moinak Sen Sarma 0000-0003-2015-4069; Arghya Samanta 0000-0002-1768-0263.

S-Editor: Liu JH
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Sandahl TD**, Laursen TL, Munk DE, Vilstrup H, Weiss KH, Ott P. The Prevalence of Wilson's Disease: An Update. *Hepatology* 2020; **71**: 722-732 [PMID: 31449670 DOI: 10.1002/hep.30911]

2 **Yachha SK**, Sharma BC, Khanduri A, Srivastava A. Current spectrum of hepatobiliary disorders in northern India. *Indian Pediatr* 1997; **34**: 885-890 [PMID: 9567550]

3 **Roberts EA**, Schilsky ML; Division of Gastroenterology and Nutrition, Hospital for Sick Children, Toronto, Ontario, Canada. A practice guideline on Wilson disease. *Hepatology* 2003; **37**: 1475-1492 [PMID: 12774027 DOI: 10.1053/jhep.2003.50252]

4 **Stremmel W**, Weiskirchen R. Therapeutic strategies in Wilson disease: pathophysiology and mode of action. *Ann Transl Med* 2021; **9**: 732 [PMID: 33987430 DOI: 10.21037/atm-20-3090]

5 **Ala A**, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet* 2007; **369**: 397-408 [PMID: 17276780 DOI: 10.1016/S0140-6736(07)60196-2]

6 **Nagral A**, Sarma MS, Matthai J, Kukkle PL, Devarbhavi H, Sinha S, Alam S, Bavdekar A, Dhiman RK, Eapen CE, Goyal V, Mohan N, Kandadai RM, Sathiyasekaran M, Poddar U, Sibal A, Sankaranarayanan S, Srivastava A, Thapa BR, Wadia PM, Yachha SK, Dhawan A. Wilson's Disease: Clinical Practice Guidelines of the Indian National Association for Study of the Liver, the Indian Society of Pediatric Gastroenterology, Hepatology and Nutrition, and the Movement Disorders Society of India. *J Clin Exp Hepatol* 2019; **9**: 74-98 [PMID: 30765941 DOI: 10.1016/j.jceh.2018.08.009]

7 **Wu F**, Wang J, Pu C, Qiao L, Jiang C. Wilson's disease: a comprehensive review of the molecular mechanisms. *Int J Mol Sci* 2015; **16**: 6419-6431 [PMID: 25803104 DOI: 10.3390/ijms16036419]

8 **Schilsky ML**, Roberts EA, Bronstein JM, Dhawan A, Hamilton JP, Rivard AM, Washington MK, Weiss KH, Zimbrean PC. A multidisciplinary approach to the diagnosis and management of Wilson disease: Executive summary of the 2022 Practice Guidance on Wilson disease from the American Association for the Study of Liver Diseases. *Hepatology* 2023; **77**: 1428-1455 [PMID: 36152019 DOI: 10.1002/hep.32805]

9 **Boga S**, Ala A, Schilsky ML. Hepatic features of Wilson disease. *Handb Clin Neurol* 2017; **142**: 91-99 [PMID: 28433114 DOI: 10.1016/B978-0-444-63625-6.00009-4]

10 **European Association for Study of Liver**. EASL Clinical Practice Guidelines: Wilson's disease. *J Hepatol* 2012; **56**: 671-685 [PMID: 22340672 DOI: 10.1016/j.jhep.2011.11.007]

11 **Walshe JM**; Clinical Investigations Standing Committee of the Association of Clinical Biochemists. Wilson's disease: the importance of measuring serum caeruloplasmin non-immunologically. *Ann Clin Biochem* 2003; **40**: 115-121 [PMID: 12662398 DOI: 10.1258/000456303763046021]

12 **Merle U**, Eisenbach C, Weiss KH, Tuma S, Stremmel W. Serum ceruloplasmin oxidase activity is a sensitive and highly specific diagnostic marker for Wilson's disease. *J Hepatol* 2009; **51**: 925-930 [PMID: 19720421 DOI: 10.1016/j.jhep.2009.06.022]

13 **Mak CM**, Lam CW, Tam S. Diagnostic accuracy of serum ceruloplasmin in Wilson disease: determination of sensitivity and specificity by ROC curve analysis among ATP7B-genotyped subjects. *Clin Chem* 2008; **54**: 1356-1362 [PMID: 18556333 DOI: 10.1373/clinchem.2008.103432]

14 **Lu X**, Li S, Zhang W, Lin Y, Lu Z, Cai Y, Su X, Shao Y, Liu Z, Sheng H, Huang Y, Liu L, Zeng C. Assessment of the diagnostic value of serum ceruloplasmin for Wilson's disease in children. *BMC Gastroenterol* 2022; **22**: 124 [PMID: 35296237 DOI: 10.1186/s12876-022-02186-0]

15 **Ferenci P**, Członkowska A, Merle U, Ferenc S, Gromadzka G, Yurdaydin C, Vogel W, Bruha R, Schmidt HT, Stremmel W. Late-onset Wilson's disease. *Gastroenterology* 2007; **132**: 1294-1298 [PMID: 17433323 DOI: 10.1053/j.gastro.2007.02.057]

16 **Lackner C**, Denk H. Histopathology of Wilson disease. In: Kerkar N, Roberts E, editors. Clinical and translational perspectives on Wilson disease. New York: Academic Press; 2018; 257-270 [DOI: 10.1016/B978-0-12-810532-0.00023-9]

17 **Jain S**, Scheuer PJ, Archer B, Newman SP, Sherlock S. Histological demonstration of copper and copper-associated protein in chronic liver diseases. *J Clin Pathol* 1978; **31**: 784-790 [PMID: 80410 DOI: 10.1136/jcp.31.8.784]

18 **Pilloni L**, Lecca S, Van Eyken P, Flore C, Demelia L, Pilleri G, Nurchi AM, Farci AM, Ambu R, Callea F, Faa G. Value of histochemical stains for copper in the diagnosis of Wilson's disease. *Histopathology* 1998; **33**: 28-33 [PMID: 9726045 DOI: 10.1046/j.1365-2559.1998.00455.x]

19 **Goldfischer S**, Sternlieb I. Changes in the distribution of hepatic copper in relation to the progression of Wilson's disease (hepatolenticular degeneration). *Am J Pathol* 1968; **53**: 883-901 [PMID: 4177374]

20 **Mounajjed T**, Oxentenko AS, Qureshi H, Smyrk TC. Revisiting the topic of histochemically detectable copper in various liver diseases with special focus on venous outflow impairment. *Am J Clin Pathol* 2013; **139**: 79-86 [PMID: 23270902 DOI: 10.1309/AJCPDZR4OHDQNG3L]

21 **Woimant F**, Djebrani-Oussedik N, Poujois A. New tools for Wilson's disease diagnosis: exchangeable copper fraction. *Ann Transl Med* 2019; **7**: S70 [PMID: 31179307 DOI: 10.21037/atm.2019.03.02]

22 **McMillin GA**, Travis JJ, Hunt JW. Direct measurement of free copper in serum or plasma ultrafiltrate. *Am J Clin Pathol* 2009; **131**: 160-165 [PMID: 19141375 DOI: 10.1309/AJCP7Z9KBFINVGYF]

23 **Rodriguez-Castro KI**, Hevia-Urrutia FJ, Sturniolo GC. Wilson's disease: A review of what we have learned. *World J Hepatol* 2015; **7**: 2859-2870 [PMID: 26692151 DOI: 10.4254/wjh.v7.i29.2859]

24 **Poujois A**, Poupon J, Woimant F. Direct determination of non-ceruloplasmin-bound copper in plasma. In: Kerkar N, Eve A. Clinical and translational perspectives on Wilson disease. Elsevier, 2019; 249-255

25 **Poujois A**, Trocello JM, Djebrani-Oussedik N, Poupon J, Collet C, Girardot-Tinant N, Sobesky R, Habès D, Debray D, Vanlemmens C, Fluchère F, Ory-Magne F, Labreuche J, Preda C, Woimant F. Exchangeable copper: a reflection of the neurological severity in Wilson's disease. *Eur J Neurol* 2017; **24**: 154-160 [PMID: 27739240 DOI: 10.1111/ene.13171]

26 **El Balkhi S**, Trocello JM, Poupon J, Chappuis P, Massicot F, Girardot-Tinant N, Woimant F. Relative exchangeable copper: a new highly sensitive and highly specific biomarker for Wilson's disease diagnosis. *Clin Chim Acta* 2011; **412**: 2254-2260 [PMID: 21878323 DOI: 10.1016/j.cca.2011.08.019]

27 **Trocello JM**, El Balkhi S, Woimant F, Girardot-Tinant N, Chappuis P, Lloyd C, Poupon J. Relative exchangeable copper: a promising tool for family screening in Wilson disease. *Mov Disord* 2014; **29**: 558-562 [PMID: 24375554 DOI: 10.1002/mds.25763]

28 **Nagral A**, Jhaveri A, Nalawade S, Momaya N, Chakkarwar V, Malde P. Kayser-Fleischer rings or bile pigment rings? *Indian J Gastroenterol* 2015; **34**: 410-412 [PMID: 26541339 DOI: 10.1007/s12664-015-0603-2]

29 **Sethi M**, Madan S, Beri S. Fading Kayser-Fleischer ring revisited. *Saudi J Ophthalmol* 2021; **35**: 146-148 [PMID: 35391817 DOI: 10.4103/1319-4534.337854]

30 **Taly AB**, Meenakshi-Sundaram S, Sinha S, Swamy HS, Arunodaya GR. Wilson disease: description of 282 patients evaluated over 3 decades. *Medicine (Baltimore)* 2007; **86**: 112-121 [PMID: 17435591 DOI: 10.1097/MD.0b013e318045a00e]

31 **Sridhar MS**, Rangaraju A, Anbarasu K, Reddy SP, Daga S, Jayalakshmi S, Shaik B. Evaluation of Kayser-Fleischer ring in Wilson disease by anterior segment optical coherence tomography. *Indian J Ophthalmol* 2017; **65**: 354-357 [PMID: 28573989 DOI: 10.4103/ijo.IJO_400_16]

32 **Broniek-Kowalik K**, Dzieżyc K, Litwin T, Członkowska A, Szaflik JP. Anterior segment optical coherence tomography (AS-OCT) as a new method of detecting copper deposits forming the Kayser-Fleischer ring in patients with Wilson disease. *Acta Ophthalmol* 2019; **97**: e757-e760 [PMID: 30635971 DOI: 10.1111/aos.14009]

33 **Korman JD**, Volenberg I, Balko J, Webster J, Schiodt FV, Squires RH Jr, Fontana RJ, Lee WM, Schilsky ML; Pediatric and Adult Acute Liver Failure Study Groups. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. *Hepatology* 2008; **48**: 1167-1174 [PMID: 18798336 DOI: 10.1002/hep.22446]

34 **Shaver WA**, Bhatt H, Combes B. Low serum alkaline phosphatase activity in Wilson's disease. *Hepatology* 1986; **6**: 859-863 [PMID: 3758940 DOI: 10.1002/hep.1840060509]

35 **Sinha S**, Taly AB, Ravishankar S, Prashanth LK, Venugopal KS, Arunodaya GR, Vasudev MK, Swamy HS. Wilson's disease: cranial MRI observations and clinical correlation. *Neuroradiology* 2006; **48**: 613-621 [PMID: 16752136 DOI: 10.1007/s00234-006-0101-4]

36 **Stefano Zagami A**, Boers PM. Disappearing "face of the giant panda". *Neurology* 2001; **56**: 665 [PMID: 11245722 DOI: 10.1212/WNL.56.5.665]

37 **Akhan O**, Akpinar E, Oto A, Köroglu M, Ozmen MN, Akata D, Bijan B. Unusual imaging findings in Wilson's disease. *Eur Radiol* 2002; **12** Suppl 3: S66-S69 [PMID: 12522607 DOI: 10.1007/s00330-002-1589-6]

38 **Barbosa ER**, Caramelli P, Bacheschi LA, Haddad MS, Magalhães AC, Menezes JR, Scaff M, Canelas HM. Wilson's disease: magnetic resonance imaging (MRI) with clinical correlations in 16 cases. *Rev Paul Med* 1993; **111**: 407-411 [PMID: 8108634]

39 **Chang IJ**, Hahn SH. The genetics of Wilson disease. *Handb Clin Neurol* 2017; **142**: 19-34 [PMID: 28433102 DOI: 10.1016/B978-0-444-63625-6.00003-3]

40 **Merle U**, Schaefer M, Ferenci P, Stremmel W. Clinical presentation, diagnosis and long-term outcome of Wilson's disease: a cohort study. *Gut* 2007; **56**: 115-120 [PMID: 16709660 DOI: 10.1136/gut.2005.087262]

41 **Ferenci P**, Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142 [PMID: 12955875 DOI: 10.1034/j.1600-0676.2003.00824.x]

42 **Sözeri E**, Feist D, Ruder H, Schärer K. Proteinuria and other renal functions in Wilson's disease. *Pediatr Nephrol* 1997; **11**: 307-311 [PMID: 9203178 DOI: 10.1007/s004670050282]

43 **Litin RB**, Randall RV, Goldstein NP, Power MH, Diessner GR. Hypercalciuria in hepatolenticular degeneration (Wilson's disease). *Am J Med Sci* 1959; **238**: 614-620 [PMID: 14417517]

44 **Reynolds HV**, Talekar CR, Bellapart J, Leggett BA, Boots RJ. Copper removal strategies for Wilson's disease crisis in the ICU. *Anaesth Intensive Care* 2014; **42**: 253-257 [PMID: 24580393]

45 **Mensing B**, Nowak A, Zweifel S, Terracciano L, Bernsmeier C, Filipowicz Sinnreich M. [Wilson's disease or hepatolenticular degeneration]. *Ther Umsch* 2018; **75**: 241-248 [PMID: 30468117 DOI: 10.1024/0040-5930/a000995]

46 **Leu ML**, Strickland GT, Gutman RA. Renal function in Wilson's disease: response to penicillamine therapy. *Am J Med Sci* 1970; **260**: 381-398 [PMID: 5511397 DOI: 10.1097/00000441-197012000-00007]

47 **Zhuang XH**, Mo Y, Jiang XY, Chen SM. Analysis of renal impairment in children with Wilson's disease. *World J Pediatr* 2008; **4**: 102-105 [PMID: 18661763 DOI: 10.1007/s12519-008-0019-5]

48 **Kapoor N**, Cherian KE, Sajith KG, Thomas M, Eapen CE, Thomas N, Paul TV. Renal Tubular Function, Bone Health and Body Composition in Wilson's Disease: A Cross-Sectional Study from India. *Calcif Tissue Int* 2019; **105**: 459-465 [PMID: 31317233 DOI: 10.1007/s00223-019-00588-z]

49 **Wolff SM**. Renal Lesions in Wilson's Disease. *Lancet* 1964; **1**: 843-845 [PMID: 14129796 DOI: 10.1016/s0140-6736(64)91573-9]

50 **Elsas LJ**, Hayslett JP, Spargo BH, Durant JL, Rosenberg LE. Wilson's disease with reversible renal tubular dysfunction. Correlation with proximal tubular ultrastructure. *Ann Intern Med* 1971; **75**: 427-433 [PMID: 5315082 DOI: 10.7326/0003-4819-75-3-427]

51 **Azizi E**, Eshel G, Aladjem M. Hypercalciuria and nephrolithiasis as a presenting sign in Wilson disease. *Eur J Pediatr* 1989; **148**: 548-549 [PMID: 2744017 DOI: 10.1007/BF00441555]

52 **Palkar AV**, Shrivastava MS, Padwal NJ, Padhiyar RN, Moulick N. Renal tubular acidosis due to Wilson's disease presenting as metabolic bone disease. *BMJ Case Rep* 2011; **2011** [PMID: 22688476 DOI: 10.1136/bcr.04.2011.4121]

53 **Gündüz Z**, Düşünsel R, Anarat A. Wilson cirrhosis associated with membranoproliferative glomerulonephritis. *Nephron* 1996; **74**: 497-498 [PMID: 8893211 DOI: 10.1159/000189412]

54 **Acharya R**, Zeng X, Clapp WL, Upadhyay K. IgA vasculitis with nephritis in cirrhotic Wilson disease: Is there an association? *Clin Nephrol Case Stud* 2020; **8**: 80-84 [PMID: 33062584 DOI: 10.5414/CNCS110268]

55 **Begum A**, Rahman MA, Akter AM, Akter M, Islam MA, Uddin GM. Nephrotic Syndrome after Treatment with D-Penicillamine in a Patient with Wilson's Disease- A Case Report. J Ped Nephrol [Internet]. 2016 Jul. 4 [cited 2023 Sep. 22]; 4: 70-73

56 **Davison AM**, Day AT, Golding JR, Thomson D. Effect of penicillamine on the kidney. *Proc R Soc Med* 1977; **70** Suppl 3: 109-113 [PMID: 122655]

57 **Mao H**, Qin J, Kang T, Ou S. Wilson disease and IgA nephropathy: accidental or related? *Int Urol Nephrol* 2022; **54**: 2441-2443 [PMID: 35260977 DOI: 10.1007/s11255-022-03159-8]

58 **Litwin T**, Członkowska A, Socha P. Oral chelator treatment of Wilson Disease: d-penicillamine. InClinical and translational perspectives on Wilson disease 2019; 357-364

59 **Hall CL**, Jawad S, Harrison PR, MacKenzie JC, Bacon PA, Klouda PT, MacIver AG. Natural course of penicillamine nephropathy: a long term study of 33 patients. *Br Med J (Clin Res Ed)* 1988; **296**: 1083-1086 [PMID: 3132218 DOI: 10.1136/bmj.296.6629.1083]

60 **Habib GS**, Saliba W, Nashashibi M, Armali Z. Penicillamine and nephrotic syndrome. *Eur J Intern Med* 2006; **17**: 343-348 [PMID: 16864010 DOI: 10.1016/j.ejim.2006.03.001]

61 **Sternlieb I**. Penicillamine and the nephrotic syndrome. *JAMA* 1966; **198**: 1311-1312 [PMID: 5953529 DOI: 10.1001/jama.198.12.1311]

62 **Jaffe IA**, Treser G, Suzuki Y, Ehrenreich T. Nephropathy induced by D-penicillamine. *Ann Intern Med* 1968; **69**: 549-556 [PMID: 4175137

DOI: 10.7326/0003-4819-69-3-549]
63 **Neild GH**, Gärtner HV, Bohle A. Letter: D-penicillamine-induced membranous glomerulonephritis. *Lancet* 1975; **1**: 1201-1202 [PMID: 48830 DOI: 10.1016/S0140-6736(75)93197-9]
64 **Bacon PA**, Tribe CR, Mackenzie JC, Verrier-Jones J, Cumming RH, Amer B. Penicillamine nephropathy in rheumatoid arthritis. A clinical, pathological and immunological study. *Q J Med* 1976; **45**: 661-684 [PMID: 1005658]
65 **Jaffe IA**. Induction of auto-immune syndromes by penicillamine therapy in rheumatoid arthritis and other diseases. *Springer Semin Immunopathol* 1981; **4**: 193-207 [PMID: 7034252 DOI: 10.1007/BF01857095]
66 **Walshe JM**. Management of penicillamine nephropathy in Wilson's disease: a new chelating agent. *Lancet* 1969; **2**: 1401-1402 [PMID: 4188283 DOI: 10.1016/S0140-6736(69)90940-4]
67 **Siafakas CG**, Jonas MM, Alexander S, Herrin J, Furuta GT. Early onset of nephrotic syndrome after treatment with D-penicillamine in a patient with Wilson's disease. *Am J Gastroenterol* 1998; **93**: 2544-2546 [PMID: 9860423 DOI: 10.1111/j.1572-0241.1998.00715.x]
68 **Ross JH**, McGinty F, Brewer DG. Penicillamine nephropathy. *Nephron* 1980; **26**: 184-186 [PMID: 7432580 DOI: 10.1159/000181984]
69 **Hill H**, Hill A, Davison AM. Resumption of treatment with penicillamine after proteinuria. *Ann Rheum Dis* 1979; **38**: 229-231 [PMID: 485579 DOI: 10.1136/ard.38.3.229]
70 **Lee Y**, Lee ST, Cho H. D-penicillamine-induced ANA (+) ANCA (+) vasculitis in pediatric patients with Wilson's disease. *Clin Nephrol* 2016; **85**: 296-300 [PMID: 26784915 DOI: 10.5414/CN108763]
71 **Hoxha E**, Harendza S, Zahner G, Panzer U, Steinmetz O, Fechner K, Helmchen U, Stahl RA. An immunofluorescence test for phospholipase-A_2-receptor antibodies and its clinical usefulness in patients with membranous glomerulonephritis. *Nephrol Dial Transplant* 2011; **26**: 2526-2532 [PMID: 21633097 DOI: 10.1093/ndt/gfr247]
72 **Kumar RP**, Prasad ND, Tirumavalavan S, Fernando ME. D-penicillamine-induced membranous nephropathy. *Indian J Nephrol* 2014; **24**: 195-196 [PMID: 25120302 DOI: 10.4103/0971-4065.132024]
73 **DeSilva RN**, Eastmond CJ. Management of proteinuria secondary to penicillamine therapy in rheumatoid arthritis. *Clin Rheumatol* 1992; **11**: 216-219 [PMID: 1617896 DOI: 10.1007/BF02207960]
74 **Walshe JM**. The liver in Wilson's disease. In: Schiff L, Schiff ER, editors. Diseases of the Liver. 6th ed. Philadelphia: Lippincott; 1987; 1037-1050
75 **Proost R**, Cassiman D, Levtchenko E, Morava-Kozicz E, Neirynck J, Witters P. Fulminant Wilson Disease in Children: Recovery After Plasma Exchange Without Transplantation. *J Pediatr Gastroenterol Nutr* 2020; **71**: 720-725 [PMID: 32804913 DOI: 10.1097/MPG.0000000000002894]
76 **Steen VD**, Blair S, Medsger TA Jr. The toxicity of D-penicillamine in systemic sclerosis. *Ann Intern Med* 1986; **104**: 699-705 [PMID: 2938530 DOI: 10.7326/0003-4819-104-5-699]
77 **Jaffe IA**. Adverse effects profile of sulfhydryl compounds in man. *Am J Med* 1986; **80**: 471-476 [PMID: 2937293 DOI: 10.1016/0002-9343(86)90722-9]
78 **Day AT**, Golding JR, Lee PN, Butterworth AD. Penicillamine in rheumatoid disease: a long-term study. *Br Med J* 1974; **1**: 180-183 [PMID: 4590670 DOI: 10.1136/bmj.1.5900.180]
79 **Kay AG**. Myelotoxicity of D-penicillamine. *Ann Rheum Dis* 1979; **38**: 232-236 [PMID: 485580 DOI: 10.1136/ard.38.3.232]
80 **Socha P**, Janczyk W, Dhawan A, Baumann U, D'Antiga L, Tanner S, Iorio R, Vajro P, Houwen R, Fischler B, Dezsofi A, Hadzic N, Hierro L, Jahnel J, McLin V, Nobili V, Smets F, Verkade HJ, Debray D. Wilson's Disease in Children: A Position Paper by the Hepatology Committee of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2018; **66**: 334-344 [PMID: 29341979 DOI: 10.1097/MPG.0000000000001787]
81 **Taly AB**, Prashanth LK, Sinha S. Wilson's disease: An Indian perspective. *Neurol India* 2009; **57**: 528-540 [PMID: 19934550 DOI: 10.4103/0028-3886.57789]
82 **Ferenci P**, Stremmel W, Członkowska A, Szalay F, Viveiros A, Stättermayer AF, Bruha R, Houwen R, Pop TL, Stauber R, Gschwantler M, Pfeiffenberger J, Yurdaydin C, Aigner E, Steindl-Munda P, Dienes HP, Zoller H, Weiss KH. Age and Sex but Not ATP7B Genotype Effectively Influence the Clinical Phenotype of Wilson Disease. *Hepatology* 2019; **69**: 1464-1476 [PMID: 30232804 DOI: 10.1002/hep.30280]
83 **Dusek P**, Litwin T, Członkowska A. Neurologic impairment in Wilson disease. *Ann Transl Med* 2019; **7**: S64 [PMID: 31179301 DOI: 10.21037/atm.2019.02.43]
84 **Brewer GJ**, Terry CA, Aisen AM, Hill GM. Worsening of neurologic syndrome in patients with Wilson's disease with initial penicillamine therapy. *Arch Neurol* 1987; **44**: 490-493 [PMID: 3579660 DOI: 10.1001/archneur.1987.00520170020016]
85 **Ranjan A**, Kalita J, Kumar V, Misra UK. MRI and oxidative stress markers in neurological worsening of Wilson disease following penicillamine. *Neurotoxicology* 2015; **49**: 45-49 [PMID: 26004675 DOI: 10.1016/j.neuro.2015.05.004]
86 **Litwin T**, Dzieżyc K, Karliński M, Chabik G, Czepiel W, Członkowska A. Early neurological worsening in patients with Wilson's disease. *J Neurol Sci* 2015; **355**: 162-167 [PMID: 26071888 DOI: 10.1016/j.jns.2015.06.010]
87 **Chen DB**, Feng L, Lin XP, Zhang W, Li FR, Liang XL, Li XH. Penicillamine increases free copper and enhances oxidative stress in the brain of toxic milk mice. *PLoS One* 2012; **7**: e37709 [PMID: 22629446 DOI: 10.1371/journal.pone.0037709]
88 **Miki M**. Penicillamine as antioxidant. *Methods Enzymol* 1994; **234**: 542-547 [PMID: 7808330 DOI: 10.1016/0076-6879(94)34126-5]
89 **Mohr I**, Weiss KH. Current anti-copper therapies in management of Wilson disease. *Ann Transl Med* 2019; **7**: S69 [PMID: 31179306 DOI: 10.21037/atm.2019.02.48]
90 **Klein D**, Lichtmannegger J, Heinzmann U, Summer KH. Dissolution of copper-rich granules in hepatic lysosomes by D-penicillamine prevents the development of fulminant hepatitis in Long-Evans cinnamon rats. *J Hepatol* 2000; **32**: 193-201 [PMID: 10707858 DOI: 10.1016/S0168-8278(00)80063-X]
91 **Netter P**, Bannwarth B, Péré P, Nicolas A. Clinical pharmacokinetics of D-penicillamine. *Clin Pharmacokinet* 1987; **13**: 317-333 [PMID: 3319347 DOI: 10.2165/00003088-198713050-00003]
92 **Shimizu N**, Yamaguchi Y, Aoki T. Treatment and management of Wilson's disease. *Pediatr Int* 1999; **41**: 419-422 [PMID: 10453198 DOI: 10.1046/j.1442-200x.1999.01093.x]
93 **Brewer GJ**, Yuzbasiyan-Gurkan V, Johnson V, Dick RD, Wang Y. Treatment of Wilson's disease with zinc: XI. Interaction with other anticopper agents. *J Am Coll Nutr* 1993; **12**: 26-30 [PMID: 8440814 DOI: 10.1080/07315724.1993.10718278]
94 **Levy RS**, Fisher M, Alter JN. Penicillamine: review and cutaneous manifestations. *J Am Acad Dermatol* 1983; **8**: 548-558 [PMID: 6222087 DOI: 10.1016/S0190-9622(83)70062-9]

95 **Ishak R**, Abbas O. Penicillamine revisited: historic overview and review of the clinical uses and cutaneous adverse effects. *Am J Clin Dermatol* 2013; **14**: 223-233 [PMID: 23605177 DOI: 10.1007/s40257-013-0022-z]

96 **Kalita J**, Kumar V, Chandra S, Kumar B, Misra UK. Worsening of Wilson disease following penicillamine therapy. *Eur Neurol* 2014; **71**: 126-131 [PMID: 24356057 DOI: 10.1159/000355276]

97 **Kumar M**, Murugan TP, Lionel AP, Thomas MM, Mannam P, Yoganathan S. Management of Children and Adolescents with Wilson Disease and Neurological Worsening Following D-Penicillamine Therapy: A Single Centre Experience. *Ann Indian Acad Neurol* 2022; **25**: 698-702 [PMID: 36211139 DOI: 10.4103/aian.aian_519_21]

98 **Lu J**. Triethylenetetramine pharmacology and its clinical applications. *Mol Cancer Ther* 2010; **9**: 2458-2467 [PMID: 20660601 DOI: 10.1158/1535-7163.MCT-10-0523]

99 **Woimant F**, Debray D, Morvan E, Obadia MA, Poujois A. Efficacy and Safety of Two Salts of Trientine in the Treatment of Wilson's Disease. *J Clin Med* 2022; **11** [PMID: 35887738 DOI: 10.3390/jcm11143975]

100 **Seetharaman J**, Sarma MS. Chelation therapy in liver diseases of childhood: Current status and response. *World J Hepatol* 2021; **13**: 1552-1567 [PMID: 34904029 DOI: 10.4254/wjh.v13.i11.1552]

101 **Weiss KH**, Thompson C, Dogterom P, Chiou YJ, Morley T, Jackson B, Amin N, Kamlin COF. Comparison of the Pharmacokinetic Profiles of Trientine Tetrahydrochloride and Trientine Dihydrochloride in Healthy Subjects. *Eur J Drug Metab Pharmacokinet* 2021; **46**: 665-675 [PMID: 34357516 DOI: 10.1007/s13318-021-00704-1]

102 **Arnon R**, Calderon JF, Schilsky M, Emre S, Shneider BL. Wilson disease in children: serum aminotransferases and urinary copper on triethylene tetramine dihydrochloride (trientine) treatment. *J Pediatr Gastroenterol Nutr* 2007; **44**: 596-602 [PMID: 17460493 DOI: 10.1097/MPG.0b013e3180467715]

103 **Taylor RM**, Chen Y, Dhawan A; EuroWilson Consortium. Triethylene tetramine dihydrochloride (trientine) in children with Wilson disease: experience at King's College Hospital and review of the literature. *Eur J Pediatr* 2009; **168**: 1061-1068 [PMID: 19066958 DOI: 10.1007/s00431-008-0886-8]

104 **Weiss KH**, Kruse C, Manolaki N, Zuin M, Ferenci P, van Scheppingen D, Wijnberg L, de Koning CE, Dhawan A. Multicentre, retrospective study to assess long-term outcomes of chelator based treatment with trientine in Wilson disease patients withdrawn from therapy with d -penicillamine. *Eur J Gastroenterol Hepatol* 2022; **34**: 940-947 [PMID: 35482910 DOI: 10.1097/MEG.0000000000002387]

105 **Weiss KH**, Thurik F, Gotthardt DN, Schäfer M, Teufel U, Wiegand F, Merle U, Ferenci-Foerster D, Maieron A, Stauber R, Zoller H, Schmidt HH, Reuner U, Hefter H, Trocello JM, Houwen RH, Ferenci P, Stremmel W; EUROWILSON Consortium. Efficacy and safety of oral chelators in treatment of patients with Wilson disease. *Clin Gastroenterol Hepatol* 2013; **11**: 1028-35.e1 [PMID: 23542331 DOI: 10.1016/j.cgh.2013.03.012]

106 **Brewer GJ**, Askari F, Lorincz MT, Carlson M, Schilsky M, Kluin KJ, Hedera P, Moretti P, Fink JK, Tankanow R, Dick RB, Sitterly J. Treatment of Wilson disease with ammonium tetrathiomolybdate: IV. Comparison of tetrathiomolybdate and trientine in a double-blind study of treatment of the neurologic presentation of Wilson disease. *Arch Neurol* 2006; **63**: 521-527 [PMID: 16606763 DOI: 10.1001/archneur.63.4.521]

107 **Camarata MA**, Ala A, Schilsky ML. Zinc Maintenance Therapy for Wilson Disease: A Comparison Between Zinc Acetate and Alternative Zinc Preparations. *Hepatol Commun* 2019; **3**: 1151-1158 [PMID: 31388634 DOI: 10.1002/hep4.1384]

108 **Tang S**, Bai L, Hou W, Hu Z, Chen X, Zhao J, Liang C, Zhang W, Duan Z, Zheng S. Comparison of the Effectiveness and Safety of d-Penicillamine and Zinc Salt Treatment for Symptomatic Wilson Disease: A Systematic Review and Meta-Analysis. *Front Pharmacol* 2022; **13**: 847436 [PMID: 35370752 DOI: 10.3389/fphar.2022.847436]

109 **Brewer GJ**, Dick RD, Yuzbasiyan-Gurkin V, Tankanow R, Young AB, Kluin KJ. Initial therapy of patients with Wilson's disease with tetrathiomolybdate. *Arch Neurol* 1991; **48**: 42-47 [PMID: 1986725 DOI: 10.1001/archneur.1991.00530130050019]

110 **Stremmel W**. Bis-choline Tetrathiomolybdate as Old Drug in a New Design for Wilson's Disease: Good for Brain and Liver? *Hepatology* 2019; **69**: 901-903 [PMID: 30070374 DOI: 10.1002/hep.30130]

111 **Brewer GJ**, Hedera P, Kluin KJ, Carlson M, Askari F, Dick RB, Sitterly J, Fink JK. Treatment of Wilson disease with ammonium tetrathiomolybdate: III. Initial therapy in a total of 55 neurologically affected patients and follow-up with zinc therapy. *Arch Neurol* 2003; **60**: 379-385 [PMID: 12633149 DOI: 10.1001/archneur.60.3.379]

112 **Weiss KH**, Askari FK, Czlonkowska A, Ferenci P, Bronstein JM, Bega D, Ala A, Nicholl D, Flint S, Olsson L, Plitz T, Bjartmar C, Schilsky ML. Bis-choline tetrathiomolybdate in patients with Wilson's disease: an open-label, multicentre, phase 2 study. *Lancet Gastroenterol Hepatol* 2017; **2**: 869-876 [PMID: 28988934 DOI: 10.1016/S2468-1253(17)30293-5]

113 **Brewer GJ**, Askari F, Dick RB, Sitterly J, Fink JK, Carlson M, Kluin KJ, Lorincz MT. Treatment of Wilson's disease with tetrathiomolybdate: V. Control of free copper by tetrathiomolybdate and a comparison with trientine. *Transl Res* 2009; **154**: 70-77 [PMID: 19595438 DOI: 10.1016/j.trsl.2009.05.002]

114 **Schilsky ML**, Czlonkowska A, Zuin M, Cassiman D, Twardowschy C, Poujois A, Gondim FAA, Denk G, Cury RG, Ott P, Moore J, Ala A, D'Inca R, Couchonnal-Bedoya E, D'Hollander K, Dubois N, Kamlin COF, Weiss KH; CHELATE trial investigators. Trientine tetrahydrochloride versus penicillamine for maintenance therapy in Wilson disease (CHELATE): a randomised, open-label, non-inferiority, phase 3 trial. *Lancet Gastroenterol Hepatol* 2022; **7**: 1092-1102 [PMID: 36183738 DOI: 10.1016/S2468-1253(22)00270-9]

115 **Medici V**, Trevisan CP, D'Incà R, Barollo M, Zancan L, Fagiuoli S, Martines D, Irato P, Sturniolo GC. Diagnosis and management of Wilson's disease: results of a single center experience. *J Clin Gastroenterol* 2006; **40**: 936-941 [PMID: 17063115 DOI: 10.1097/01.mcg.0000225670.91722.59]

116 **Czlonkowska A**, Gajda J, Rodo M. Effects of long-term treatment in Wilson's disease with D-penicillamine and zinc sulphate. *J Neurol* 1996; **243**: 269-273 [PMID: 8936358 DOI: 10.1007/BF00868525]

117 **Weiss KH**, Gotthardt DN, Klemm D, Merle U, Ferenci-Foerster D, Schaefer M, Ferenci P, Stremmel W. Zinc monotherapy is not as effective as chelating agents in treatment of Wilson disease. *Gastroenterology* 2011; **140**: 1189-1198.e1 [PMID: 21185835 DOI: 10.1053/j.gastro.2010.12.034]

118 **Dhawan A**, Taylor RM, Cheeseman P, De Silva P, Katsiyiannakis L, Mieli-Vergani G. Wilson's disease in children: 37-year experience and revised King's score for liver transplantation. *Liver Transpl* 2005; **11**: 441-448 [PMID: 15776453 DOI: 10.1002/lt.20352]

119 **Askari FK**, Greenson J, Dick RD, Johnson VD, Brewer GJ. Treatment of Wilson's disease with zinc. XVIII. Initial treatment of the hepatic decompensation presentation with trientine and zinc. *J Lab Clin Med* 2003; **142**: 385-390 [PMID: 14713890 DOI: 10.1016/S0022-2143(03)00157-4]

120 **Santos Silva EE**, Sarles J, Buts JP, Sokal EM. Successful medical treatment of severely decompensated Wilson disease. *J Pediatr* 1996; **128**:

285-287 [PMID: 8636833 DOI: 10.1016/S0022-3476(96)70412-2]

121 **Chen JC**, Chuang CH, Wang JD, Wang CW. Combination Therapy Using Chelating Agent and Zinc for Wilson's Disease. *J Med Biol Eng* 2015; **35**: 697-708 [PMID: 26692828 DOI: 10.1007/s40846-015-0087-7]

122 **Gupta P**, Choksi M, Goel A, Zachariah U, Sajith KG, Ramachandran J, Chandy G, Kurian G, Rebekah G, Eapen CE. Maintenance zinc therapy after initial penicillamine chelation to treat symptomatic hepatic Wilson's disease in resource-constrained setting. *Indian J Gastroenterol* 2018; **37**: 31-38 [PMID: 29457214 DOI: 10.1007/s12664-018-0829-x]

123 **Panda K**, Lal BB, Sood V, Khanna R, Alam S. Incidence of Relapse Following Withdrawal of D-Penicillamine from Combination (D-Penicillamine+ Zinc) Therapy in Previously Symptomatic Hepatic Wilson Disease. *Journal of Clinical and Experimental Hepatology* 2022; **12**: S80 [DOI: 10.1016/j.jceh.2023.07.222]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1127-1139

DOI: 10.4254/wjh.v15.i10.1127

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Liver disease epidemiology and burden in patients with alterations in plasma protein metabolism: German retrospective insurance claims analysis

Nils Picker, May Hagiwara, Severin Baumann, Ed G Marins, Thomas Wilke, Kaili Ren, Ulf Maywald, Chitra Karki, Pavel Strnad

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Gaspar R, Portugal; Ren WR, China; Zhang Y, China

Received: May 22, 2023
Peer-review started: May 22, 2023
First decision: July 8, 2023
Revised: August 11, 2023
Accepted: September 18, 2023
Article in press: September 18, 2023
Published online: October 27, 2023

Nils Picker, Severin Baumann, Real-World Evidence, Cytel Inc. Ingress-Health HWM GmbH, Wismar 23966, Germany

May Hagiwara, Chitra Karki, R&D, Global Evidence and Outcomes, Takeda Development Center Americas, Inc., Cambridge, MA 02139, United States

Ed G Marins, Global Medical Affairs, Takeda Development Center Americas, Inc., Cambridge, MA 02139, United States

Thomas Wilke, IPAM Institute, IPAM E.V., Wismar 23966, Germany

Kaili Ren, Statistics and Quantitative Sciences, Data Science Institute, Takeda Development Center Americas, Inc., Cambridge, MA 02139, United States

Ulf Maywald, Drug Department, AOK PLUS, Dresden 01058, Germany

Pavel Strnad, Medical Clinic III, Gastroenterology, Metabolic Diseases and Intensive Care, University Hospital RWTH Aachen, Aachen 52074, Germany

Corresponding author: Pavel Strnad, MD, Professor, Medical Clinic III, Gastroenterology, Metabolic Diseases and Intensive Care, University Hospital RWTH Aachen, 30 Pauwelsstraße, Aachen 52074, Germany. pstrnad@ukaachen.de

Abstract

BACKGROUND

Alpha-1 antitrypsin deficiency is a rare genetic disease and a leading cause of inherited alterations in plasma protein metabolism (APPM).

AIM

To understand the prevalence, burden and progression of liver disease in patients with APPM including alpha-1 antitrypsin deficiency.

METHODS

We conducted a retrospective analysis of anonymized patient-level claims data from a German health insurance provider (AOK PLUS). The APPM cohort comprised patients with APPM (identified using the German Modification of the

International Classification of Diseases-10th Revision [ICD-10-GM] code E88.0 between 01/01/2010-30/09/2020) and incident liver disease (ICD-10-GM codes K74, K70.2-3 and K71.7 between 01/01/2012-30/09/2020). The control cohort comprised patients without APPM but with incident liver disease. Outcomes were incidence/prevalence of liver disease in patients with APPM, demographics/baseline characteristics, diagnostic procedures, progression-free survival (PFS), disease progression and mortality.

RESULTS

Overall, 2680 and 26299 patients were included in the APPM (fibrosis, 96; cirrhosis, 2584) and control (fibrosis, 1444; cirrhosis, 24855) cohorts, respectively. Per 100000 individuals, annual incidence and prevalence of APPM and liver disease was 10-15 and 36-51, respectively. In the APPM cohort, median survival was 4.7 years [95% confidence interval (CI): 3.5-7.0] and 2.5 years (95%CI: 2.3-2.8) in patients with fibrosis and cirrhosis, respectively. A higher proportion of patients in the APPM cohort experienced disease progression (92.0%) compared with the control cohort (67.2%). Median PFS was shorter in the APPM cohort (0.9 years, 95%CI: 0.7-1.1) compared with the control cohort (3.7 years, 95%CI: 3.6-3.8; $P < 0.001$). Patients with cirrhosis in the control cohort had longer event-free survival for ascites, hepatic encephalopathy, hepatic failure and esophageal/gastric varices than patients with cirrhosis in the APPM cohort ($P < 0.001$). Patients with fibrosis in the control cohort had longer event-free survival for ascites, cirrhosis, hepatic failure and esophageal/gastric varices than patients with fibrosis in the APPM cohort ($P < 0.001$). In the APPM cohort, the most common diagnostic procedures within 12 mo after the first diagnosis of liver disease were imaging procedures (66.3%) and laboratory tests (51.0%).

CONCLUSION

Among patients with liver disease, those with APPM experience substantial burden and earlier liver disease progression than patients without APPM.

Key Words: Alpha-1 antitrypsin deficiency; Epidemiology; Germany; Liver diseases; Retrospective study

Core Tip: This was a retrospective analysis of anonymized, patient-level, insurance claims data from a German health insurance provider (AOK PLUS), which demonstrated that a diagnosis of alterations in plasma protein metabolism (APPM) (E88.0) in patients with liver disease was associated with a substantial burden and higher rate of liver disease progression compared with patients with liver disease but without APPM. To enable accurate diagnosis and inform disease management, it is important to have specific diagnostic codes that differentiate between genetic liver disease and liver manifestations from other causes.

Citation: Picker N, Hagiwara M, Baumann S, Marins EG, Wilke T, Ren K, Maywald U, Karki C, Strnad P. Liver disease epidemiology and burden in patients with alterations in plasma protein metabolism: German retrospective insurance claims analysis. *World J Hepatol* 2023; 15(10): 1127-1139
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1127.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1127

INTRODUCTION

Alterations in plasma protein metabolism (APPM) can either be inherited or acquired[1]. As hepatocytes are responsible for the majority of protein production (approximately 10-20 g/d)[2], liver disease is frequently observed among patients with APPM[1].

Alpha-1 antitrypsin (AAT) deficiency (AATD) is the most common form of inherited APPM and is caused by mutations in serpin family A member 1 (*SERPINA1*), which encodes AAT, a serum protein produced primarily by hepatocytes that protects the lungs from protease-mediated degradation[3]. A homozygous mutation (Glu342Lys) in *SERPINA1,* named protease inhibitor (Pi) ZZ, is estimated to affect approximately 1 in 2000-5000 newborn infants in Europe and North America[4]. Mutations in *SERPINA1* cause a reduction in serum AAT levels and promote the development of respiratory diseases, such as emphysema or chronic obstructive pulmonary disease[5]. In addition, AATD can result in liver diseases such as liver cirrhosis or hepatocellular carcinoma (HCC) due to the accumulation of hepatic AAT, which can trigger proteotoxic stress and lead to hepatocyte death and liver injury[6,7]. Approximately 20%-36% of patients with a PiZZ genotype develop significant fibrosis and approximately 10%-15% develop advanced fibrosis [8]. The only available curative treatment for end-stage liver disease in patients with AATD is liver transplantation[9]. Liver transplants in Germany have been allocated based on urgency according to the model for end-stage liver disease scoring system[10].

Given the lack of information on the natural history and epidemiology of AATD, we conducted a retrospective analysis of insurance claims data from Germany to better understand overall prevalence, burden and progression of liver disease in patients with APPM, including AATD. To improve our understanding of the natural history of APPM and liver disease, we compared patients with APPM and liver disease with a control cohort of patients with liver disease but without APPM.

MATERIALS AND METHODS

Data source and sample selection

This retrospective study used anonymized, patient-level, insurance claims data from 01 January 2010 to 30 September 2020, provided by the German regional health insurance provider, AOK PLUS. This data set covers approximately 3.3 million individuals from the federal states of Saxony and Thuringia, accounting for approximately 4.5% of the German statutory health insured (SHI) population in 2020. The age and comorbidity characteristics of patients insured by AOK PLUS are similar to those in the general German population who are insured by sickness funds[11-14].

The cohort of patients with APPM and incident liver disease was identified using the German Modification of the International Classification of Diseases-10th Revision (ICD-10-GM) code E88.0 for disorders of plasma protein metabolism (which includes AATD and other metabolic disorders such as plasminogen deficiency and bisalbuminaemia), and codes K74, K70.2-3 and K71.7 for liver disease.

Patients were included in the APPM cohort if they had APPM (diagnosed between 01 January 2010 and the end of the study) and incident liver disease (diagnosed between 01 January 2012 and the end of the study; Supplementary Figure 1). Patients were excluded if their liver disease was diagnosed in 2010 or 2011, to guarantee a liver disease-free period of 2 years. Continuous insurance coverage (no interruption of insurance for > 30 d) in 2010 and/or 2011 was required. Patients were observed from the date of the first diagnosis of liver disease (index date) until death, loss to follow-up due to end of insurance or end of the study. In patients with incident fibrosis who developed cirrhosis after 01 January 2012, the index date was defined as the date of the first diagnosis of cirrhosis for analyses of the subgroup of patients with cirrhosis. Patients were included in the control cohort if they did not have a diagnosis of APPM, but had incident liver disease. All patients with incident liver disease in both cohorts were further divided into two sub-cohorts: (1) Those with fibrosis at index date (ICD-10-GM codes: K74.0-2 and K70.2); and (2) those with cirrhosis at index date or those with fibrosis who developed cirrhosis during the study period (ICD-10-GM codes: K74.3-7, K70.3 and K71.7).

Outcomes

The study evaluated point prevalence and cumulative incidence of patients with APPM and liver disease, stratified by sex. In addition, demographics and baseline disease characteristics, including the Charlson Comorbidity Index, were measured at index date, with comorbidities identified during the previous 12 mo. Comorbidities were identified based on three-digit ICD-10-GM codes and evidence of confirmatory diagnosis/diagnoses (at least one inpatient or at least two outpatient diagnoses). Diagnostic procedures (liver biopsy, imaging, laboratory tests, AAT phenotyping and liver function tests) in the 12 mo after the index date were identified based on German procedure codes (Operationen-und Prozedurenschlüssel and einheitlicher Bewertungsmaßstab). A composite endpoint of progression-free survival (PFS) was defined as the time from index date until the first date with selected liver disease-related clinical events (acute peritonitis, ascites, cirrhosis, only among patients with fibrosis), esophageal/gastric varices, HCC, hepatic encephalopathy, hepatic failure, liver transplantation or all-cause death (used to assess mortality). Disease progression events were also analyzed separately, and comprised the following (one inpatient or one confirmed outpatient diagnosis): Ascites, esophageal/gastric varices, acute peritonitis, hepatic encephalopathies, gastrointestinal bleeding (*e.g.*, melaena or hematemesis), hepatic failure, malignant neoplasm of the liver and intrahepatic bile ducts and HCC. Procedure-related events were analyzed, which comprised liver incision, liver resection, other operations on the liver, failure or rejection of transplant, and infection due to prosthesis, implant or graft.

Statistical analysis

To calculate the point prevalence of liver disease in patients with APPM, the denominator was the number of individuals insured by AOK PLUS on 01 January of the respective calendar year (2011-2020) and during the preceding 12 mo. The numerator was the number of patients alive on the 1st January of each year, with evidence of confirmatory diagnosis/diagnoses of APPM (made in two different quarters within the same year) and a diagnosis of liver disease during the previous year, and with continuous insurance coverage during that year.

The cumulative incidence of liver disease in patients with APPM was estimated for 2012-2019 by dividing the number of new cases in a calendar year by the total number of insured patients at risk of liver disease (*i.e.*, those with no current evidence of liver disease) at the beginning of the same year. The numerator was the number of patients with APPM diagnosed at any point and with liver disease diagnosed during the year of the index date, but without any liver disease diagnosis within the previous 2 years and with continuous insurance coverage during this period. The denominator was the number of patients alive at the beginning of the respective calendar year for whom no liver disease diagnosis was documented in the 24 mo before index date, and with continuous insurance over this period.

Point prevalence and cumulative incidence were adjusted for age and sex differences compared with the German SHI population.

Patient demographics and baseline characteristics were analyzed using summary statistics (mean, standard deviation, median and interquartile range) for continuous variables and frequency statistics for categorical variables.

Time to disease progression was estimated using the Kaplan-Meier method. Patients were censored if they were lost to follow-up or had reached the end of the study. In addition to the number of patients with a progression event, the following Kaplan-Meier estimates were reported: median follow-up time in patients without an event, the 25th, 50th and 75th percentiles of time without an event, and event rates at years 1, 3 and 5 post-baseline. All time to event analyses were compared *via* a log-rank test.

Time from index date to all-cause death was estimated for patients with APPM and incident fibrosis or cirrhosis, and separately for patients with APPM and cirrhosis with and without a previous documented diagnosis of fibrosis.

RESULTS

Overview of analyzed patients

In total, 45503 patients had confirmatory diagnosis(es) of liver disease between 01 January 2012 and the end of the study (Figure 1). Of these, 2680 fulfilled the criteria to be included in the APPM cohort. In total, 96 of these patients had fibrosis and 2584 had cirrhosis at their index date (between 01 January 2012 and the end of the study).

In total, 26299 patients were included in the corresponding control cohort with no diagnosis of APPM. Of these, 1444 had fibrosis and 24855 had cirrhosis at their index date (Figure 1).

Epidemiology

Between 2012 and 2019, the annual cumulative incidence of liver disease per 100000 individuals with APPM was 10-15, and was higher in males (15-22) than females (5-9; Supplementary Figure 2). When adjusted for age and sex differences *vs* the SHI population, the cumulative incidence was 8-13 per 100000 individuals. Between 2011 and 2020, the point prevalence of liver disease per 100000 individuals with APPM was 36-51 and was again higher in males (52-74) than females (22-32; Supplementary Figure 3). When adjusted for age and sex differences compared with the SHI population, the point prevalence was 33-47 per 100000 individuals.

Demographics and baseline characteristics

Demographics and baseline characteristics were similar between cohorts (Table 1). In the APPM and control cohorts, respectively, 840 patients (31.3%) and 8595 patients (32.7%) were female (P = 0.159 between cohorts). Patients in the APPM cohort were significantly younger than in the control cohort, with a median age (interquartile range) of 63 years (54-73) and 65 years (56-76; P < 0.001), respectively. The most common liver-related comorbidities in the APPM and control cohorts, respectively, were "other liver disease" [679 (25.3%) and 6298 (23.9%)], non-alcoholic steatohepatitis [17 (0.6%) and 150 (0.6%)] and chronic hepatitis [12 (0.5%) and 163 (0.6%)]. The proportion of patients with respiratory system-related comorbidities was similar between the APPM [827 (30.9%)] and control [8333 (31.7%)] cohorts.

Minor differences in demographics and baseline characteristics were observed when the APPM cohort was stratified by the presence of fibrosis or cirrhosis. There was a higher proportion of females with fibrosis [41 (42.7%)] compared with cirrhosis [815 (31.0%)] and alcohol-related disorders were more common in patients with cirrhosis [649 (24.7%)] compared with fibrosis [17 (17.7%)].

Diagnostic procedures

The most common diagnostic procedures within 12 mo after the index date in the APPM cohort were imaging procedures [1778 (66.3%)] and laboratory tests [1366 (51.0%); Table 2]. Only 55 patients (2.1%) underwent AAT phenotyping.

Disease progression and mortality

A higher proportion of patients in the APPM cohort experienced disease progression [2465 (92.0%)] compared with the control cohort [17682 (67.2%)]. Median PFS (composite endpoint) was significantly shorter in the APPM cohort [0.9 years (95%CI: 0.7-1.1)] compared with the control cohort [3.7 years (95%CI: 3.6-3.8); P < 0.001; Supplementary Figure 4]. The most common disease progression events were ascites, hepatic failure, esophageal/gastric varices, and hepatic encephalopathies (Figure 2). Patients with cirrhosis but without APPM had significantly longer event-free survival for ascites, hepatic failure, esophageal/gastric varices, and hepatic encephalopathy compared with those with APPM and cirrhosis (all P < 0.001; Figure 3). Similarly, patients with fibrosis but without APPM had significantly longer event-free survival for ascites, cirrhosis, hepatic failure and esophageal/gastric varices compared with those with APPM and fibrosis (all P < 0.001; Supplementary Figure 5). In the subgroup of 96 patients in the APPM cohort with fibrosis, median time from fibrosis to cirrhosis was 2.9 years (95%CI: 1.2-not reached; Supplementary Figure 5B). In the control cohort, median time from fibrosis to cirrhosis was not reached.

The most common procedures and procedure-related events indicating disease progression were liver resection, liver transplantation, failure and rejection of liver transplant and other operations on the liver (Supplementary Figure 6). A significantly higher proportion of patients with APPM and fibrosis had a liver resection [15 (15.6%) *vs* 93 (6.4%); P = 0.025] and a liver transplantation [6 (6.3%) *vs* 3 (0.2%); P < 0.001] compared with patients without APPM but with fibrosis (Table 3). Similarly, a significantly higher proportion of patients with APPM and cirrhosis had a liver resection [87 (3.3%) *vs* 488 (1.9%); P < 0.001] and a liver transplantation [78 (3.0%) *vs* 31 (0.1%); P < 0.001] indicating greater disease progression compared with patients without APPM but with cirrhosis (Table 3; Supplementary Figure 6).

Table 1 Demographics and baseline characteristics, *n* (%)

	Patients with APPM and liver disease: APPM cohort, *n* = 2680			Patients without APPM and with liver disease: Control cohort, *n* = 26299
	All patients, *n* = 2680	Patients with fibrosis, *n* = 96	Patients with cirrhosis, *n* = 2626[1]	
Total observed patient-years	6118	283	5944	70261
Median follow-up, yr (IQR)	1.5 (0.4-3.7)	2.3 (0.8-4.7)	1.5 (0.4-3.7)	2.0 (0.5-4.4)
Female sex[2]	840 (31.3)	41 (42.7)	815 (31.0)	8595 (32.7)
Median age, yr (IQR)[3]	63.0 (54.0-73.0)	64.5 (53.0-76.5)	63.0 (54.0-73.0)	65.0 (56.0-76.0)
0-14 yr	14 (0.5)	6 (6.3)	10 (0.4)	49 (0.2)
15-29 yr	10 (0.4)	0	10 (0.4)	156 (0.6)
30-44 yr	183 (6.8)	10 (10.4)	177 (6.7)	1420 (5.4)
45-59 yr	818 (30.5)	21 (21.9)	814 (31.0)	7516 (28.6)
60-74 yr	1046 (39.0)	31 (32.3)	1029 (39.2)	9564 (36.4)
75-89 yr	577 (21.5)	25 (26.0)	557 (21.2)	7090 (27.0)
≥ 90 yr	32 (1.2)	3 (3.1)	29 (1.1)	504 (1.9)
With care needs[4]	547 (20.4)	33 (34.3)	528 (20.1)	4934 (18.8)
Median CCI[5] (IQR)	3.0 (1.0-5.0)	4.0 (1.5-6.0)	3.0 (1.0-5.0)	3.0 (1.0-5.0)
Most common liver-related comorbidities				
"Other" liver disease[6]	679 (25.3)	26 (27.1)	669 (25.5)	6298 (23.9)
Non-alcoholic steatohepatitis	17 (0.6)	1 (1.0)	16 (0.6)	150 (0.6)
Chronic hepatitis	12 (0.5)	0	12 (0.5)	163 (0.6)
Respiratory system-related comorbidities	827 (30.9)	41 (42.7)	806 (30.7)	8333 (31.7)
Most common comorbidities				
Hypertension	1751 (65.3)	64 (66.7)	1710 (65.1)	17278 (65.7)
Type 2 diabetes mellitus	1103 (41.2)	40 (41.7)	1082 (41.2)	10766 (40.9)
Dyslipidaemia	832 (31.0)	35 (36.5)	813 (31.0)	8932 (34.0)
Alcohol-related disorders[7]	650 (24.3)	17 (17.7)	649 (24.7)	6127 (23.3)
Heart failure	605 (22.6)	21 (21.9)	591 (22.5)	6071 (23.1)

Data are *n* (%) unless otherwise indicated.

[1]In total, 42 patients with fibrosis progressed to cirrhosis during follow-up and are included in both fibrosis and cirrhosis subgroups.

[2]Patients in the alterations in plasma protein metabolism (AAPM) cohort were significantly younger than patients in the control cohort (Wilcoxon rank-sum test: $P < 0.001$).

[3]There was no statistically significant difference in the proportion of females between cohorts (Wilcoxon rank-sum test: $P = 0.159$).

[4]Dummy variable for patients who were classified in any of the five care levels which determine the benefits from long-term care insurance in Germany.

[5]Assessed based on diagnoses observed in the 12 mo before the index date.

[6]Captured using the K76 code in the German Modification of the International Classification of Diseases-10th Revision, and includes central hemorrhagic necrosis of the liver, chronic congestive liver, fatty liver (not otherwise classified), hepatorenal syndrome, liver infarction, peliosis hepatis, portal hypertension, veno-occlusive liver disease and other/unspecified liver disease.

[7]Patients with alcohol-related disorders diagnosed before [1172 (43.7%)] and after [1042 (38.9%)] diagnosis of APPM were detected in the cohort with APPM and liver disease.

CCI: Charlson comorbidity index; IQR: Interquartile range.

Median survival was shorter in the APPM cohort [2.6 years (95%CI: 2.3-2.8)] than in the control cohort [4.3 years (95%CI: 4.2-4.5)]. Median survival was 4.7 years (95%CI: 3.5-7.0) in patients with APPM and fibrosis and 2.5 years (95%CI: 2.3-2.8) in patients with APPM and cirrhosis (Figure 4). In the 42 patients in the APPM cohort with fibrosis who developed cirrhosis during the follow-up period, median survival was 4.1 years (95%CI: 2.2-7.1). In the 2584 patients in the APPM cohort with cirrhosis and without previous fibrosis, the median survival was 2.5 years (95%CI: 2.2-2.7).

Table 2 Diagnostic procedures within 12 mo after the index date, *n* (%)

Diagnostic procedure	Patients with APPM and liver disease: APPM cohort, *n* = 2680
Imaging procedure[1]	1778 (66.3)
Laboratory test[2]	1366 (51.0)
Liver biopsy	207 (7.7)
AAT phenotyping	55 (2.1)
Liver function test[3]	13 (0.5)

Data are *n* (%).
[1]Included sonographies, computed tomography scans, magnetic resonance imaging procedures, angiographies of the abdomen and magnetic resonance elastographies.
[2]Included albumin and bilirubin measurements.
[3]Liver function test with intravenous application of a ^{13}C-labeled substrate.
AAT: Alpha-1 antitrypsin; APPM: Alterations in plasma protein metabolism.

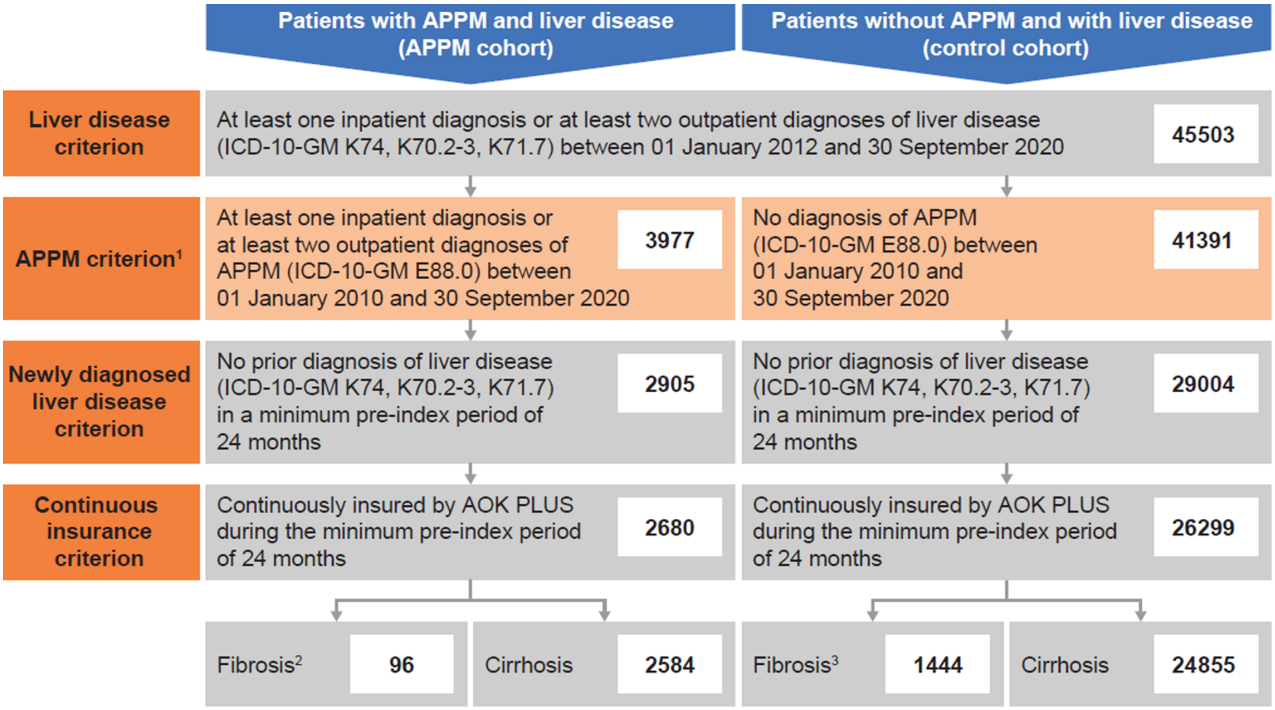
Table 3 Procedures indicating disease progression stratified by fibrosis or cirrhosis

Parameter	Patients with fibrosis				Patients with cirrhosis			
	Liver resection		Liver transplantation		Liver resection		Liver transplantation	
	Patients with APPM, *n* = 96	Patients without APPM, *n* = 1445	Patients with APPM, *n* = 96	Patients without APPM, *n* = 1445	Patients with APPM, *n* = 2626	Patients without APPM, *n* = 25134	Patients with APPM, *n* = 2626	Patients without APPM, *n* = 25134
Patients with event during follow-up	15 (15.6)	93 (6.4)	6 (6.3)	3 (0.2)	87 (3.3)	488 (1.9)	78 (3.0)	31 (0.1)
Patients included in the KM analysis[1]	85 (88.5)	1373 (95.0)	94 (97.9)	1442 (99.8)	2606 (99.2)	24849 (98.9)	2621 (99.8)	25034 (99.6)
Median follow-up in patients without an event, yr (IQR)[2]	2.3 (0.7-5.0)	3.0 (1.2-5.2)	2.3 (0.7-4.7)	2.8 (1.2-5.1)	1.5 (0.4-3.7)	1.9 (0.5-4.4)	1.5 (0.4-3.7)	1.9 (0.5-4.4)
Failure rate								
After 1 yr	3 (4.2)	15 (1.2)	1 (1.2)	1 (0.1)	40 (2.0)	198 (1.0)	26 (1.4)	8 (< 0.1)
After 3 yr	3 (4.2)	18 (1.5)	3 (4.8)	1 (0.1)	56 (3.4)	258 (1.5)	53 (3.6)	23 (0.2)
After 5 yr	3 (4.2)	20 (1.9)	4 (7.3)	1 (0.1)	64 (4.7)	289 (1.9)	66 (5.6)	25 (0.2)
P value (log-rank test)	0.025		< 0.001		< 0.001		< 0.001	

Data are *n* (%) unless otherwise indicated.
[1]Patients with the event on the index date were excluded from the Kaplan-Meier (KM) analysis. As there were no patients with liver incision in the alterations in plasma protein metabolism cohort, and only one patient in the control cohort, this outcome is not reported.
[2]There were no patients with fibrosis who had an infection/inflammatory reaction due to prothesis/implant/graft. For one patient in the control cohort, the index date and end date were the same; this patient was automatically excluded from the KM analysis. Median follow-up in patients without the event was estimated *via* the reverse KM method.
IQR: Interquartile range; APPM: Alterations in plasma protein metabolism.

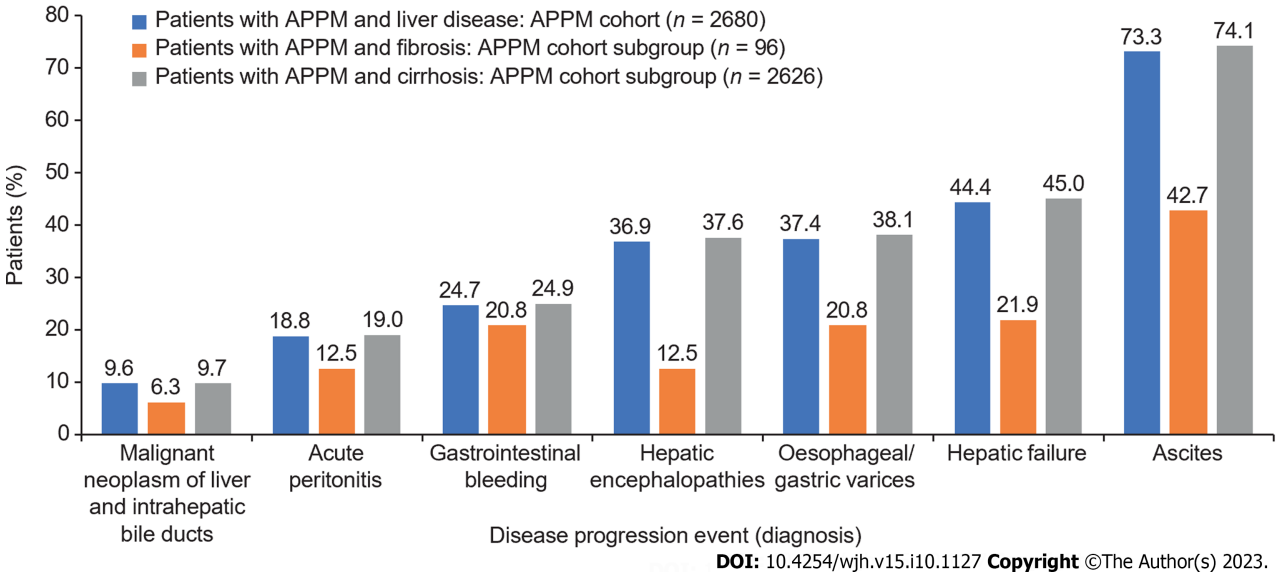
DISCUSSION

This retrospective insurance claims-based study demonstrated that, per 100000 individuals, the annual incidence (2012-2019) and point prevalence (2011-2020) of APPM was 10-15 and 36-51, respectively, with higher rates in males than in females. Patients in the APPM cohort experienced shorter PFS, higher mortality and a higher rate of liver decompensation events compared with patients in the control cohort. In addition, patients with fibrosis in the APPM cohort had significantly shorter cirrhosis-free survival compared with patients with fibrosis in the control cohort ($P < 0.001$). This may have been because APPM is indicative of a more advanced stage of fibrosis that is more likely to decompensate, or alternatively may reflect how the E88.0 code is used in clinical practice. Liver cirrhosis is often associated with decreased plasma levels of hepatocyte-derived proteins[15]. Some of these proteins, such as albumin and transferrin, are well-



DOI: 10.4254/wjh.v15.i10.1127

Figure 1 Selection of patient cohorts. [1]In total, 135 patients had only one outpatient alterations in plasma protein metabolism (APPM) diagnosis and therefore did not meet these criteria; [2]In total, 42 patients with fibrosis in the APPM cohort progressed to cirrhosis during follow-up; and [3]In total, 280 patients with fibrosis in the control cohort progressed to cirrhosis during follow-up. ICD-10-GM: German Modification of the International Classification of Diseases-10th Revision.



DOI: 10.4254/wjh.v15.i10.1127

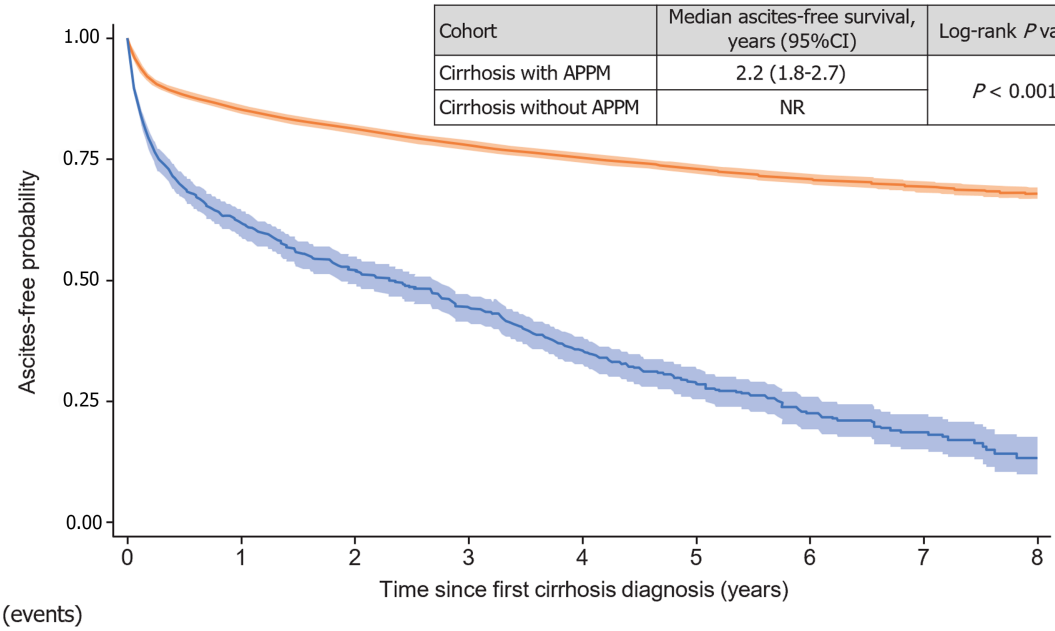
Figure 2 Disease progression events. Disease progression events that occurred after the index date until the end of the study. APPM: Alterations in plasma protein metabolism.

established indicators of poor prognosis[1]. Previous studies have demonstrated that patients with cirrhosis and a heterozygous Pi mutation, PiMZ, in *SERPINA1* decompensate faster than patients with cirrhosis but without AATD[16,17]. However, only 2.1% of patients in the APPM cohort underwent AAT phenotyping in our study, and therefore we were unable to further evaluate the association between decompensation and AATD genotype. The median age of patients in the APPM cohort was 2 years younger than in the control cohort, yet the APPM cohort had a higher risk of liver disease-related clinical events. This supports that patients with APPM are at a higher risk of liver disease-related clinical events than patients without APPM irrespective of age.

As AATD is also associated with the development of respiratory system comorbidities such as chronic obstructive pulmonary disease[18], it was anticipated that the APPM cohort would have a higher incidence of such comorbidities compared with the control cohort. However, we observed a similar incidence in the APPM and control cohorts (30.9%

A

Cohort	Median ascites-free survival, years (95%CI)	Log-rank *P* value
Cirrhosis with APPM	2.2 (1.8-2.7)	*P* < 0.001
Cirrhosis without APPM	NR	



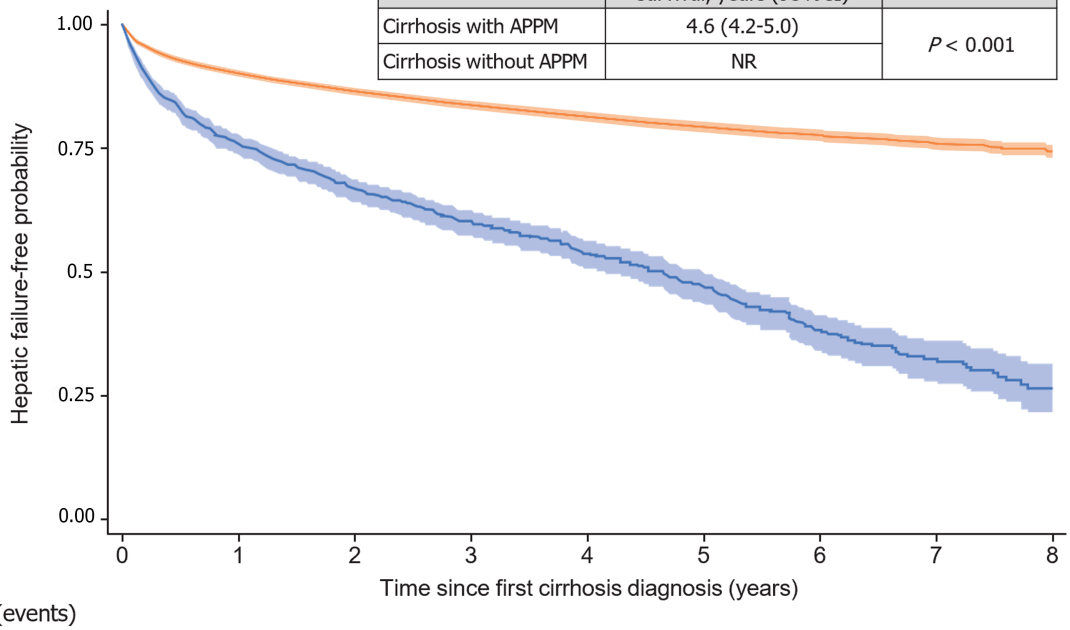
Number at risk (events)

Cirrhosis with APPM	1556	(544)	740	(112)	524	(71)	364	(66)	234	(40)	144	(26)	74	(11)	36	(8)	12
Cirrhosis without APPM	19318	(2566)	12229	(530)	9359	(343)	7175	(230)	5377	(146)	3834	(92)	2575	(48)	1462	(20)	535

95%CI Cirrhosis with APPM 95%CI Cirrhosis without APPM

B

Cohort	Median hepatic failure-free survival, years (95%CI)	Log-rank *P* value
Cirrhosis with APPM	4.6 (4.2-5.0)	*P* < 0.001
Cirrhosis without APPM	NR	



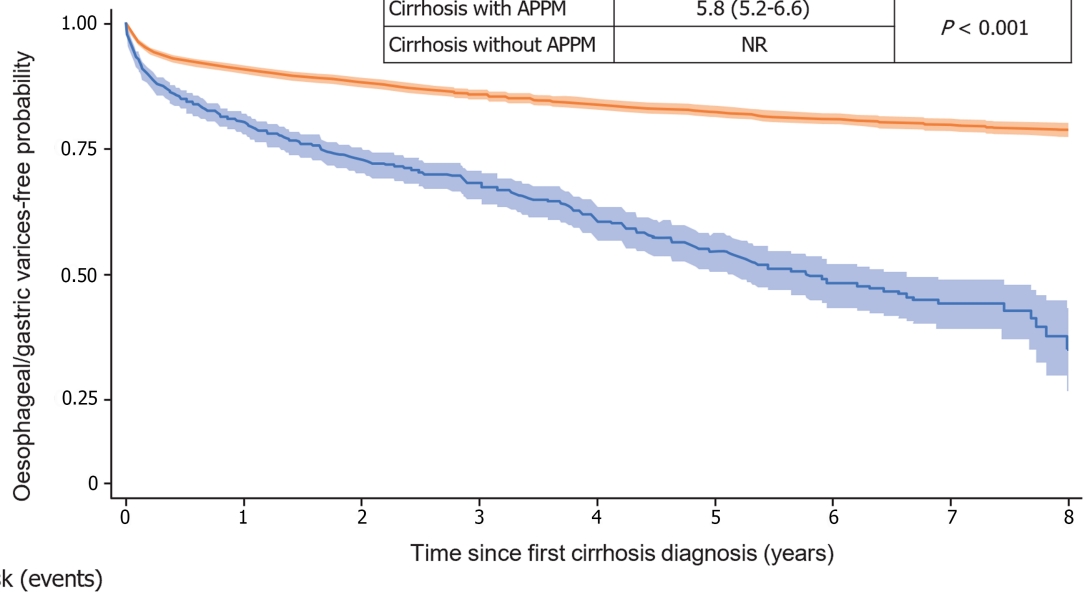
Number at risk (events)

Cirrhosis with APPM	2253	(460)	1160	(124)	806	(70)	558	(53)	382	(40)	243	(38)	135	(16)	63	(10)	25
Cirrhosis without APPM	22915	(1908)	14289	(523)	10774	(321)	8174	(196)	6080	(129)	4315	(87)	2873	(46)	1637	(22)	595

95%CI Cirrhosis with APPM 95%CI Cirrhosis without APPM

C

Cohort	Median oesophageal/gastric varices-free survival, years (95%CI)	Log-rank *P* value
Cirrhosis with APPM	5.8 (5.2-6.6)	*P* < 0.001
Cirrhosis without APPM	NR	



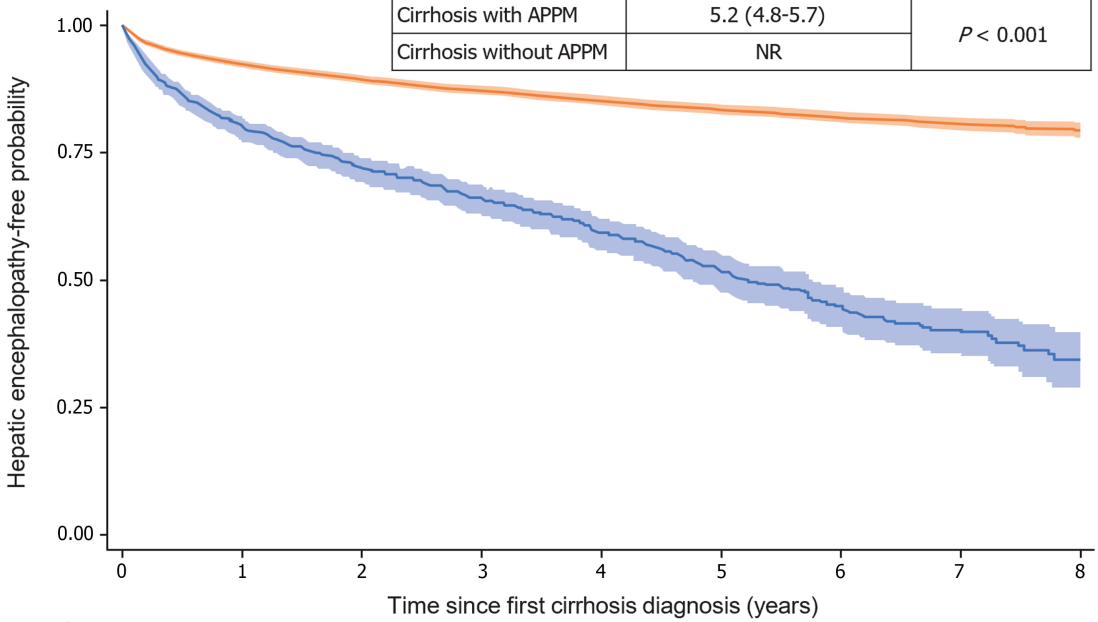
Number at risk (events)

Cirrhosis with APPM	2218	(359)	1065	(86)	717	(42)	496	(45)	332	(29)	213	(20)	121	(7)	50	(5)	14
Cirrhosis without APPM	22620	(1758)	13576	(340)	10255	(261)	7767	(161)	5808	(86)	4142	(61)	2774	(33)	1568	(14)	574

95%CI — Cirrhosis with APPM; 95%CI — Cirrhosis without APPM

D

Cohort	Median hepatic encephalopathy-free survival, years (95%CI)	Log-rank *P* value
Cirrhosis with APPM	5.2 (4.8-5.7)	*P* < 0.001
Cirrhosis without APPM	NR	



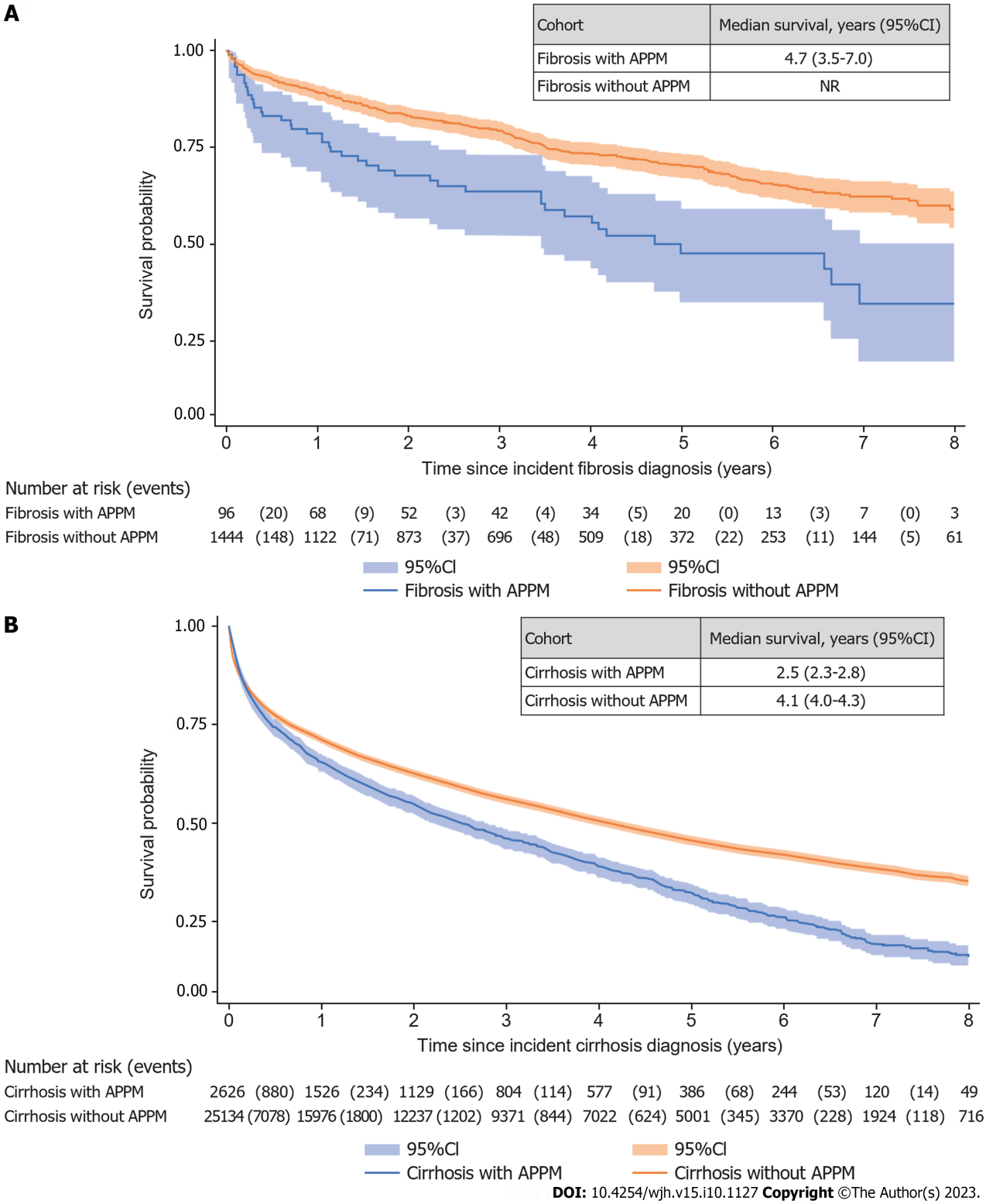
Number at risk (events)

Cirrhosis with APPM	2341	(383)	1221	(112)	853	(61)	596	(53)	412	(44)	259	(28)	152	(13)	71	(9)	28
Cirrhosis without APPM	23466	(1512)	14630	(416)	11052	(249)	8404	(177)	6245	(103)	4445	(72)	2968	(35)	1686	(17)	613

95%CI — Cirrhosis with APPM; 95%CI — Cirrhosis without APPM

DOI: 10.4254/wjh.v15.i10.1127

Figure 3 Event-free survival for disease progression events in patients with cirrhosis with and without alterations in plasma protein metabolism. A: Ascites; B: Hepatic failure; C: Esophageal and gastric varices; D: Hepatic encephalopathy. APPM: Alterations in plasma protein metabolism; CI: Confidence interval; NR: Not reached.



DOI: 10.4254/wjh.v15.i10.1127

Figure 4 Survival in patients with fibrosis and cirrhosis. A: Fibrosis; B: Cirrhosis. APPM: Alterations in plasma protein metabolism; CI: Confidence interval; NR: Not reached.

and 31.7%), which might indicate that the APPM cohort included a substantial number of patients without AATD. The E88.0 code we used to identify patients with APPM cannot be equated with AATD as the code includes a broad range of acquired and inherited APPM disorders, such as plasminogen deficiency. The European Commission Expert Group on Rare Diseases currently recommends the Orphanet nomenclature of rare diseases (ORPHA) codes to identify rare disorders[19]. The adoption of ORPHA codes is expected to facilitate the transition to ICD-11 codes, which include an expanded set of rare disorder codes compared with ICD-10[19]. In addition, developments of the ICD coding system, such as the addition of the E88.0A code for AATD, could improve the identification of patients with AATD in future administrative insurance claims analyses. In a recent registry-based cohort study of the prevalence, incidence and

mortality associated with AATD in Denmark using the E88.0A code, a sensitivity analysis demonstrated a predominance of AATD in the E88.0 category for APPM and a near complete shift to the more specific E88.0A code for AATD between 2000 and 2018[20]. The adoption of diagnosis codes specific to patients with AATD may facilitate earlier diagnosis and improved patient management, which may, in turn, contribute to slowing disease progression and decreasing the burden of disease in these patients with a rare, chronic disease.

The limitations of this study are typical of those seen in other insurance claims-based analyses. As noted previously, we were unable to determine the proportion of patients included in the study who had AATD due to the limitations of the ICD-10-GM coding system. In addition, as AATD is a highly underdiagnosed disease[21], we cannot exclude the possibility that some cases may have been included in the control cohort. The general lack of laboratory test results, direct clinical measures and biomarkers in the database confounded our ability to analyze the AATD genotype distribution. Only 96 patients in the APPM cohort and 1444 in the control cohort were recorded as having fibrosis, which was lower than anticipated, likely owing to underdiagnosis (patients are often asymptomatic in the early stages of fibrosis) and/or underreporting[22]. Furthermore, a substantial number of patients with fibrosis developed cirrhosis and liver decompensation events, which suggests that these patients were more likely to be at an advanced stage of fibrosis. Therefore, the development and implementation of structured early screening programs may be useful to increase the early detection of fibrosis in the general population[23]. Fibrosis stage data were not available in the database, which together with the low number of patients in this category constitutes a significant limitation as survival prognosis is thought to be highly linked to fibrosis stage[24]. In addition, regional differences in morbidity and mortality may exist, and our data may not be representative of geographic regions outside of Germany. However, in Germany, approximately 73.3 million people were insured by an SHI fund in 2020, which equates to approximately 88% of the general population. Owing to the uniform structure of SHI funds in all regions of Germany, we consider the data to be representative of the German population[25]. Lastly, as this was a retrospective insurance claims-based study that collected data using ICD-10-GM codes, we were unable to assess body weight/body mass index, which are known risk factors for early progression to advanced liver disease[26], and unable to determine the exact procedures involved in the diagnosis of liver disease.

CONCLUSION

Among patients with liver disease in Germany, those with APPM experience substantial burden and a higher rate of liver disease progression than patients without APPM. To enable accurate diagnosis and inform disease management, it is important to have specific diagnostic codes that differentiate between genetic liver disease and liver manifestations from other causes.

ARTICLE HIGHLIGHTS

Research background

Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disease that can result in the development of liver and/or lung disease, and is a leading cause of inherited alterations in plasma protein metabolism (APPM).

Research motivation

Currently, there is a lack of information on the natural history and epidemiology of AATD.

Research objectives

To understand the prevalence, burden and progression of liver disease in patients with APPM, which includes patients diagnosed with AATD, in Germany.

Research methods

A retrospective analysis of anonymized, patient-level, insurance claims data from a German health insurance provider (AOK PLUS) was conducted. The APPM cohort comprised patients with APPM (01/01/2010-30/09/2020) and incident liver disease (01/01/2012-30/09/2020) and the control cohort comprised patients without APPM but with incident liver disease. Outcomes were incidence/prevalence of liver disease in patients with APPM, demographics/baseline characteristics, disease progression, progression-free survival, mortality, and diagnostic procedures.

Research results

Overall, 2680 and 26299 patients were included in the APPM [fibrosis (96); cirrhosis (2584)] and control [fibrosis (1444); cirrhosis (24855)] cohorts, respectively. The annual incidence and prevalence of APPM and liver disease was 10-15/100000 and 36-51/100000, respectively. Median survival was shorter in the APPM cohort (2.6 years) than in the control cohort (4.3 years). In patients in the APPM cohort with fibrosis and cirrhosis, respectively, median survival was 4.7 years and 2.5 years. More patients in the APPM cohort (92.0%) experienced liver disease progression than in the control cohort (67.2%). Median progression-free survival was shorter in the APPM cohort [0.9 years (95%CI: 0.7-1.1)] compared with the control cohort [3.7 years (95%CI: 3.6-3.8); $P < 0.001$]. In patients with cirrhosis, event-free survival for ascites, hepatic encephalopathy, hepatic failure, and esophageal/gastric varices was longer in the control cohort than in the APPM cohort

($P < 0.001$). In patients with fibrosis, event-free survival for ascites, cirrhosis, hepatic failure, and esophageal/gastric varices was longer in the control cohort than in the APPM cohort ($P < 0.001$). The most common diagnostic procedures within 12 mo after the first diagnosis of liver disease in the APPM cohort were imaging procedures (66.3%) and laboratory tests (51.0%).

Research conclusions

In Germany, patients with APPM and liver disease experience substantial burden and a higher rate of and earlier liver disease progression than patients without APPM.

Research perspectives

The adoption of diagnosis codes specific to AATD should enable differentiation of this disease from other APPM disorders and facilitate earlier diagnosis and patient management. This should contribute to slowing disease progression and decreasing the burden of disease in patients with this rare, chronic disease.

ACKNOWLEDGEMENTS

Medical writing and submission assistance was provided by Matthew Reynolds of Oxford PharmaGenesis, Oxford, United Kingdom and was supported by Takeda Development Center Americas, Inc.

FOOTNOTES

Author contributions: Picker N, Hagiwara M, Baumann S, Marins EG, Wilke T, Ren K, Maywald U, Karki C, and Strnad P provided substantial contribution to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work, and drafted the work or revised it critically for important intellectual content; all authors provided final approval of the version to be published; all authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Institutional review board statement: Institutional review board approval was not required for this retrospective analysis of anonymized data.

Informed consent statement: Signed informed consent forms were not required as this was a retrospective analysis of anonymized data.

Conflict-of-interest statement: Dr. Strnad reports grants and other from CSL Behring, grants and other from Grifols, grants and other from Arrowhead Pharmaceuticals, grants and other from Dicerna Pharmaceuticals, grants from Vertex Pharmaceuticals, other from Albireo, other from GlaxoSmithKline, other from Intellia Pharmaceuticals, other from Ono Pharmaceuticals, other from Takeda Pharmaceuticals, during the conduct of the study.

Data sharing statement: The data that support the findings of this study are available from AOK PLUS. Restrictions apply to the availability of these data, which were used under license for this study.

Country/Territory of origin: Germany

ORCID number: Pavel Strnad 0000-0002-7122-6379.

S-Editor: Chen YL
L-Editor: Filipodia
P-Editor: Cai YX

REFERENCES

1 **Kuscuoglu D**, Janciauskiene S, Hamesch K, Haybaeck J, Trautwein C, Strnad P. Liver - master and servant of serum proteome. *J Hepatol* 2018; **69**: 512-524 [PMID: 29709680 DOI: 10.1016/j.jhep.2018.04.018]

2 **Quinlan GJ**, Martin GS, Evans TW. Albumin: biochemical properties and therapeutic potential. *Hepatology* 2005; **41**: 1211-1219 [PMID: 15915465 DOI: 10.1002/hep.20720]

3 **Ferrarotti I**, Carroll TP, Ottaviani S, Fra AM, O'Brien G, Molloy K, Corda L, Medicina D, Curran DR, McElvaney NG, Luisetti M. Identification and characterisation of eight novel SERPINA1 Null mutations. *Orphanet J Rare Dis* 2014; **9**: 172 [PMID: 25425243 DOI: 10.1186/s13023-014-0172-y]

4 **O'Brien ML**, Buist NR, Murphey WH. Neonatal screening for alpha1-antitrypsin deficiency. *J Pediatr* 1978; **92**: 1006-1010 [PMID: 307054 DOI: 10.1016/S0022-3476(78)80388-6]

5 **Strnad P**, McElvaney NG, Lomas DA. Alpha(1)-Antitrypsin Deficiency. *N Engl J Med* 2020; **382**: 1443-1455 [PMID: 32268028 DOI: 10.1056/NEJMra1910234]

6 **Townsend SA**, Edgar RG, Ellis PR, Kantas D, Newsome PN, Turner AM. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. *Aliment Pharmacol Ther* 2018; **47**: 877-885 [PMID: 29446109 DOI: 10.1111/apt.14537]

7 **Fromme M**, Schneider CV, Trautwein C, Brunetti-Pierri N, Strnad P. Alpha-1 antitrypsin deficiency: A re-surfacing adult liver disorder. *J Hepatol* 2022; **76**: 946-958 [PMID: 34848258 DOI: 10.1016/j.jhep.2021.11.022]

8 **Hamesch K**, Mandorfer M, Pereira VM, Moeller LS, Pons M, Dolman GE, Reichert MC, Schneider CV, Woditsch V, Voss J, Lindhauer C, Fromme M, Spivak I, Guldiken N, Zhou B, Arslanow A, Schaefer B, Zoller H, Aigner E, Reiberger T, Wetzel M, Siegmund B, Simões C, Gaspar R, Maia L, Costa D, Bento-Miranda M, van Helden J, Yagmur E, Bzdok D, Stolk J, Gleiber W, Knipel V, Windisch W, Mahadeva R, Bals R, Koczulla R, Barrecheguren M, Miravitlles M, Janciauskiene S, Stickel F, Lammert F, Liberal R, Genesca J, Griffiths WJ, Trauner M, Krag A, Trautwein C, Strnad P; European Alpha1-Liver Study Group. Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology* 2019; **157**: 705-719.e18 [PMID: 31121167 DOI: 10.1053/j.gastro.2019.05.013]

9 **Karatas E**, Di-Tommaso S, Dugot-Senant N, Lachaux A, Bouchecareilh M. Overview of alpha-1 antitrypsin deficiency-mediated liver disease. *EMJ Hepatol* 2019; **7**: 65-79 [DOI: 10.33590/emjhepatol/10314658]

10 **Schlitt HJ**, Loss M, Scherer MN, Becker T, Jauch KW, Nashan B, Schmidt H, Settmacher U, Rogiers X, Neuhaus P, Strassburg C. [Current developments in liver transplantation in Germany: MELD-based organ allocation and incentives for transplant centres]. *Z Gastroenterol* 2011; **49**: 30-38 [PMID: 21225535 DOI: 10.1055/s-0029-1245946]

11 **Flemming R**. Patterns of pregabalin prescribing in four German federal states: analysis of routine data to investigate potential misuse of pregabalin. *BMJ Open* 2022; **12**: e060104 [PMID: 35879005 DOI: 10.1136/bmjopen-2021-060104]

12 **Datzmann T**, Schmitt J, Fuhrmann S, Roessler M, Meier F, Schoffer O. Implementation and Effectiveness of Novel Therapeutic Substances for Advanced Malignant Melanoma in Saxony, Germany, 2010-2020-Cohort Study Based on Administrative Data. *Cancers (Basel)* 2021; **13**: 6150 [PMID: 34944771 DOI: 10.3390/cancers13246150]

13 **Markevych I**, Tesch F, Datzmann T, Romanos M, Schmitt J, Heinrich J. Outdoor air pollution, greenspace, and incidence of ADHD: A semi-individual study. *Sci Total Environ* 2018; **642**: 1362-1368 [PMID: 30045516 DOI: 10.1016/j.scitotenv.2018.06.167]

14 **Trautmann F**, Schuler M, Schmitt J. Burden of soft-tissue and bone sarcoma in routine care: Estimation of incidence, prevalence and survival for health services research. *Cancer Epidemiol* 2015; **39**: 440-446 [PMID: 25801944 DOI: 10.1016/j.canep.2015.03.002]

15 **Gurbuz B**, Guldiken N, Reuken P, Fu L, Remih K, Preisinger C, Brůha R, Leníček M, Petrtýl J, Reissing J, Aly M, Fromme M, Zhou B, Karkossa I, Schubert K, von Bergen M, Stallmach A, Bruns T, Strnad P. Biomarkers of hepatocellular synthesis in patients with decompensated cirrhosis. *Hepatol Int* 2023; **17**: 698-708 [PMID: 36652164 DOI: 10.1007/s12072-022-10473-x]

16 **Chen VL**, Burkholder DA, Moran IJ, DiBattista JV, Miller MJ, Chen Y, Du X, Oliveri A, Cushing KC, Lok AS, Speliotes EK. Hepatic decompensation is accelerated in patients with cirrhosis and alpha-1 antitrypsin Pi*MZ genotype. *JHEP Rep* 2022; **4**: 100483 [PMID: 35571533 DOI: 10.1016/j.jhepr.2022.100483]

17 **Balcar L**, Tonon M, Semmler G, Calvino V, Hartl L, Incicco S, Jachs M, Bauer D, Hofer BS, Gambino CG, Accetta A, Brocca A, Trauner M, Mandorfer M, Piano S, Reiberger T; Baveno Cooperation: an EASL consortium. Risk of further decompensation/mortality in patients with cirrhosis and ascites as the first single decompensation event. *JHEP Rep* 2022; **4**: 100513 [PMID: 35845294 DOI: 10.1016/j.jhepr.2022.100513]

18 **Sandhaus R**, Strange C, Stone G, Runken MC, Blanchette CM, Howden R. Comorbidity Associations with AATD Among Commercially Insured and Medicare Beneficiaries with COPD in the US. *Int J Chron Obstruct Pulmon Dis* 2020; **15**: 2389-2397 [PMID: 33116454 DOI: 10.2147/COPD.S263297]

19 **Aymé S**, Bellet B, Rath A. Rare diseases in ICD11: making rare diseases visible in health information systems through appropriate coding. *Orphanet J Rare Dis* 2015; **10**: 35 [PMID: 25887186 DOI: 10.1186/s13023-015-0251-8]

20 **Acquavella J**, Vágó E, Sorensen HT, Horváth-Puhó E, Hess GP. Registry-based cohort study of alpha-1 antitrypsin deficiency prevalence, incidence and mortality in Denmark 2000-2018. *BMJ Open Respir Res* 2022; **9**: e001281 [PMID: 36549785 DOI: 10.1136/bmjresp-2022-001281]

21 **Barrecheguren M**, Monteagudo M, Simonet P, Llor C, Rodriguez E, Ferrer J, Esquinas C, Miravitlles M. Diagnosis of alpha-1 antitrypsin deficiency: a population-based study. *Int J Chron Obstruct Pulmon Dis* 2016; **11**: 999-1004 [PMID: 27274221 DOI: 10.2147/COPD.S108505]

22 **Karlsen TH**, Sheron N, Zelber-Sagi S, Carrieri P, Dusheiko G, Bugianesi E, Pryke R, Hutchinson SJ, Sangro B, Martin NK, Cecchini M, Dirac MA, Belloni A, Serra-Burriel M, Ponsioen CY, Sheena B, Lerouge A, Devaux M, Scott N, Hellard M, Verkade HJ, Sturm E, Marchesini G, Yki-Järvinen H, Byrne CD, Targher G, Tur-Sinai A, Barrett D, Ninburg M, Reic T, Taylor A, Rhodes T, Treloar C, Petersen C, Schramm C, Flisiak R, Simonova MY, Pares A, Johnson P, Cucchetti A, Graupera I, Lionis C, Pose E, Fabrellas N, Ma AT, Mendive JM, Mazzaferro V, Rutter H, Cortez-Pinto H, Kelly D, Burton R, Lazarus JV, Ginès P, Buti M, Newsome PN, Burra P, Manns MP. The EASL-Lancet Liver Commission: protecting the next generation of Europeans against liver disease complications and premature mortality. *Lancet* 2022; **399**: 61-116 [PMID: 34863359 DOI: 10.1016/S0140-6736(21)01701-3]

23 **Labenz C**, Arslanow A, Nguyen-Tat M, Nagel M, Wörns MA, Reichert MC, Heil FJ, Mainz D, Zimper G, Römer B, Binder H, Farin-Glattacker E, Fichtner U, Graf E, Stelzer D, Van Ewijk R, Ortner J, Velthuis L, Lammert F, Galle PR. Structured Early detection of Asymptomatic Liver Cirrhosis: Results of the population-based liver screening program SEAL. *J Hepatol* 2022; **77**: 695-701 [PMID: 35472313 DOI: 10.1016/j.jhep.2022.04.009]

24 **Dulai PS**, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, Sebastiani G, Ekstedt M, Hagstrom H, Nasr P, Stal P, Wong VW, Kechagias S, Hultcrantz R, Loomba R. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology* 2017; **65**: 1557-1565 [PMID: 28130788 DOI: 10.1002/hep.29085]

25 **Dornquast C**, Kroll LE, Neuhauser HK, Willich SN, Reinhold T, Busch MA. Regionale Unterschiede in der Prävalenz kardiovaskulärer Erkrankungen. *Deutsches Ärzteblatt* 2016; **42**: 704-11

26 **Bowlus CL**, Willner I, Zern MA, Reuben A, Chen P, Holladay B, Xie L, Woolson RF, Strange C. Factors associated with advanced liver disease in adults with alpha1-antitrypsin deficiency. *Clin Gastroenterol Hepatol* 2005; **3**: 390-396 [PMID: 15822045 DOI: 10.1016/S1542-3565(05)00082-0]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1140-1152

DOI: 10.4254/wjh.v15.i10.1140

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Prevalence and risk factors of lymphatic dysfunction in cirrhosis patients with refractory ascites: An often unconsidered mechanism

Rahul Arya, Ramesh Kumar, Tarun Kumar, Sudhir Kumar, Utpal Anand, Rajeev Nayan Priyadarshi, Tanmoy Maji

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Giacomelli L, Italy; Ullah K, Pakistan

Received: August 17, 2023
Peer-review started: August 17, 2023
First decision: September 5, 2023
Revised: September 14, 2023
Accepted: October 8, 2023
Article in press: October 8, 2023
Published online: October 27, 2023

Rahul Arya, Ramesh Kumar, Sudhir Kumar, Tanmoy Maji, Department of Gastroenterology, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Tarun Kumar, Department of Pathology, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Utpal Anand, Department of Surgical Gastroenterology, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Rajeev Nayan Priyadarshi, Department of Radiodiagnosis, All India Institute of Medical Sciences, Patna 801507, Bihar, India

Corresponding author: Ramesh Kumar, MD, Additional Professor, Department of Gastroenterology, All India Institute of Medical Sciences, Phulwari Sharif, Patna 801507, Bihar, India. docrameshkr@gmail.com

Abstract

BACKGROUND

The lymphatic system is crucial in maintaining the body fluid homeostasis. A dysfunctional lymphatic system may contribute to the refractoriness of ascites and edema in cirrhosis patients. Therefore, assessment of lymphatic dysfunction in cirrhosis patients with refractory ascites (RA) can be crucial as it would call for using different strategies for fluid mobilization.

AIM

To assessing the magnitude, spectrum, and clinical associations of lymphatic dysfunction in liver cirrhosis patients with RA.

METHODS

This observational study included 155 consecutive cirrhosis patients with RA. The presence of clinical signs of lymphedema, such as peau d'orange appearance and positive Stemmer sign, intestinal lymphangiectasia (IL) on duodenal biopsy seen as dilated vessels in the lamina propria with strong D2-40 immunohistochemistry, and chylous ascites were used to diagnose the overt lymphatic dysfunctions.

RESULTS

A total of 69 (44.5%) patients out of 155 had evidence of lymphatic dysfunction.

Peripheral lymphedema, found in 52 (33.5%) patients, was the most common manifestation, followed by IL in 42 (27.0%) patients, and chylous ascites in 2 (1.9%) patients. Compared to patients without lymphedema, those with lymphedema had higher mean age, median model for end-stage liver disease scores, mean body mass index, mean ascitic fluid triglyceride levels, and proportion of patients with hypoproteinemia (serum total protein < 5 g/dL) and lymphocytopenia (< 15% of total leukocyte count). Patients with IL also had a higher prevalence of lymphocytopenia and hypoproteinemia (28.6% *vs.* 9.1%, $P = 0.004$). Seven (13%) patients with lymphedema had lower limb cellulitis compared to none in those without it. On multivariate regression analysis, factors independently associated with lymphatic dysfunction included obesity [odds ratio (OR): 4.2, 95% confidence intervals (95%CI): 1.1–15.2, $P = 0.027$], lymphocytopenia [OR: 6.2, 95%CI: 2.9–13.2, $P < 0.001$], and hypoproteinemia [OR: 3.7, 95%CI: 1.5–8.82, $P = 0.003$].

CONCLUSION

Lymphatic dysfunction is common in cirrhosis patients with RA. Significant indicators of its presence include hypoproteinemia and lymphocytopenia, which are likely due to the loss of lymphatic fluid from the circulation. Future efforts to mobilize fluid in these patients should focus on methods to improve lymphatic drainage.

Key Words: Cirrhosis; Lymphedema; Lymphangicetasia; Refractory ascites; Chylous ascites; Lymphocytopenia

Core Tip: Lymphatic dysfunction is often underappreciated in advanced cirrhosis patients. Our study evaluated the magnitude, spectrum, and associations of lymphatic dysfunction in cirrhosis patients with refractory ascites (RA). Nearly half (44.5%) of the studied population ($n = 155$) revealed evidence of overt lymphatic dysfunction in the forms of peripheral lymphedema (33.5%), intestinal lymphangiectasia (27.0%), and chylous ascites (1.9%). Obesity, hypoproteinemia, and lymphocytopenia were independently associated with lymphatic dysfunction in said patients. From a therapeutic standpoint, it can be extremely important to evaluate lymphatic dysfunction in cirrhosis patients with RA since it would call for using different strategies for fluid mobilization.

Citation: Arya R, Kumar R, Kumar T, Kumar S, Anand U, Priyadarshi RN, Maji T. Prevalence and risk factors of lymphatic dysfunction in cirrhosis patients with refractory ascites: An often unconsidered mechanism. *World J Hepatol* 2023; 15(10): 1140-1152
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1140.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1140

INTRODUCTION

The lymphatic system is crucial in maintaining the body fluid homeostasis[1]. By recirculating surplus tissue fluid back into the bloodstream, the lymphatic system prevents tissues from becoming edematous. In patients with cirrhosis and portal hypertension (PHT), the production of lymph from the liver and intestines is significantly increased[2,3]. Increased production of lymph promotes lymphangiogenesis, which in turn tends to improve the functional capacity of the lymphatics. However, as cirrhosis progresses, these compensatory mechanisms become overwhelmed, leading to the development of ascites and edema[2-4]. Subsequently, functional impairment of the lymphatic system also sets in, resulting in further worsening of the fluid accumulation[4,5]. The consequent lymphatic flow stagnation and leakage lay the ground for lymphedema development, which is the deposition of protein-rich lymph fluid within the tissues[6]. The gut lymphatics are important for maintaining the abdominal fluid balance. Studies have shown that patients with cirrhosis have much higher abdominal lymph production (up to 30-fold) and lymph flow in the thoracic duct (8-9 L/day) [7,8]. Moreover, a persistently high lymphatic pressure associated with PHT has the potential to cause intestinal lymphangiectasia (IL) and chylous ascites (CA) in cirrhosis patients[9-11]. Rupture of IL with subsequent loss of lymph can result in hypoproteinemia, lymphocytopenia, and malabsorption of fat[4,12].

Lymphatic dysfunction occurs in patients with cirrhosis, although the amount of published data on this subject is extremely limited[2-5]. There have been only a few reports of CA and IL in these patients[10,11]. Lymphedema has not even been considered in cirrhosis patients with persistent peripheral edema. The structural and functional changes in gut lymphatics, which are vital in splanchnic lymph drainage, remain an unexplored area in cirrhosis patients. Despite being a significant factor in maintaining fluid homeostasis, the lymphatic system is commonly ignored when assessing the pathophysiology of refractory ascites (RA) in cirrhosis patients. RA, which represents an extreme form of fluid accumulation, eventually develops in about 10% of cirrhosis patients with advanced decompensation[13].

Over the past two decades, a better understanding of the lymphatic vascular system has emerged; however, little is known about how lymphatic dysfunction contributes to the pathophysiology of advanced cirrhosis. Given the significant role the lymphatic system plays in maintaining the balance of body fluids, it is reasonable to assume that a dysfunctional lymphatic system may contribute to the refractoriness of ascites and edema in cirrhosis patients. Therefore, assessing

lymphatic dysfunction in such patients can provide a novel approach to tissue decongestion. Case studies have shown dietary changes to be effective in controlling ascites and improving liver stiffness in cirrhosis patients with IL[10,14].

In a recent study, treatment with recombinant vascular endothelial growth factor (VEGF) C, a lymphatic-specific growth factor, resulted in decreased portal pressure, enhanced lymphatic drainage, and decreased ascites in cirrhotic rats [15]. However, techniques for assessing lymphatic structure and function in cirrhosis are limited. Lymphography and lymphoscintigraphy lack sufficient accuracy and are not readily available. Lymphedema, IL, and CA are significant surrogate markers of lymphatic dysfunction that need to be investigated in patients with cirrhosis. Hence, this study assessed the magnitude, spectrum, and clinical associations of lymphatic dysfunction in patients with liver cirrhosis and RA using surrogate markers.

MATERIALS AND METHODS

This observational study was conducted in the Department of Gastroenterology, All India Institute of Medical Sciences, Patna, a tertiary care medical center in India. The protocol was approved by the institute's research board, and investigations were conducted according to the Declaration of Helsinki principles. Consecutive adult liver cirrhosis patients between 18 years and 75 years admitted with RA from December 2021 to March 2023 were screened according to the inclusion criteria. RA was diagnosed as ascites that could not be mobilized or the early recurrence of which could not be prevented because of a lack of response to a maximum dose of diuretic treatment or because patients developed complications that precluded the use of an effective dose of diuretics[16]. Liver cirrhosis was diagnosed by clinical features, imaging characteristics, and endoscopic findings. Patients with low serum ascites albumin gradient (< 1.1) ascites, congestive heart failure, primary or metastatic abdominal malignancy, history of radiation therapy, concomitant tuberculosis, history of abdominal surgery, and filariasis were excluded from the study. Cirrhosis patients with severe sepsis, advanced hepatic encephalopathy, and respiratory failure were also excluded from the study.

Demographic and clinical data, including the degree of ascites and duration of RA, were noted at baseline. Estimates of dry weight were made for the corrected body mass index calculation by deducting 15% of the actual weight due to grade-3 ascites and an additional 5% because of peripheral edema. Per the Asian standard, body mass index > 25 kg/m^2 was considered obese. Routine investigations, including hemograms, liver function tests, kidney function tests, international normalized ratio, fasting blood sugar, and ascitic fluid analysis, including ascitic fluid triglyceride estimation, were performed in all patients, who also underwent a complete etiological workup. The severity of cirrhosis was assessed by Child-Pugh classification and model for end-stage liver disease (MELD) scores. Standard medical therapy, including etiology-specific treatment, was given to all patients.

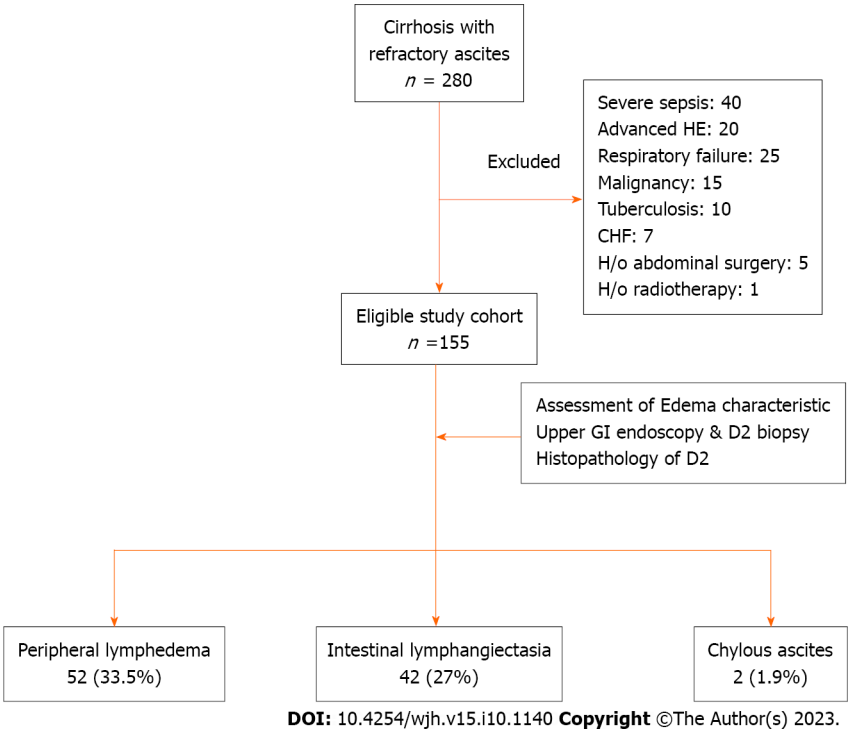
Assessment of lymphatic dysfunction

The lymphatic dysfunction was ascertained by the presence of one or more of the following surrogate markers: (1) Features of peripheral lymphedema, as evident by physical characteristics such as painless leg edema, pitting or non-pitting, showing orange peel (peau d'orange) appearance, and positive Stemmer sign; (2) Presence of IL on endoscopy and/or histopathological examination of duodenal biopsy specimens; and (3) Presence of CA, as indicated by milky white ascites with elevated triglyceride level > 110 mg/dL.

The diagnosis of IL on endoscopy was based on the presence of swollen mucosa with scattered white spots suggestive of dilated lacteals. Portal hypertensive duodenopathy (PHD) was considered in the presence of swollen duodenal mucosa with varying degrees of erythema, erosions, friability, and telangiectasia. Irrespective of endoscopic evidence of PHD, biopsies were obtained from the second part of the duodenum (D2), distal to the ampulla of Vater, using standard endoscopic biopsy forceps in all patients. The biopsies obtained were submitted in a vial containing diluted formalin for fixation. Histological examination (hematoxylin and eosin) was performed by an expert pathologist. A markedly dilated vessel in the lamina propria, which on immunohistochemistry showed strong D2-40 positivity, confirmed the presence of IL. Hypoproteinemia was considered when total serum protein was < 5 g/dL, with a decrease in both albumin and globulin. Lymphocytopenia was considered when the proportion of blood lymphocytes was < 15% of the total leukocyte count.

Statistical analysis

Because the magnitude and impact of lymphatic dysfunction in cirrhosis are not clearly defined, we conducted this observational study as an exploratory research project with a cross-sectional design intended to include a minimum of 100 eligible patients. Continuous variables, depending on the normalcy of distribution, were expressed as mean ± standard deviation or median (range). Categorical data were represented as proportion. To compare the normal covariates between patients with and without lymphatic dysfunction, independent sample *t*-test or Mann-Whitney *U* test was used when applicable. Comparisons in categorical variables were performed using χ^2 or Fisher's test. Multivariate regression analysis (MVA) was used to determine independent associates of lymphatic dysfunction. The relevant variables in the univariate analysis with $P < 0.10$ were considered for MVA. However, highly correlated variables were excluded to avoid multicollinearity in regression analysis. The odds ratio (OR) and 95% confidence interval (95%CI) for all significant variables in MVA were reported. Data were analyzed using SPSS software version 23.0 (SPSS, Chicago, IL, United States), wherein $P < 0.05$ was taken as significant.



DOI: 10.4254/wjh.v15.i10.1140

Figure 1 Flow-diagram showing selection, evaluation, and categorization of study population based on lymphatic dysfunction. A total of 280 cirrhosis patients with refractory ascites were screened, and eventually 155 patients were found to be eligible based on the inclusion and exclusion criteria. Subsequent to evaluation, 69 (44.5%) patients were found to have evidence of lymphatic dysfunction in the form of lymphedema, intestinal lymphangiectasia, and chylous ascites, either alone or in combination. CHF: Congestive heart failure; D2: Second part of duodenum; GI: Gastrointestinal; HE: Hepatic encephalopathy; H/o: History of.

RESULTS

A total of 280 cirrhosis patients with RA were screened during the study, but 125 of them were found to be ineligible based on the inclusion and exclusion criteria (Figure 1). The study cohort ultimately consisted of 155 cirrhosis patients with RA.

Cohort characteristics

The mean age of the patients was 49.0 ± 13.1 years with a predominance of male subjects (77.0%). The predominant etiology of cirrhosis involved alcohol (37.4%), followed by non-alcoholic steatohepatitis (35.4%). The median Child-Pugh and MELD scores were 10.3 and 18.1, respectively. Twenty-seven (17.4%) patients were diabetic, 16 (10.3%) patients were obese, and 9 (5.8%) patients had functional chronic kidney disease. All patients had grade-3 ascites, and the median duration of RA was 5 (3-48) mo. On endoscopy, large esophageal varices were found in 60 (38.0%) patients, and severe portal hypertensive gastropathy (PHG) was noted in 35 (22.6%) patients. Additionally, 31 (20.0%) patients had evidence of PHD. The presence of PHD was independent of the severity of PHG as 70.0% of patients with PHD had mild PHG. Other baseline characteristics are specified in Table 1.

Evaluation for lymphatic dysfunction

Edema characteristics: The physical appearance of peripheral edema revealed two distinct patterns (Figure 2). In 52 (33.5%) patients, edema was severe, pitting or non-pitting, with skin texture showing exaggerated dorsal skin creases, hyperkeratosis, and peau d'orange appearance. Moreover, these patients had a positive Stemmer sign, suggesting the presence of lymphedema. In the remaining 103 (66.5%) patients, edema was of the pitting type, skin texture was smooth, and the Stemmer sign was negative.

Endoscopic and histopathological evidence of IL: All patients tolerated the endoscopic procedure with D2 biopsy irrespective of coagulopathy and thrombocytopenia. Eight (5.1%) patients had endoscopic evidence of IL as a whitish enlarged villi on swollen mucosa (Figure 3). On histopathological examination of D2 biopsy specimens, 42 (27.0%) patients revealed markedly dilated vessels in the lamina propria, which on immunohistochemistry with D2-40 confirmed the presence of IL (Figure 4). Thus, 34 (22%) patients revealed IL on histopathological examination without endoscopic evidence of the same.

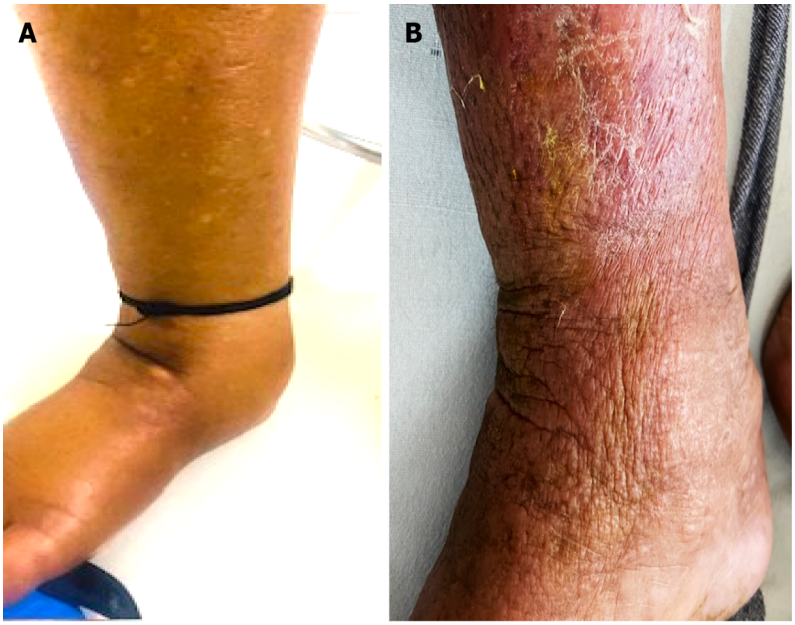
CA: The median level of triglyceride in ascitic fluid was 19.5 (0–224.0) mg/dL. Based on a physical examination of ascetic fluid and triglyceride levels, 2 patients (1.29%) were found to have CA.

Table 1 Clinical and laboratory characteristics of study population at baseline, *n* (%)

Parameters	*n* = 155
Age in yr, mean ± SD	49.0 ± 13.1
Male	119 (77.0)
Etiology of cirrhosis	
Alcohol	58 (37.4)
Hepatitis B	23 (14.8)
Hepatitis C	7 (4.5)
NASH	55 (35.4)
Others	14 (9.0)
Duration of RA in mo, median (range)	5 (-48)
MELD scores, median (range)	18.1 ± 8.6, 17(6-42)
Child-Pugh score, mean ± SD	10.3 ± 1.8
Diabetes mellitus	27 (17.4)
Hypertension	12 (7.7)
Corrected body mass index in kg/m^2, mean ± SD	20.25 ± 3.40
Obesity	16 (10.3)
Chronic kidney disease	9 (5.8)
Serum total bilirubin in mg/dL, median (range)	2.7 (0.3-28.5)
Serum AST, median (range) U/L	60 (18-672)
Serum ALT, median (range) U/L	32 (4-250)
Serum total protein, mean ± SD mg/dL	5.8 ± 0.7
Serum albumin, mean ± SD mg/dL	2.5 ± 0.5
Hypoproteinemia	42 (27.0%)
INR, median (range)	1.6 (0.4-6.7)
Serum sodium, mean ± SD meq/L	130.0 ± 6.5
Serum potassium, mean ± SD meq/L	4.07 ± 2.40
Serum urea in mg/dL, median (range)	34 (12-191)
Creatinine in mg/dL, median (range)	0.9 (0.3-3.7)
Hemoglobin in g/dL, mean ± SD	8.8 (4.2-65.0)
Total leukocyte count as /cmm, median (range)	6130 (1750-34300)
Lymphocytopenia	72 (46.5)
Esophageal varices	
None	3 (1.9)
Small grade	92 (59.4)
Large grade	60 (38.0)
PHG	
Mild	118 (76.0)
Severe	35 (22.6)
PHD	31 (20.0)
Ascitic fluid analysis	
Cell count as /μL, median (range)	100 (0-11200)
Protein median in gm/dL, median (range)	1.1 (0.1-3.5)

ADA in U/L, median (range)	10 (1-33)
Triglycerides in mg/dL, median (range)	19.5 (0-224.0)
SAAG, median (range)	2.0 (1.2-4.0)

ADA: Adenosine deaminase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; cmm: Cubic millimeter; INR: International normalized ratio; MELD: Model for end-stage liver disease; NASH: Non-alcoholic steatohepatitis; PHG: Portal hypertensive gastropathy; PHD: Portal hypertensive duodenopathy; RA: Refractory ascites; SAAG: Serum ascites albumin gradient.



DOI: 10.4254/wjh.v15.i10.1140

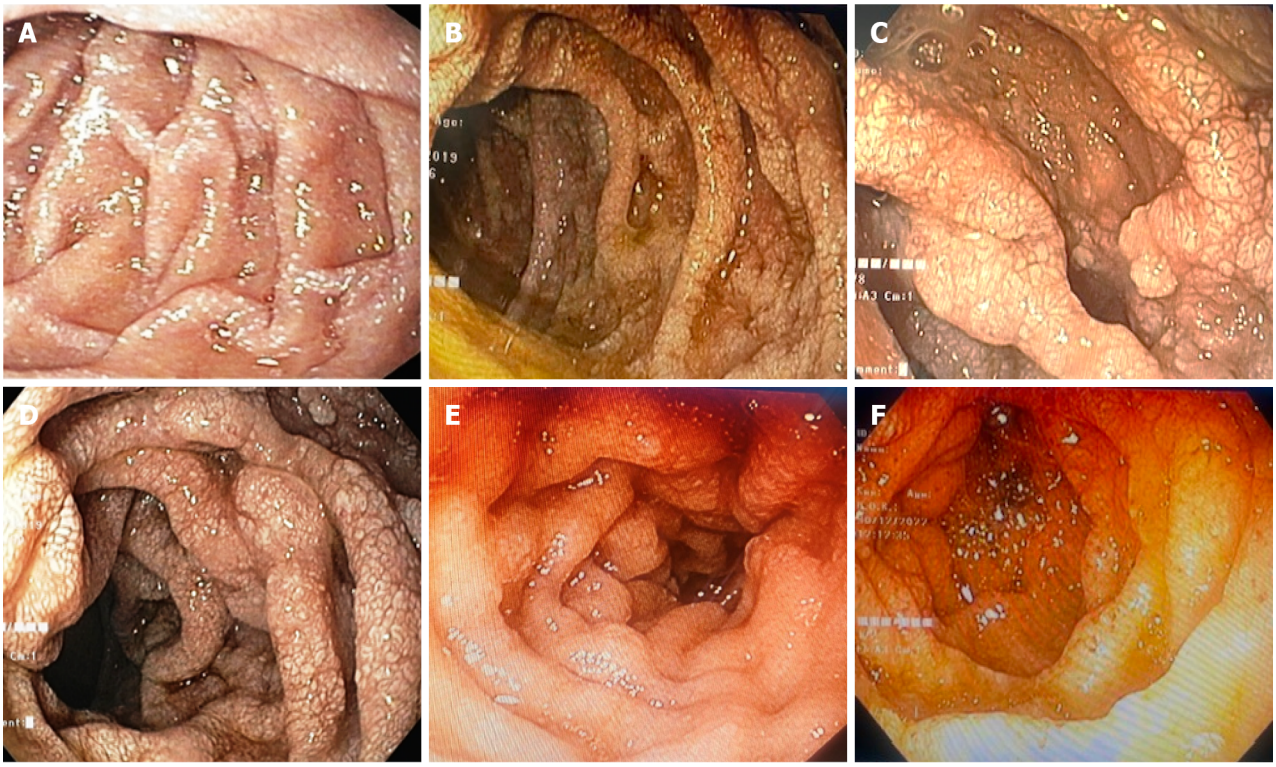
Figure 2 Representative images showing two distinct patterns of peripheral edema among cirrhosis patients with refractory ascites. A and B: Physical appearance of peripheral edema revealed two distinct patterns. In the majority of patients, edema was pitting type and skin texture was smooth (A), which is a finding consistent with edema due to the hydrostatic-oncotic pressure imbalance. In about one-third of patients, edema was severe, mostly non-pitting type with skin texture showing exaggerated dorsal skin creases, hyperkeratosis, and peau d'orange appearance (B), suggesting lymphedema. Some patients with lymphedema also had evidence of cellulitis.

Thus, a total of 69 (44.5%) cirrhosis patients with RA had evidence of lymphatic dysfunction in the forms of lymphedema, IL, and CA either alone or in combination. Lymphedema, present in 52 (33.5%) patients, was the most common manifestation of lymphatic dysfunction. Twenty-six patients had only lymphedema, 18 patients had only IL, 24 patients had both lymphedema and IL, and 2 patients had lymphedema as well as IL and CA.

Clinical characteristics and association of lymphatic dysfunction

Clinically, lymphedema was the most relevant marker of lymphatic dysfunction in the study population. Compared to patients without lymphedema, those with lymphedema were older (mean age of 53.3 years *vs* 47.7 years, $P = 0.007$), had more severe liver disease (median MELD scores of 21 *vs* 14, $P < 0.001$), and had a greater proportion of obese cirrhosis (21.0% *vs* 5.8%, $P = 0.012$). Patients with lymphedema had a higher prevalence of PHD (32.7% *vs* 13.6%, $P = 0.001$) and histopathological evidence of IL (46.2% *vs* 17.5%, $P < 0.001$) than those without it. Furthermore, the mean ascitic fluid triglyceride levels ($P = 0.006$), the proportion of lymphocytopenia (73.0% *vs* 33.0%, $P < 0.001$), and hypoproteinemia (50.0% *vs* 15.5%, $P < 0.001$) were significantly higher in patients with lymphedema than those without it (Supplementary Table 1). Lower limb cellulitis was noted in 7 (13.0%) patients with lymphedema *vs* none in patients where lymphedema was absent.

Patients with IL had a higher prevalence of lymphedema than those without IL (57.1% *vs* 23.6%, $P < 0.001$). The frequency of lymphocytopenia and hypoproteinemia was higher in patients with IL than those without it (28.6% *vs* 9.1%, $P = 0.004$). However, IL was not associated with age, severity of liver cirrhosis, or metabolic comorbidities. With regard to the only 2 patients with CA, the levels of triglyceride in their ascitic fluid were observed at 137 mg/dL and 224 mg/dL. Both patients were male, diabetic, and had high Child-Pugh scores (13 and 12). Both had evidence of IL on endoscopy and D2 biopsy.



DOI: 10.4254/wjh.v15.i10.1140

Figure 3 Endoscopic evaluation of study population. A-F: Representative endoscopic images of duodenum of study population showing portal hypertensive duodenopathy with evidence of intestinal lymphangiectasia visualized as scattered white spots suggestive of dilated lacteals.

Comparison of clinical and laboratory characteristics of patients with and without lymphatic dysfunctions

The mean age (52.0 ± 13.8 *vs* 47.8 ± 12.3, $P < 0.04$) and proportion of obese cirrhosis (16.0% *vs* 5.8%) were higher in patients with lymphatic dysfunction than those without it. The median duration of RA (6 mo *vs* 4 mo, $P = 0.02$), median MELD score (18 *vs* 14, $P = 0.003$), and mean Child-Pugh score (10.0 ± 1.9 *vs* 9.9±1.6) were similarly higher in patients with lymphatic dysfunction (Table 2). Among the patients with lymphatic dysfunction, lymphopenia was noted in 49 (79.0%) patients, hypoproteinemia in 29 (42.0%) patients, and a combined hypoproteinemia plus lymphopenia in 21 (30.0%) patients. The values of serum bilirubin, serum aspartate aminotransferase, serum alanine aminotransferase, and international normalized ratio were higher, while serum sodium was lower in patients with lymphatic dysfunction compared to those without it.

Independent associations of lymphatic dysfunction

On MVA (Table 3), factors independently associated with lymphatic dysfunction included obesity (OR: 4.2, 95%CI: 1.1–15.2, $P = 0.027$), lymphocytopenia (OR: 6.2, 95%CI: 2.9–13.2, $P < 0.001$), and hypoproteinemia (OR: 3.7, 95%CI: 1.5–8.8, $P = 0.003$). When independent predictors of only lymphedema were assessed by MVA, age (OR: 1.06, $P = 0.002$) and Child-Pugh scores (OR: 1.82, $P = 0.005$) were found to be significant, apart from obesity (OR: 6.3, $P = 0.012$), lymphocytopenia (OR: 3.5, $P = 0.01$), and hypoproteinemia (OR: 7.1, $P = 0.001$) (Table 4).

DISCUSSION

Our study was the first to assess the characteristics of lymphatic dysfunction in liver cirrhosis patients who have RA, an extreme form of fluid accumulation. We found evidence of lymphatic dysfunction in nearly half (44.5%) of the patients. The spectrum of dysfunctions included peripheral lymphedema (33.5%), IL (27.0%), and CA (1.3%). Obesity, lymphocytopenia, and hypoproteinemia independently predicted the presence of lymphatic dysfunction in such patients. Additionally, higher mean ages and Child-Pugh scores were independently associated with peripheral lymphedema, the most common manifestation of lymphatic dysfunctions.

Portal pressure in cirrhosis patients positively correlated with lymphatic flow[17,18]. As cirrhosis progresses, functional deficiencies in the lymphatic system emerge, causing flow stagnation and leakage of lymph from the ectatic lymphatic system[4,5]. These changes lay the ground for the lymphedema development. Our study detected evidence of lymphedema in one-third of cirrhosis patients with RA. Lymphedema should be common in patients with advanced cirrhosis, given the lymphatic failure that often follows cirrhosis, yet there is a dearth of research on it in the existing literature. It is difficult to distinguish early lymphedema from edema due to the change in plasma hydrostatic-oncotic pressure balance. The presence of physical signs, such as a peau d'orange appearance and the positive Stemmer sign, may

Table 2 Comparison of clinical and laboratory characteristics of patients with and without clinically evident lymphatic dysfunction (Univariate analysis)

Parameters	LD present, *n* = 69	LD absent, *n* = 86	*P* value
Age in yr, mean ± SD	52.0 ± 13.8	47.8 ± 12.3	0.040
Male, *n* (%)	56 (81.2%)	63 (73.3%)	0.320
Etiology of cirrhosis, *n* (%)			0.050
Alcohol	22 (31.8%)	36 (41.8%)	
Hepatitis	14 (20.2%)	9 (10.4%)	
Hepatitis C	01 (1.4%)	6 (7.0%)	
NASH	28 (40.5%)	27 (31.3%)	
Others	04 (5.7%)	10 (11.6%)	
Duration of RA in mo, median (range)	06 (5-48)	4 (3-36)	0.020
MELD scores, median (range)	18 (7-42)	14 (6-38)	0.003
Child-Pugh score, mean ± SD	10 ± 1.9	9.9 ± 1.6	0.001
Diabetes mellitus, *n* (%)	15 (21.7%)	12 (14.0%)	0.280
Hypertension, *n* (%)	06 (8.7%)	6 (7.0%)	0.760
Corrected BMI in kg/m^2, mean ± SD	22.2 ± 2.3	20.8 ± 4.6	0.060
Obesity, *n* (%)	11 (16.0%)	5 (5.8%)	0.030
Chronic kidney disease, *n* (%)	4 (5.8%)	5 (5.8%)	1.000
Serum total bilirubin in mg/dL, median (range)	3.6 (0.3-28.5)	2.1 (0.4-26.4)	0.040
Serum AST in U/L, median (range)	67 (18-672)	56 (22-238)	0.010
Serum ALT in U/L, median (range)	34 (4-250)	30 (7-202)	0.020
Serum total protein in mg/dL, mean ± SD	5.6 ± 0.6	6.0 ± 0.8	0.001
Severe hypoproteinemia, *n* (%)	29 (42.0%)	13 (15.1%)	< 0.001
Albumin in mg/dL, mean ± SD	2.4 ± 0.5	2.6 ± 0.5	0.005
INR, median (range)	1.8 (0.8-3.8)	1.5 (0.4-6.7)	0.007
Serum sodium in meq/L, mean ± SD	128.0 ± 7.2	131.0 ± 5.5	0.001
Serum potassium in meq/L, mean ± SD	4.2 ± 1.2	3.9 ± 2.4	0.130
Serum urea, median (range) mg/dL	42 (12-191)	31 (12-188)	0.060
Creatinine in mg/dL, median (range)	1.0 (0.4-2.9)	0.9 (0.3-3.7)	0.160
Hemoglobin in g/dL, mean ± SD	8.6 (5.3-12.3)	8.8 (4.2-15)	0.360
Total leukocyte count as /cmm, median (range)	7000 (1750-34300)	5550 (2050-12680)	0.960
Lymphocytopenia, *n* (%)	49 (79.0%)	23 (26.7%)	< 0.001
Combined lymphocytopenia and hypoproteinemia, *n* (%)	21 (30.4%)	2 (2.3%)	< 0.001
Esophageal varices, *n* (%)			0.610
None	02 (2.9%)	1 (1.2%)	
Small grade	40 (58.0%)	52 (60.5%)	
Large grade	27 (39.1%)	33 (38.4%)	
PHG, *n* (%)			0.025
Mild	46 (66.7%)	72 (84.0%)	
Severe	21 (30.4%)	14 (16.0%)	
PHD, *n* (%)	22 (31.8%)	9 (10.4%)	

Ascitic fluid analysis			
Cell count as /μL, median (range)	150 (0-3500)	100 (0-1120)	0.970
Protein in gm/dL, median (range)	0.9 (0.1-2.5)	1.1 (0.6-3.5)	0.370
ADA in U/L, median (range)	8.5 (1.0-33.0)	11.0 (2.0-30.0)	0.200
Triglycerides in mg/dL, median (range)	23 (2-224)	18 (0-64)	0.070
SAAG, median (range)	1.8 (1.2-4.0)	2.1 (1.2-3.5)	0.390
Lower limb cellulitis	7 (10.0%)	0	0.003

ADA: Adenosine deaminase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; BMI: Body mass index; cmm: Cubic millimeter; INR: International normalized ratio; LD: Lymphatic dysfunction; MELD: Model for end-stage liver disease; NASH: Non-alcoholic steatohepatitis; PHG: Portal hypertensive gastropathy; PHD: Portal hypertensive duodenopathy; RA: Refractory ascites; SAAG: Serum ascites albumin gradient.

Table 3 Multivariate regression analysis for determining independent associations of lymphatic dysfunction in cirrhosis patients with refractory ascites

Parameters	*P* value	OR	95%CI
Obesity	0.027	4.2	1.1-15.2
Lymphocytopenia	< 0.001	6.2	2.9-13.2
Hypoproteinemia	0.003	3.7	1.5-8.8

95%CI: 95% Confidence interval; OR: Odds ratio.

Table 4 Multivariate analysis for determining independent associations of lymphedema

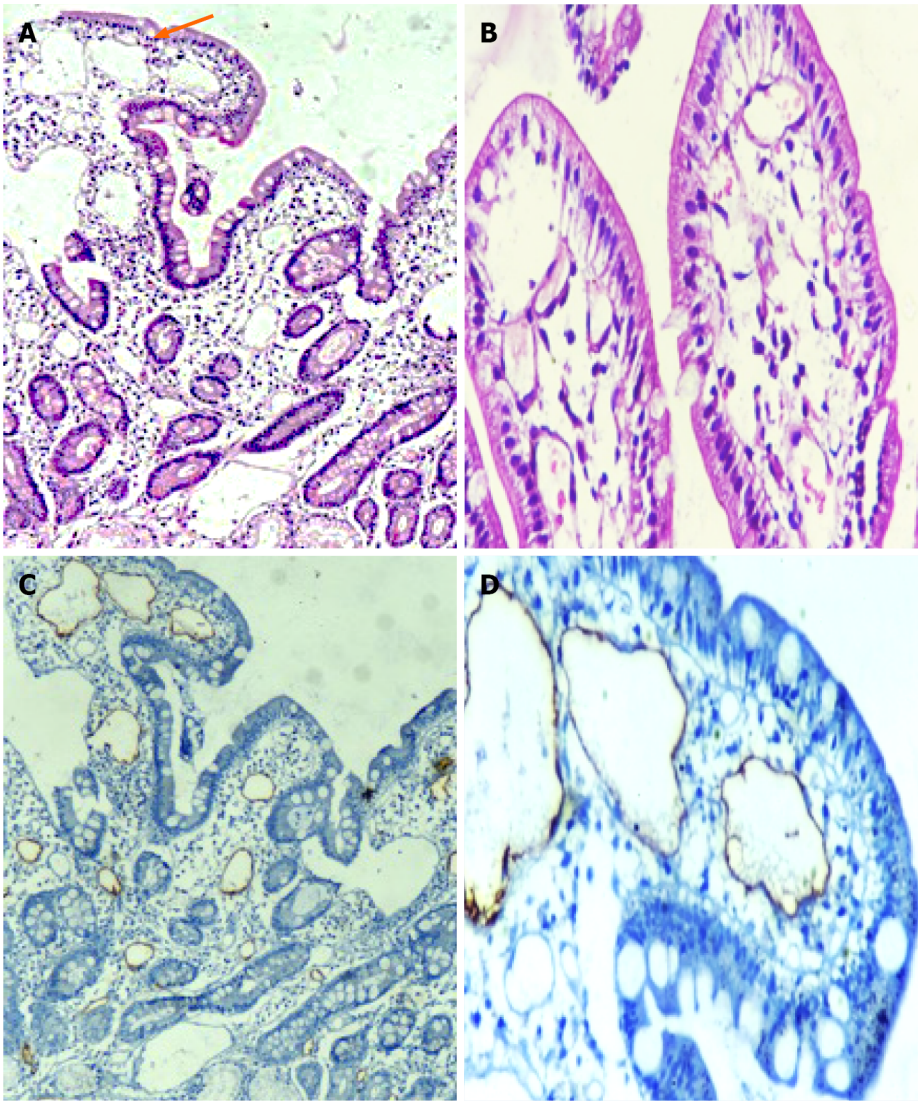
Parameters	*P* value	OR	95%CI
Age	0.002	1.06	1.02-1.10
Child-Pugh score	0.005	1.82	1.19-2.79
Obesity	0.012	6.33	1.49-26.90
Hypoproteinemia	0.001	7.10	2.44-20.70
Lymphocytopenia	0.011	3.55	1.34-9.38

95%CI: 95% Confidence interval; OR: Odds ratio.

reflect the advanced stage of lymphedema. Patients with lymphedema are more susceptible to cellulitis due to their hyperkeratotic surface, deep fissures, stagnant lymph, and weakened immunity. In our study, 13% of lymphedema patients had lower limb cellulitis, compared to none in those who did not have lymphedema. In fact, lymphedema is considered the most important risk factor for cellulitis[19].

In our study, 31 (20.0%) patients revealed evidence of PHD on endoscopy, though only 8 (5.1%) patients had macroscopic evidence of IL. PHD in cirrhosis has been reported at 8.4% by Menchén *et al*[20], 14% by Misra *et al*[21], and 51% by Barakat *et al*[22] in earlier studies. No study has so far reported the endoscopic prevalence of IL in cirrhosis patients. Notably, 27.0% of the study subjects had evidence of IL only on histological examination of D2 biopsy specimens. Very few studies have examined histopathological changes in the duodenum of cirrhosis patients[15,21,22]. Barakat *et al*[22] reported marked capillary congestion and capillary angiogenesis in duodenal mucosa of cirrhosis patients. The changes were mostly marked in the subepithelial location, which on the immunohistochemical stain was CD34 positive. Notably, CD34 is a pan-endothelial marker that can also be positive in the lymphatic endothelium[23]. As a selective marker of lymphatic endothelium (D2-40) was not used in that study, it is possible that IL could have been misinterpreted as capillary congestion and angiogenesis. In a recent prospective study, IL was found to be significantly higher in patients with decompensated cirrhosis than in compensated cirrhosis, and the density of IL on duodenal biopsy was associated with systemic inflammation and 3-mo mortality[24]. It is believed that IL in cirrhosis results from a persistent rise in lymphatic pressure secondary to PHT. However, it is unclear why IL does not manifest in all cirrhotic patients despite sustained PHT.

Because intestinal lymph contains proteins, chylomicrons, and lymphocytes, rupture of IL with subsequent leakage of lymph into the intestine can lead to hypoproteinemia and lymphocytopenia[4,5]. Regardless of the presence of IL, study



DOI: 10.4254/wjh.v15.i10.1140

Figure 4 Histological examination of second part of duodenum biopsy specimens. A-D: Representative histopathological images (hematoxylin and eosin) of duodenum showing markedly dilated vessels in the lamina propria (A and B), which on immunohistochemistry showing strong D2-40 positivity (C and D), confirming the presence of intestinal lymphangiectasia.

participants with lymphatic dysfunction displayed lymphocytopenia in 79.0% of cases and hypoproteinemia in 42.0% of the cases, indicating that lymphatic dysfunction at non-enteric sites can also result in loss of lymphocytes and protein from circulation. CA, which was observed in 2 patients, is a rare complication caused by rupture of subserosal lymphatic vessels secondary to a sustained high portal pressure[25]. As intestinal lymph contains triglyceride-rich fat droplets (chylomicrons), CA appear milky in color. Notably, the rupture of hepatic lymphatics does not produce CA as it is devoid of fat droplets. Although < 1% of cirrhosis patients develop CA, cirrhosis has been attributed to 11% of atraumatic CA cases[11,26,27].

Our study revealed several risk factors and indicators of lymphatic dysfunction, including older age, higher MELD and Child-Pugh scores, obesity, lymphocytopenia, and hypoproteinemia, which may point to lymphatic dysfunction in a given patient. It is well recognized that aging alters the structure and function of the lymphatic system. Important aging-related lymphatic alterations include impaired contractile function, decreased nitric oxide lymphatic collectors, and loss of endothelial glycocalyx[28]. Similarly, recent evidence suggests that obesity can significantly impair lymphatic function and increase risk of lymphedema, whereas losing weight can enhance lymphatic functioning[29]. Moreover, lymphatic dysfunction is also involved in the pathogenesis of obesity and obesity-related chronic inflammation[30].

The pathophysiological process underlying lymphatic dysfunction in patients with cirrhosis needs to be explored at the molecular level. In addition to old age and obesity, other variables affecting lymphatic function in patients with cirrhosis include diabetes, dyslipidemia, neurohormonal alterations, and chronic inflammation[4,31,32]. Intestinal lymphatic function may be impacted by intestinal dysmotility and intestinal dysbiosis typically found in advanced cirrhosis[33,34]. Ribera *et al*[5] discovered that excess nitric oxide production by lymphatic endothelial cells was responsible for poor lymphatic drainage in cirrhotic rats with ascites. Interestingly, when these rats were given a nitric oxide synthase inhibitor, lymphatic drainage was improved and the ascitic volume was much reduced, suggesting an influential role for nitric oxide in the dysfunction of the lymphatic system.

From a therapeutic standpoint, the lymphatic dysfunction in cirrhosis patients with RA are worth evaluating because it would call for using different strategies for fluid mobilization. Some clinical and experimental studies have found improved ascites with dietary changes, VEGF C, and nitric oxide synthase inhibitors in cirrhosis subjects with evident lymphatic dysfunction[8,14,15]. Thus, future efforts to mobilize fluid in these patients might focus on methods to improve lymphatic drainage. Unfortunately, there is no recommendation on how to diagnose and evaluate lymphatic functions in patients with cirrhosis. There are many imaging techniques, such as lymphoscintigraphy and magnetic resonance lymphography, but they are frequently constrained by poor resolution, a lack of standardization, the need for invasive procedures, the danger of radiation exposure, and a lack of accessibility[4]. Low attenuation rims surrounding the portal veins and the intrahepatic vena cava on the computed tomography scan correspond to the lymph congestion secondary to impaired lymphatic drainage[35]. However, the clinical implications of these findings in cirrhotic patients need to be determined. In light of these limitations, surrogate markers of lymphatic dysfunction based on physical examination, routine blood investigations, and endoscopy can be an important leap forward.

Our study data was novel, relevant, and generalizable. It sheds light on a well-known but understudied area that calls for further research to determine the role of lymphatics in the complication of liver cirrhosis. It would be interesting to further investigate whether use of prolymphangiogenic substances like VEGF C and D can enhance lymphatic functioning and fluid mobilization in patients with advanced cirrhosis. It would also be worthwhile investigating whether the placement of a transjugular intrahepatic portosystemic shunt aids in improving lymphatic functions in cirrhosis patients, given the involvement of PHT in lymphatic stasis and leakage.

There were some limitations in our study. First, our study was an association study, so causal inference cannot be drawn. We also did not use any lymphangiographic methods to demonstrate lymphatic stasis or leakage. Further, the diagnosis of lymphedema based on physical characteristics has inherent limitations and is at risk of subjective bias and misclassification error in borderline cases. Hence, further studies are recommended to address such gaps.

CONCLUSION

In conclusion, patients with advanced liver cirrhosis frequently exhibit signs of overt lymphatic dysfunction. Given the crucial role of the lymphatic system in volume management, its failure may be at the root of many complications of cirrhosis, including RA. Therefore, addressing the lymphatic system in patients with liver cirrhosis may offer a novel strategy in decongesting tissue and improving outcomes.

ARTICLE HIGHLIGHTS

Research background

Lymphatic dysfunction occurs in patients with liver cirrhosis, although the published data on this subject is extremely limited. Given the significant role the lymphatic system plays in maintaining the balance of body fluids, it is reasonable to assume that a dysfunctional lymphatic system may contribute to the refractoriness of ascites and edema in cirrhosis patients.

Research motivation

From a therapeutic standpoint, it can be extremely important to evaluate lymphatic dysfunction in cirrhosis patients with refractory ascites (RA) since it would call for using different strategies for fluid mobilization.

Research objectives

The objectives of this study were to assess the magnitude, spectrum, and clinical associations of lymphatic dysfunction in cirrhosis patients with RA using surrogate markers such as lymphedema, intestinal lymphangiectasia (IL), and chylous ascites (CA).

Research methods

This observational study was conducted as an exploratory project with a cross-sectional design and included 155 consecutive cirrhosis patients with RA. The presence of clinical signs of lymphedema, IL on duodenal biopsy, and CA were used as surrogate markers of lymphatic dysfunction.

Research results

The study found evidence of lymphatic dysfunction in nearly half of the cirrhosis patients with RA. The spectrum of dysfunction included peripheral lymphedema in 33.5%, intestinal IL in 27.0%, and CA in 1.3%. Obesity, lymphocytopenia, and hypoproteinemia were independently associated with the presence of lymphatic dysfunction in such patients.

Research conclusions

Lymphatic dysfunction is common in cirrhosis patients with RA. Hypoproteinemia and lymphocytopenia are significant indicators of its presence.

Research perspectives

Evaluation of lymphatic dysfunction in cirrhosis patients with RA can serve as a guide for future research into novel approaches for tissue decongestion.

FOOTNOTES

Author contributions: Arya R and Kumar R designed the manuscript, collected data, and wrote the manuscript; Kumar T contributed to the histopathological examination and data collection; Kumar S, Anand U, Priyadarshi RN, and Maji T collected data and provided critical input for the manuscript.

Institutional review board statement: The study was reviewed and approved by the Institute research board (Reference No: AIIMS/Pat/IRC/2020/PGTh/Jan 21-22).

Informed consent statement: Informed written consent was obtained from all study participants.

Conflict-of-interest statement: The authors declare that they have no conflicts of interest for this article.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement checklist of items, and the manuscript was prepared and revised according to the STROBE Statement checklist of items.

Country/Territory of origin: India

ORCID number: Rahul Arya 0000-0003-3714-0990; Tarun Kumar 0000-0001-5554-3975; Sudhir Kumar 0000-0001-7117-1382; Utpal Anand 0000-0003-0653-4129; Rajeev Nayan Priyadarshi 0000-0003-2890-8910; Tanmoy Maji 0000-0002-5115-3563.

S-Editor: Lin C
L-Editor: Filipodia
P-Editor: Lin C

REFERENCES

1 **Olszewski WL**. The lymphatic system in body homeostasis: physiological conditions. *Lymphat Res Biol* 2003; **1**: 11-21; discussion 21 [PMID: 15624317 DOI: 10.1089/15396850360495655]
2 **Vollmar B**, Wolf B, Siegmund S, Katsen AD, Menger MD. Lymph vessel expansion and function in the development of hepatic fibrosis and cirrhosis. *Am J Pathol* 1997; **151**: 169-175 [PMID: 9212743]
3 **Chung C**, Iwakiri Y. The lymphatic vascular system in liver diseases: its role in ascites formation. *Clin Mol Hepatol* 2013; **19**: 99-104 [PMID: 23837133 DOI: 10.3350/cmh.2013.19.2.99]
4 **Kumar R**, Anand U, Priyadarshi RN. Lymphatic dysfunction in advanced cirrhosis: Contextual perspective and clinical implications. *World J Hepatol* 2021; **13**: 300-314 [PMID: 33815674 DOI: 10.4254/wjh.v13.i3.300]
5 **Ribera J**, Pauta M, Melgar-Lesmes P, Tugues S, Fernández-Varo G, Held KF, Soria G, Tudela R, Planas AM, Fernández-Hernando C, Arroyo V, Jiménez W, Morales-Ruiz M. Increased nitric oxide production in lymphatic endothelial cells causes impairment of lymphatic drainage in cirrhotic rats. *Gut* 2013; **62**: 138-145 [PMID: 22267600 DOI: 10.1136/gutjnl-2011-300703]
6 **Browse NL**, Stewart G. Lymphoedema: pathophysiology and classification. *J Cardiovasc Surg (Torino)* 1985; **26**: 91-106 [PMID: 3884629]
7 **Aller MA**, Prieto I, Argudo S, de Vicente F, Santamaría L, de Miguel MP, Arias JL, Arias J. The interstitial lymphatic peritoneal mesothelium axis in portal hypertensive ascites: when in danger, go back to the sea. *Int J Inflam* 2010; **2010**: 148689 [PMID: 21152120 DOI: 10.4061/2010/148689]
8 **Tanaka M**, Iwakiri Y. The Hepatic Lymphatic Vascular System: Structure, Function, Markers, and Lymphangiogenesis. *Cell Mol Gastroenterol Hepatol* 2016; **2**: 733-749 [PMID: 28105461 DOI: 10.1016/j.jcmgh.2016.09.002]
9 **Chindaratana K**, Tanpowpong P, Lertudomphonwanit C, Treepongkaruna S. Gastrointestinal protein loss in children with portal hypertension. *Indian J Gastroenterol* 2021; **40**: 333-337 [PMID: 32970314 DOI: 10.1007/s12664-020-01079-y]
10 **Kumar R**, Kumar T, Anand U, Priyadarshi RN. Intestinal Lymphangiectasia Associated With Refractory Ascites in a Cirrhosis Patient. *Cureus* 2021; **13**: e12567 [PMID: 33564554 DOI: 10.7759/cureus.12567]
11 **Cheng WS**, Gough IR, Ward M, Croese J, Powell LW. Chylous ascites in cirrhosis: a case report and review of the literature. *J Gastroenterol Hepatol* 1989; **4**: 95-99 [PMID: 2490947 DOI: 10.1111/j.1440-1746.1989.tb00811.x]
12 **Freeman HJ**, Nimmo M. Intestinal lymphangiectasia in adults. *World J Gastrointest Oncol* 2011; **3**: 19-23 [PMID: 21364842 DOI: 10.4251/wjgo.v3.i2.19]
13 **Wong F**. Management of refractory ascites. *Clin Mol Hepatol* 2023; **29**: 16-32 [PMID: 35676862 DOI: 10.3350/cmh.2022.0104]

14 **Milazzo L**, Peri AM, Lodi L, Gubertini G, Ridolfo AL, Antinori S. Intestinal lymphangiectasia and reversible high liver stiffness. *Hepatology* 2014; **60**: 759-761 [PMID: 24449480 DOI: 10.1002/hep.27025]

15 **Juneja P**, Ruhina Rahman SN, Jakhar D, Mourya AK, Tripathi DM, Kaur I, Tiwari V, Rohilla S, Gupta A, Rawal P, Baweja S, Rastogi A, Naidu VGM, Sarin SK, Banerjee S, Kaur S. Recombinant VEGF-C (Cys156Ser) improves mesenteric lymphatic drainage and gut immune surveillance in experimental cirrhosis. *JHEP Rep* 2023; **5**: 100816 [PMID: 37663117 DOI: 10.1016/j.jhepr.2023.100816]

16 **Fukui H**, Kawaratani H, Kaji K, Takaya H, Yoshiji H. Management of refractory cirrhotic ascites: challenges and solutions. *Hepat Med* 2018; **10**: 55-71 [PMID: 30013405 DOI: 10.2147/HMER.S136578]

17 **Barrowman JA**, Granger DN. Effects of experimental cirrhosis on splanchnic microvascular fluid and solute exchange in the rat. *Gastroenterology* 1984; **87**: 165-172 [PMID: 6724260]

18 **Yokomori H**, Oda M, Kaneko F, Kawachi S, Tanabe M, Yoshimura K, Kitagawa Y, Hibi T. Lymphatic marker podoplanin/D2-40 in human advanced cirrhotic liver--re-evaluations of microlymphatic abnormalities. *BMC Gastroenterol* 2010; **10**: 131 [PMID: 21059220 DOI: 10.1186/1471-230X-10-131]

19 **Dupuy A**, Benchikhi H, Roujeau JC, Bernard P, Vaillant L, Chosidow O, Sassolas B, Guillaume JC, Grob JJ, Bastuji-Garin S. Risk factors for erysipelas of the leg (cellulitis): case-control study. *BMJ* 1999; **318**: 1591-1594 [PMID: 10364117 DOI: 10.1136/bmj.318.7198.1591]

20 **Menchén L**, Ripoll C, Marín-Jiménez I, Colón A, Gómez-Camarero J, González-Asanza C, Menchén P, Cos E, Bañares R. Prevalence of portal hypertensive duodenopathy in cirrhosis: clinical and haemodynamic features. *Eur J Gastroenterol Hepatol* 2006; **18**: 649-653 [PMID: 16702855 DOI: 10.1097/00042737-200606000-00012]

21 **Misra V**, Misra SP, Dwivedi M, Gupta SC. Histomorphometric study of portal hypertensive enteropathy. *Am J Clin Pathol* 1997; **108**: 652-657 [PMID: 9384446 DOI: 10.1093/ajcp/108.6.652]

22 **Barakat M**, Mostafa M, Mahran Z, Soliman AG. Portal hypertensive duodenopathy: clinical, endoscopic, and histopathologic profiles. *Am J Gastroenterol* 2007; **102**: 2793-2802 [PMID: 17900330 DOI: 10.1111/j.1572-0241.2007.01536.x]

23 **Basha BM**, Hsi ED. Immunohistochemical Analysis of Endothelial Cells in Vascular Transformation of Lymph Node Sinuses: Vascular or Lymphatic Differentiation? *Appl Immunohistochem Mol Morphol* 2019; **27**: 482-489 [PMID: 29629951 DOI: 10.1097/PAI.0000000000000661]

24 **Juneja P**, Sharma A, Shasthry SM, Kumar G, Tripathi DM, Rajan V, Rastogi A, Sarin SK, Kaur S. Podoplanin-positive dilated lymphatic vessels in duodenum associates with three-month mortality in patients with cirrhosis. *Front Physiol* 2023; **14**: 1045983 [PMID: 37304826 DOI: 10.3389/fphys.2023.1045983]

25 **Lizaola B**, Bonder A, Trivedi HD, Tapper EB, Cardenas A. Review article: the diagnostic approach and current management of chylous ascites. *Aliment Pharmacol Ther* 2017; **46**: 816-824 [PMID: 28892178 DOI: 10.1111/apt.14284]

26 **Tsauo J**, Shin JH, Han K, Yoon HK, Ko GY, Ko HK, Gwon DI. Transjugular Intrahepatic Portosystemic Shunt for the Treatment of Chylothorax and Chylous Ascites in Cirrhosis: A Case Report and Systematic Review of the Literature. *J Vasc Interv Radiol* 2016; **27**: 112-116 [PMID: 26723922 DOI: 10.1016/j.jvir.2015.09.022]

27 **Steinemann DC**, Dindo D, Clavien PA, Nocito A. Atraumatic chylous ascites: systematic review on symptoms and causes. *J Am Coll Surg* 2011; **212**: 899-905.e1 [PMID: 21398159 DOI: 10.1016/j.jamcollsurg.2011.01.010]

28 **Shang T**, Liang J, Kapron CM, Liu J. Pathophysiology of aged lymphatic vessels. *Aging (Albany NY)* 2019; **11**: 6602-6613 [PMID: 31461408 DOI: 10.18632/aging.102213]

29 **Kataru RP**, Park HJ, Baik JE, Li C, Shin J, Mehrara BJ. Regulation of Lymphatic Function in Obesity. *Front Physiol* 2020; **11**: 459 [PMID: 32499718 DOI: 10.3389/fphys.2020.00459]

30 **Jiang X**, Tian W, Nicolls MR, Rockson SG. The Lymphatic System in Obesity, Insulin Resistance, and Cardiovascular Diseases. *Front Physiol* 2019; **10**: 1402 [PMID: 31798464 DOI: 10.3389/fphys.2019.01402]

31 **Davis MJ**, Lane MM, Davis AM, Durtschi D, Zawieja DC, Muthuchamy M, Gashev AA. Modulation of lymphatic muscle contractility by the neuropeptide substance P. *Am J Physiol Heart Circ Physiol* 2008; **295**: H587-H597 [PMID: 18539752 DOI: 10.1152/ajpheart.01029.2007]

32 **Rehal S**, Blanckaert P, Roizes S, von der Weid PY. Characterization of biosynthesis and modes of action of prostaglandin E2 and prostacyclin in guinea pig mesenteric lymphatic vessels. *Br J Pharmacol* 2009; **158**: 1961-1970 [PMID: 19922540 DOI: 10.1111/j.1476-5381.2009.00493.x]

33 **Unthank JL**, Bohlen HG. Lymphatic pathways and role of valves in lymph propulsion from small intestine. *Am J Physiol* 1988; **254**: G389-G398 [PMID: 3348405 DOI: 10.1152/ajpgi.1988.254.3.G389]

34 **Suh SH**, Choe K, Hong SP, Jeong SH, Mäkinen T, Kim KS, Alitalo K, Surh CD, Koh GY, Song JH. Gut microbiota regulates lacteal integrity by inducing VEGF-C in intestinal villus macrophages. *EMBO Rep* 2019; **20** [PMID: 30783017 DOI: 10.15252/embr.201846927]

35 **Aspestrand F**, Schrumpf E, Jacobsen M, Hanssen L, Endresen K. Increased lymphatic flow from the liver in different intra- and extrahepatic diseases demonstrated by CT. *J Comput Assist Tomogr* 1991; **15**: 550-554 [PMID: 2061465 DOI: 10.1097/00004728-199107000-00004]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1153-1163

DOI: 10.4254/wjh.v15.i10.1153

ISSN 1948-5182 (online)

SYSTEMATIC REVIEWS

Exercise training as an intervention for frailty in cirrhotic patients on the liver transplant waiting list: A systematic review

Thais Mellato Loschi, Melline D T A Baccan, Bianca Della Guardia, Paulo N Martins, Amanda P C S Boteon, Yuri L Boteon

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): 0
Grade C (Good): C
Grade D (Fair): 0
Grade E (Poor): 0

P-Reviewer: Chang KV, Taiwan

Received: August 25, 2023
Peer-review started: August 25, 2023
First decision: September 15, 2023
Revised: September 21, 2023
Accepted: October 8, 2023
Article in press: October 8, 2023
Published online: October 27, 2023

Thais Mellato Loschi, Melline D T A Baccan, Bianca Della Guardia, Amanda P C S Boteon, Yuri L Boteon, Transplant Centre, Hospital Israelita Albert Einstein, São Paulo 05652-900, Brazil

Thais Mellato Loschi, Yuri L Boteon, Instituto Israelita de Ensino e Pesquisa Albert Einstein, Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo 05652-900, Brazil

Paulo N Martins, Department of Surgery, Transplant Division, University of Massachusetts Medical School, Worcester, MA 01655, United States

Corresponding author: Yuri L Boteon, FACS, MD, PhD, Doctor, Professor, Surgeon, Transplant Centre, Hospital Israelita Albert Einstein, 2nd Floor, Building A1, Office 200B, 627/701 Albert Einstein Avenue, São Paulo 05652-900, Brazil. yuri.boteon@einstein.br

Abstract

BACKGROUND

The existing literature suggests that exercise for cirrhotic patients is safe and favours significant improvement to their physical capacity. However, exercise training for this population and how to deliver activities, especially in severe stages of the disease and while waiting for a liver transplant (LT), remain undefined.

AIM

To review the existing exercise prescriptions for cirrhotic patients on the waiting list for LT, their results for frailty evolution and their effect on clinical outcomes.

METHODS

A systematic review was performed following the Preferred Reporting Review and Meta-Analysis guidelines and searching the PubMed, MEDLINE, and Scopus databases. The keyword "liver transplant" was used in combination with the free terms "frailty" and "exercise" for the literature review. Clinical studies that evaluated the effect of a regular training program, independent of supervision or the duration or intensity of physical exercise, in cirrhotic patients on the waiting list for LT were reviewed. The data on safe physical activity prescriptions following Frequency, Intensity, Time, and Type recommendations were extracted and summarised.

RESULTS

Nine articles met the inclusion criteria for this review. Various instruments for

frailty assessment were used, frequently in combination. Five studies prescribed physical activity for patients, one in-person and four to be performed remotely and unsupervised. The remaining four studies only used a self-report instrument to assess the level of physical activity. None reported adverse events related to exercise training. The exercise frequency mainly varied from daily to a minimum of twice per week. The intensity depended on frailty and included increasing levels of activity. The type of exercise was predominantly a combination of aerobic and resistance training. The duration of exercise varied from 4 to 12 wk. Three articles evaluated the effect of the exercise program on clinical outcomes, reporting a reduction in 90-d readmission rates post-transplant and improved frailty scores, as well as improved survival of cirrhotic patients waiting for LT.

CONCLUSION

Routine frailty assessment is essential for this population. Although more robust evidence is required, the prescription of exercise is safe and can improve patients' functional capacity, improving pre- and post-LT outcomes.

Key Words: End-stage liver disease; Liver transplant; Frailty; Exercise; Rehabilitation; Sarcopenia

Core Tip: Frailty negatively affects the outcomes of patients waiting for liver transplants (LTs). However, the tools used to assess frailty and functional performance vary amongst the existing studies, limiting the estimation of the real and accurate prevalence of the condition and the effectiveness of proposed treatments. So far, existing studies suggest that exercise may improve cirrhotic patients' functional capacity and frailty while they are on the waiting list for LTs. In addition, although evidence is scarce, studies affirm that exercise training improves pre- and post-LT outcomes.

Citation: Loschi TM, Baccan MDTA, Della Guardia B, Martins PN, Boteon APCS, Boteon YL. Exercise training as an intervention for frailty in cirrhotic patients on the liver transplant waiting list: A systematic review. *World J Hepatol* 2023; 15(10): 1153-1163
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1153.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1153

INTRODUCTION

Frailty is a multidimensional clinical syndrome that encompasses physical, psychological, social, and environmental components[1,2]. The physical component relates to sarcopenia, reduced physical function, reduced aerobic function, and physical disability[2]. Frailty is defined as a clinical state of decreased physiological reserves and increased vulnerability to health stressors, which predisposes individuals to adverse clinical outcomes[1]. It develops in elderly patients and earlier in patients with debilitating chronic diseases. In patients with end-stage liver disease (ESLD) who are candidates for liver transplant (LT), frailty is associated with decreased strength, physical deconditioning, and worse clinical outcomes with a higher probability of death and dependence after LT[2-4].

Approximately 18%-43% of cirrhotic patients are expected to be considered frail[5,6]. These figures vary largely according to the definition used and the population studied. Although there are some similarities, the mechanisms of frailty in ESLD patients are distinct from those in the geriatric population. The latter entails the original concept of frailty and the multidimensional derangement of physiological systems and is also called global frailty. In ESLD patients, the aetiology of the condition is complex but predominantly driven by liver impairment, such as synthetic protein dysfunction, ammonia-associated muscle toxicity, and encephalopathy-related physical inactivity[7-9]. Therefore, it manifests mainly as physical frailty and, consequently, the tools that have gained broad acceptance in liver clinics and research settings tend to focus unidimensionally on assessing the loss of muscle function[7]. Thus, frailty in ESLD patients is closely related to sarcopenia, which involves the loss of muscle mass and is considered a poor prognostic trait[10,11]. Although both conditions are linked and share common clinical significance, they can occur in isolation.

Sarcopenia assessment involves tests to quantify muscle mass; for example, cross-sectional imaging *via* computed tomography or magnetic resonance imaging, bioelectrical impedance analysis, dual-energy X-ray absorptiometry, or ultrasound, and a deep discussion of these is beyond the scope of this review[2,10]. To assess physical frailty in ESLD patients, three tests have been used: The Fried Frailty Index[1], the Clinical Frailty Scale (CFS)[12], and the Liver Frailty Index (LFI)[13]. Other tests that are also typically used in the care of these patients to assess their physical function and the relationship between muscle strength and function are the Short Physical Performance Battery[14], the Six-Minute Walk Test (6MWT)[15], Gait Speed[16], Hand Grip Strength[17] tests. In an inpatient setting, generic instruments can be used to measure global frailty, including the Frailty Index, the Hospital Frailty Risk Score[18], Activities of Daily Living [19,20], and Karnofsky Performance Status[21]. Regardless of the instrument used for the assessment, frailty in cirrhotic patients is associated with worse pre- and post-transplant outcomes[22-25].

The identification of interventions that could modify or reverse this condition is, therefore, crucial. Although exercise training is widely recommended for people with other chronic diseases[26], evidence for this treatment lags well behind for the population of cirrhotic patients on a waiting list for LT. Perhaps this is due to concerns about exercise causing increased portal pressure[27] or the lack of a standardised exercise protocol. Exercise programs for this population remain uncommon, and debate about the optimal method to deliver exercise (in-person supervised exercise or home-based programs) continues. Regular physical activity and exercise may favour better outcomes by adding muscle mass, as sarcopenia is associated with poor prognostic outcomes[11], and improving these individuals' functional capacity - *i.e.*, reducing their frailty. Therefore, this review aims to investigate the exercise programmes and prescriptions currently used for cirrhotic patients on the waiting list for LT, their results for frailty pre- and post-intervention and their effect on clinical outcomes.

MATERIALS AND METHODS

This systematic review was performed according to the Preferred Reporting Systematic Review and Meta-Analysis protocol[28]. No review protocol was registered before this review began.

Literature search strategy and data source

Articles were identified with the keyword "liver transplant" in combination with the free terms "frailty" and "exercise". The PubMed, MEDLINE and Scopus databases were searched for this review. There was no limitation according to publication date, and the search ended in January 2023.

Method of study screening and selection

Two authors (Loschi TM and Baccan MDTA) screened the articles independently, and there were no disagreements about the studies selected. In the first step, studies with titles that were not related to the theme were excluded. The second step involved reading the full abstracts and excluding articles that did not address important topics for this review. Finally, the full text of the remaining articles was evaluated for eligibility and articles that met the criteria were included in this review. The flow chart for the literature selection process is shown in Figure 1.

Eligibility criteria

The inclusion criteria were: (1) Articles that assessed physical frailty in cirrhotic patients on waiting lists for LT and the effect of physical exercise in this setting using any measurement instrument; and (2) Articles written in English and published. Physical exercise was considered a regular training program regardless of supervision, duration, or intensity. The exclusion criteria were: (1) A study population that was not candidates for LT; (2) Review articles; (3) Editorials and pre-projects; or (4) Articles not written in English.

Data extraction and outcome measures

Details about the exercise program, method of exercise delivery (supervised or unsupervised), and results regarding frailty pre- and post-intervention and the evaluated clinical outcomes were retrieved from each manuscript and analysed. The main outcomes evaluated were the assessment instrument for physical frailty, whether any pre-established criteria for inclusion in the transplant list related to frailty assessment were included; the frequency, intensity, type, and duration of exercise performed; and the primary and secondary outcomes evaluated after the physical exercise program.

Assessment of the risk of bias

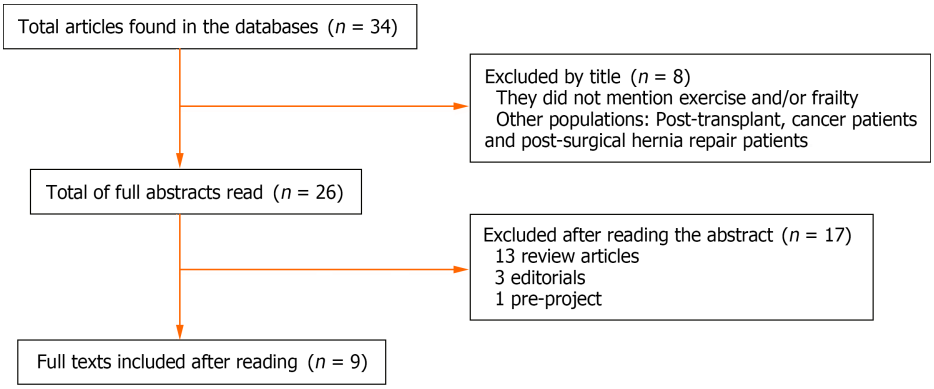
The quality of the included studies was assessed based on the National Institutes of Health Study Quality Assessment Tools. Due to the variable study designs, any identified risk of bias was discussed in the study. No simplifications or assumptions were made.

Literature quality assessment

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of the studies included in this review[29]. The tool applies a "star system" to evaluate three broad perspectives: The selection of the study groups, the comparability of the groups, and the ascertainment of exposure or assessment of the outcome of interest for case-control and cohort studies, respectively. The high-quality features of the numbered items evaluating each of the three domains are identified with a star. A study can be awarded a maximum of one star for each item across a total of four for the selection category, three for the outcome/exposure category, and one for comparability. Therefore, each article can score up to 9 points. Scores of 7 to 9, 4 to 6, and < 4 points indicate high, moderate, and low methodological quality, respectively[29].

RESULTS

The search strategy yielded nine articles that met the eligibility criteria and were, therefore, included in this systematic review.



DOI: 10.4254/wjh.v15.i10.1153

Figure 1 Flowchart of the systematic review on exercise therapy for frail cirrhotic patients on the waiting list for liver transplant. After searching the literature, texts whose titles did not fit this review and non-clinical studies were excluded. The nine articles that met the inclusion criteria were read and included in this review.

Frailty assessment or objective measurement of functional performance

The included studies used various instruments to assess frailty; most of them used a combination of instruments. The tools used were LFI (frailty for chronic liver disease), dynamometry (objective measure of handgrip strength), the Duke Activity Status Index (self-reported estimate of functional capacity), 6MWT (functional capacity), Gait Speed (functional capacity), the Rosow-Breslau scale (self-reported assessment of independence for activities), the Incremental Shuttle Walking Test (functional capacity), step count, the Fried Frailty Scale (objective score for assessing frailty), and Cardiopulmonary Exercise Testing (exercise capacity).

Exercise prescription

Of the nine studies included in this review, only one used in-person supervised exercise[30], four prescribed physical activity to be performed remotely and unsupervised[31-34], and the remaining did not specify exercise but recorded participants' self-reported levels of physical activity[35-38].

From the articles that prescribed exercise, we extracted data on the prescription of safe physical activity for this population. The acronym Frequency, Intensity, Time, and Type (FITT) was used to guide us in achieving this aim. We evaluated whether the exercise was supervised or not and whether the activity was performed in person or remotely.

The exercise frequency was 1-5 times per week for the study that prescribed in-person exercise. The studies that adopted remote exercises prescribed daily to a minimum of twice per week exercise, usually for 30 min per session. The intensity of training depended on the existence of frailty and included increasing levels of activity. The intensity progression was mainly based on professional evaluation. The type of exercise was predominantly a combination of aerobic and resistance training, although one study opted for aerobic exercise alone. The duration of exercise varied from 4 to 12 wk, and most studies included reassessment during the program. A detailed description of the exercise prescriptions is provided in Table 1.

Effect of exercise training programs on frailty status

Only one article of the nine used frailty to indicate the decision to include a cirrhotic patient on the LT waiting list[30]. In that study, patients with acceptable functional mobility were considered fit for inclusion on the list. The parameters were: a 6MWT of > 250 m, handgrip strength of > 30 kg for men and > 14 kg for women, and a Duke Activity Status Index of > 4 METS. Patients who were not listed were closely monitored, and goals related to the 6MWT were established to optimise functional mobility.

Post-transplant (length of hospital stay; hospital readmission rate) and pre-transplant (survival rate; quality of life assessment) endpoints were investigated as study outcomes. Physical activity and frailty improvement were consistently associated with favourable outcomes. Detailed information on the results is presented in Table 2**.** In addition, we used the NOS to assess article quality. The NOS scores were between 4 and 9, indicating moderate to high quality for the included articles (Table 2).

Effect of exercise training programs on clinical outcomes

Three articles evaluated the effects of exercise programs on clinical outcomes[30,31,34]. Al-Judaibi *et al*[30] in a retrospective single-centre study that analysed 458 LT patients, showed a trend towards shorter hospital stays (14 *vs* 17 d) and a reduction in 90-d readmission rates (17.9% *vs* 20%) in patients who underwent comprehensive exercise training programs before LT. Lin *et al*[31] analysed the effects of a prehabilitation strategy that involved home-based exercise in a study that included 517 patients and reported that a median improvement of 0.3 points in frail patients' LFI scores was associated with improved survival. Adherence to a physical therapy program was independently associated with increased survival. One year later, the same group reported more hospital admissions and high mortality rates among waiting list patients in the lowest quartile of the daily step count (*i.e.*, < 1200 steps per day). When adjusted by the Model for End-stage Liver Disease (MELD)-Na and the use of a physical training-dedicated smartphone application (Exercise

Table 1 Exercise prescription for cirrhotic patients on the waiting list for liver transplant

Ref.	Frequency	Intensity	Type	Time	Supervised?	In-person?
Al-Judaibi *et al*[30], 2019	In-person group: 1 to 5 times/wk. Remote group: 2 to 3 times/wk	-	In-person group: Aerobic and resistance training	-	Yes, remote group was supervised by videoconference	Yes
Lin *et al* [31], 2021	30 min a day, 5 times/wk	Frail and pre-frail: Aerobic: Encouraged to walk; purchasing a stationary bicycle or a pedal boat was suggested if the patient was at risk for walking. Resistance: Initial prescription at the time of evaluation with the physiotherapist. Weights or elastic bands can be used. Initially, 1 series of 10 repetitions, increase by 5 to 10 repetitions until reaching 30. Only then progress the load	Aerobic and resistance training	150 min of exercise per week	No	No, only if complications prevented the performance of the unsupervised exercise
Williams *et al*[32], 2019	Resistance: 20 min 2 times/wk. Aerobic: 10 min of brisk walking 3 times/d	Resisted: BORG between 12 and 14. Three levels: Low, moderate, and high. Aerobic: Increasing the number of steps	Aerobic and resistance training	Total of 12 wk, with reassessment at 6 and 12 wk and telephone contact 1 time/wk until 6 wk	No	No
Chen *et al* [33], 2020	Daily	Increase of 500 steps/d at each evaluation	Walking	Total: 8 wk, with a baseline assessment and every 2 wk	No	No
Lin *et al* [34], 2022	-	The group that used the app: The app's algorithm established the intensity after an initial assessment by a professional	-	Baseline assessment and 4 wk after	No	No

The data were extracted and reported based on the Frequency, Intensity, Time, and Type recommendation (exercise frequency, intensity, time, and type of activity) and whether the program was supervised, in-person or remote.

and Liver FITness, or EL-FIT), both hospital admissions and death were significantly associated with the lowest quartile [hazard ratio (HR) = 1.9, confidence interval (CI): 1.09-3.30 and HR = 3.42, CI: 1.23-9.68], 6MWT (HR = 0.63, CI: 0.47-0.83 and HR = 0.66, CI: 0.44-0.99 per 100 m), and Gait Speed Test (HR = 0.29, CI: 0.11-0.72 and HR = 0.21, CI: 0.05-0.84). Notably, there was a 5% reduction in the risk of admission and a 12% reduction in the risk of death for every additional 500 steps taken per day regardless of MELD-Na score and exercise program use[34]. None of the articles reported adverse events related to exercise training in this population.

DISCUSSION

Frailty is a clinical condition that negatively affects the outcomes of cirrhotic patients on waiting lists for LT[22-24]. Although the benefits of exercise training are well-established for other chronic conditions, it has not been deeply investigated in this population, mainly due to concerns for their safety[27] and the lack of a standard recommendation. In this review, we assessed the existing literature investigating the effect of exercise training on patients on the waiting list for LT, particularly the prescription of exercise. The current, extremely limited literature suggests benefits from exercise training programmes for clinical outcomes; however, frailty assessment tools lack standardisation, and the evidence for exercise therapy prescription is currently in its early stages.

In a large multicentric retrospective study in the United States that encompassed 1044 patients, frailty (defined as LFI ≥ 4.5) was associated with a 1.82-fold increase in the adjusted risk of mortality on the waiting list compared to non-frail patients[39]. Another North American study found that a 0.1 unit increase in the LFI at three months was associated with a 2-fold increased risk of death or delisting (95%CI: 1.35-3.09) regardless of baseline LFI score or MELD-Na value[22]. When applied to patients listed for LT, the 6-minute walk distance showed a moderate inverse correlation with the MELD score (r = -0.61). In addition, a 6-min walk distance of < 250 m was significantly associated with an increased risk of death on the waiting list (P = 0.0001), and every 100-m decrease in performance predicted a 2-fold increase in mortality[24]. Studies that applied other instruments to measure frailty, such as the Fried Frailty Index[23], the CFS score[5], the Gait Speed test[16], and the 6MWT[24], also found associations between high frailty and increased mortality.

Nowadays, LT specialists accept the benefits of regular physical activity and exercise to improve the functional capacity of cirrhotic patients on waiting lists for LT. The studies summarised in review articles in the literature suggested that exercise is safe for cirrhotic patients and favours a significant improvement in peak exercise capacity (VO_2), a significant increase in the distance walked in the 6MWT, improved muscle strength and function, decreased fatigue, a reduced hepatic portal venous gradient, increased lean mass, and improved quality of life[40-42]. Nevertheless, exercise

Table 2 Impact of the exercise training programs on clinical outcomes, their relationship to adverse events, and adherence to the program

Ref.	Outcomes analysed	Adverse events	Adherence	NOS quality assessment
Al-Judaibi *et al*[30], 2019	Patients who participated in the rehabilitation program showed a tendency towards a decrease in length of stay and a reduction in readmission rates in patients undergoing liver transplantation. Although not statistically significant, the observed trend towards early discharge after liver transplantation was observed in patients in the intervention group			Selection: 4*. Comparability: 0*. Outcome: 3*
Lin *et al*[31], 2021	LFI: Patients more adherent to the prehabilitation program have better results. Frailty is associated with mortality. An improvement of 0.3 in LFI is potentially associated with improved survival. 6MWT: Tendency of improvement with the pre-rehabilitation program, especially in the most adherent patients, after visit 4. Frailty is associated with mortality. Gait speed: There was no change with pre-rehabilitation. Frailty is associated with mortality. Frailty is more prevalent in females, higher BMI (as assessed by the 6MWT and GST), cirrhosis from alcohol or NASH, HB values, albumin, and bulky appearance, do not correlate with MELD. Patients with COPD and CVD are frailer by 3 frailty assessment metrics		Reposted by the patient at follow-up assessment: non-adherent < 20%, partially adherent: 20%-79%, adherent: ≥ 80%. Members: 38%. Partially adherent: 51% and non-adherent: 11%. Adherence to physical therapy was independently associated with increased survival	Selection: 2*. Comparability: 0*. Outcome: 2*
Dunn *et al* [35], 2016	Karnofsky scale and Rossow-Breslau indicated habitual physical activity performance close to normal. Comparing with objective data of counting daily steps, it showed that 75.9% of waking time was in sedentary activity. There was a significant association between the percentage of sedentary behaviour and deaths on the waiting list			Selection: 4*. Comparability: 0*. Outcome: 3*
Williams *et al*[32], 2019	SPPB, ISWT: Improved at 6 wk and no improvement at 12 wk. Step count: Improvement in the index at 12 wk. Quality of life improves in the 12th wk, mainly regarding mobility	No events	Adherence up to 6 wk: 82% step target and 90% resistance exercises. Already at 12 wk, they dropped to 53% and 78%, respectively	Selection: 4*. Comparability: 0*. Outcome: 3*
Ney *et al* [36], 2017	IPAQ: 47%, 38% and 15% of patients had low, moderate and high activity levels, respectively. The main barrier perceived for not performing physical exercise was fatigue			Selection: 2*. Comparability: 0*. Outcome: 2*
Lai *et al*[37], 2016	Fried Frailty Index > 3 frail. 6MWT: Frail with less walking distance. Frail: Less sit and stand up and lower isometric knee extension strength-tendency of critically ill patients to be more fragile			Selection: 2*. Comparability: 0*. Outcome: 2*
Chen *et al* [33], 2020	The control group had more patients who walked less than 2500 steps/d. 6MWT: Greater distance walked in the group with home exercises. CPET: No difference between groups. Computed tomography: Increased psoas muscle mass in the group with home exercises. Quality of life: No differences between groups	No events		Selection: 4*. Comparability: 2*. Outcome: 3*
Oikonomou *et al*[38], 2022	Significant correlation between LFI and physical activity level. LFI: Best in active (active: 3.75, sedentary: 4.42). No frail in the active group. Six frails in the sedentary group. Greater distance in active compared to sedentary (458.2 × 324.7). Peak VO_2 and the highest AT in the active			Selection: 3*. Comparability: 0*. Outcome: 2*
Lin *et al*[34], 2022	They were considered fragile if LFI was 4.5 or greater, 6MWT was less than 250 m, or GST was less than 0.8 m/s. Patients who walk less than 1200 steps/d have a higher LFI. Every additional 500 steps taken per day reduces the risk of hospitalisation by 5% and the risk of death by 12%. Patients in the group using an exercise app took more daily steps. Daily step count was moderately correlated with frailty metrics, and frail patients walked less than their less frail peers by any metric			

NOS: Newcastle-Ottawa Scale; LFI: Liver Frailty Index; 6MWT: Six-minute walking test; BMI: Body mass index; GST: Gait speed test; NASH: Non-alcoholic steatohepatitis; HB: Haemoglobin; MELD: Model for End-Stage Liver Disease; COPD: Chronic obstructive pulmonary disease; CVD: Cardiovascular disease; SPPB: Short Physical Performance Battery; ISWT: Incremental shuttle walk test; IPAQ: International Physical Activity Questionnaire; CPET: Cardiopulmonary exercise testing; VO_2: Oxygen uptake; AT: Anaerobic threshold.

prescriptions for this population remain poorly defined, likely because frailty assessment is predominantly based on subjective clinician assessment. These professionals focus mainly on managing the underlying disease aetiology and liver-related complications. Moreover, practical tools for clinicians to translate their knowledge into a safe and effective exercise prescription are limited. In a review article, experts suggest using the simple and time-efficient CFS and LFI scores alongside routine clinical patient evaluation to identify the patients at the highest risk for physical frailty and recommend them for prehabilitation with specialists, dieticians, and physiotherapists[2].

Therefore, one of the factors affecting the use of physical activity in this population is the physician-centred care model, which frequently delays or prevents access to professionals who prescribe exercise, such as physiotherapists and physical educators. In Brazil, few transplant centres have specialist teams to assess frailty and decreased physical performance, which can reduce the perception of the condition and, consequently, the prescription of exercise and the use of a monitoring program for these patients. In developed countries, the situation is similar. For example, a Canadian study investigating barriers to lifestyle modification in cirrhotic patients reported that only 1 in 7 centres offer their patients regular exercise programming[36]. A professional, detailed functional assessment and patient-specific exercise program that is adapted at regular follow-up sessions are desirable for optimal results. Other barriers to the adoption of prehabilitation exercise training programs are logistical - related to the distance from the transplant centre or caregiver availability due to the need to maintain a constant and continuous exercise regimen for approximately 8-12 wk - or related to the care team's fear of exacerbating or promoting an ESLD complication[42]. In addition, cost is an essential variable as that not all insurance covers more than a few sessions of physiotherapy.

While studies with cirrhotic patients generally focused on physical frailty, the tools currently used to measure frailty and functional performance varied. In addition, the method of exercise delivery encompassed in-person or remote and supervised or unsupervised approaches. Independent of the method employed, the existing studies show favourable results from exercise training in terms of improved functional capacity and frailty[30,31,34]. This finding suggests the need to encourage healthy and active lifestyles for cirrhotic patients; physicians should advise them to stay active and adhere to exercise training. This need is even more crucial in transplant centres with a predominantly physician-centred care model because adherence to these programs depends strongly on physicians raising patients' awareness of the importance of this strategy. Instruments that take objective measures, such as the LFI[13], the Short Physical Performance Battery[43], the Gait Speed test and the 6MWT[44], evaluate the strength and resistance of the lower limbs, the strength of the upper limbs, and balance. According to the literature, these are relevant predictive tools in this setting and appropriate drivers for prescribing exercise therapy.

Thus far, we know that exercise prescriptions should adhere to the FITT recommendations. However, specifications for each parameter have not been completely defined for this population. In recent review articles, the authors proposed relevant, useful orientations based mostly on expert opinions[7,45]. An exercise prescription must contain aerobic training (*e.g.*, walking), resistance exercises for the lower and upper extremities (*e.g.*, functional - stairs - or progressive weight training) and flexibility or balance training (*e.g.*, stretching and balance exercises) and use the talk test to ensure adequate intensity throughout the training[7,42].

Cirrhotic patients are more prone to remaining sedentary more frequently. Dunn *et al*[35] showed, by analysing the level of physical activity exhibited by cirrhotic patients on the transplant waiting list, that they engaged in some of the lowest reported activity of all chronic disease patients, similar to that of patients with advanced chronic lung disease or renal failure. On average, they spent 75.9% of their waking hours in sedentary activities and only 18.9% in moderate-vigorous activity[35]. In addition, Dunn *et al*[35] stated that sedentariness was associated with patient death while on the list.

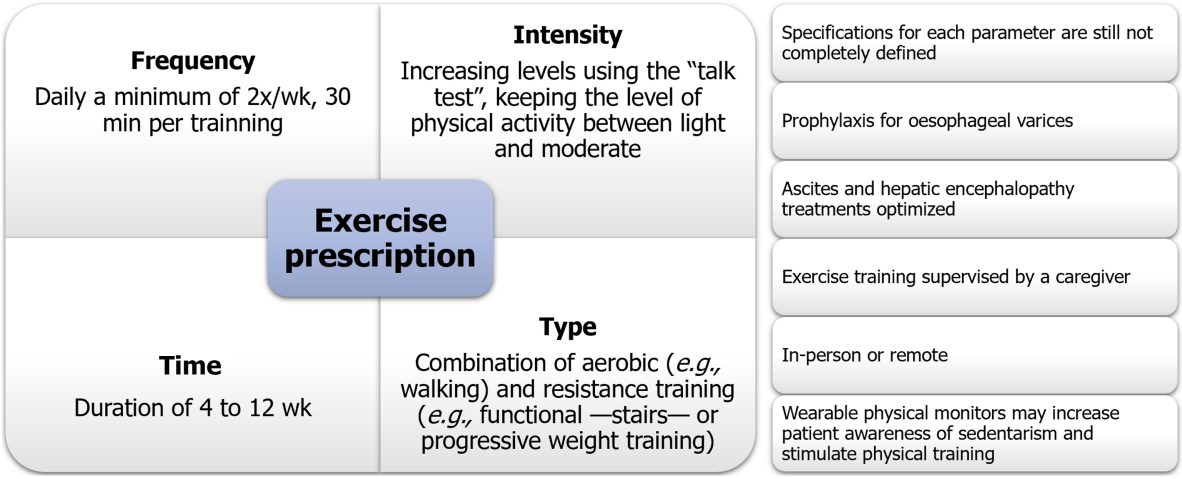
Technology may contribute to monitoring these critical patients' physical activity, increasing their compliance and response to interventions. Wearable physical monitors may provide a more realistic estimation of sedentary cirrhotic patients' activity levels as patient self-assessments and provider physical activity assessments do not reliably indicate the actual level of physical exercise[35]. In addition, the use of these gadgets may increase patients' awareness of sedentary physical activities and even stimulate their engagement in physical training, as Lin *et al*[34] showed.

None of the reviewed articles reported adverse events related to exercise training in this population, suggesting that this approach is safe. Pre-exercise safety may be assessed through disease-related safety issues, screening for cardiopulmonary diseases, and evaluating the effects of other comorbidities[40]. Uniformly, the reviewed studies excluded patients who lacked adequate primary or secondary prophylaxis for oesophageal varices. Other worrying conditions include prohibitive cardiopulmonary diseases, recent alcohol consumption, haemoglobin levels of < 11 g/dL, and incapacitating mental or physical disabilities[40]. Ascites and hepatic encephalopathy treatments should be optimised, and the exercise training should be supervised by a caregiver[7].

Regarding the format for delivering exercise, only one of the articles in this review applied an in-person approach for patients who lived near the rehabilitation centre[30]. Others adopted remote exercise training to facilitate patient engagement and showed benefits of frailty improvement[31,34]. Thus, the evidence suggests that both approaches are safe and effective, allowing the transplant centre and/or the patient to choose the modality that most fits their agenda. In a non-cirrhotic context, a randomised clinical trial of sarcopenic elders suggested that early exercise (supervised exercise followed by home-based exercise) and nutritional intervention aided in restoring lower extremity muscle mass but not physical function[46].

Although the results that these studies reported were not consistent for each criterion of the FITT principles individually, keeping the level of physical activity light-to-moderate, *i.e.*, using exercise that is not so strenuous that the patient cannot talk while performing it ("talk test"), offers a good starting point regardless of the chosen delivery modality. A summary of the exercise recommendations for cirrhotic patients waiting for LT based on the evidence in the literature is presented in Figure 2. A literature review from North America on the use of expert opinion to guide professionals in the prescription of exercise for patients with chronic liver disease recommends moderate-intensity aerobic exercise daily to achieve the goal of walking 150 min a week, which corroborates the findings of this systematic review. Experts also suggest resistance exercises with stretching focused on large muscle groups on alternate days at least twice a week with progressive intensity[40].

This review has some limitations to define. Primarily, due to the limited number of existing studies on this subject, the risk of bias and heterogeneity assessments were not strict, and we opted not to follow with a meta-analysis. Therefore, whilst an acceptable level of design was set, design-based quality checklists were not used. Given the weaknesses of the



DOI: 10.4254/wjh.v15.i10.1153

Figure 2 Summary of exercise prescription for cirrhotic patients on the waiting list for liver transplantation.

current evidence and the heterogeneity of the reviewed studies, the above recommendations should be considered carefully. In addition, this review focused on transplant candidates; therefore, extrapolation to other stages of chronic liver diseases or in-hospital patients due to decompensation must be done carefully. Exercise training in this population is intended to maintain physical function during a period of illness and prepare patients for a major surgery that requires hospitalisation and gradual rehabilitation. In addition, no studies thus far have stratified their exercise recommendations based on the severity of liver disease. Future studies should apply well-established frail assessment tools and the existing exercise recommendations, seek to achieve a clinically significant endpoint, and employ a randomly assigned control group.

CONCLUSION

Although further studies are still needed to guide the prescription of exercise and validate the use of regular physical activity to prevent and treat physical frailty in cirrhotic patients who are on the waiting list for LT, the routine assessment of frailty and practice of regular physical exercise, either in-person or remote, of low to moderate intensity is safe and can improve patients' functional capacity and pre- and post-LT outcomes.

ARTICLE HIGHLIGHTS

Research background

In patients with end-stage liver disease candidates for liver transplant (LT), frailty is associated with worse clinical outcomes and a higher probability of death. Therefore, the identification of interventions which could modify or reverse this condition is of paramount importance. Although exercise training is widely recommended for individuals with other chronic diseases, evidence still lags well behind for this population.

Research motivation

Exercise programs for cirrhotic patients are still not so frequent, and there is still debate about the optimal method to deliver exercises to them (in-person supervised exercise or home-based programs).

Research objectives

To investigate the existing exercise programmes and prescriptions for cirrhotic patients on the waiting list for liver transplantation, their results concerning frailty pre- and post-intervention and their impact on clinical outcomes.

Research methods

We searched the PubMed, MEDLINE and Scopus databases using the keyword and free terms "liver transplant", "frailty", and "exercise". The research findings, their contributions to the research in this field, and the problems that remain to be solved should be described in detail. The results were subsequently analysed for the instrument for physical frailty assessment, whether there were any pre-established criteria for inclusion in the transplant list related to frailty assessment, frequency, intensity, type and time of exercise performed, and primary and secondary outcomes evaluated after the physical exercise program.

Research results

We identified nine research articles that were included in this review. The instruments for frailty assessment varied amongst them, and five studies prescribed physical activity to patients, one in-person and four to be performed remotely and unsupervised. None reported adverse events related to exercise training. Three articles evaluated the impact of the exercise program on clinical outcomes, reporting a reduction in 90-d readmission rates post-transplant and improvement of frailty scores followed by improved survival of cirrhotic patients waiting for a transplant.

Research conclusions

We found that the routine assessment of frailty and practice of regular physical exercise, either in-person or remote, of low to moderate intensity is safe and capable of improving the patient's functional capacity and favour positive pre- and post-LT outcomes.

Research perspectives

Although further studies are still required to guide exercise prescription and validate the practice of regular physical activity for cirrhotic patients on the waiting list for LT, it may improve their outcomes.

FOOTNOTES

Author contributions: Loschi TM and Boteon YL designed this study and drafted the manuscript; Loschi TM and Baccan MDTA performed the literature review and analysis; Loschi TM, Baccan MDTA, Della Guardia B, Martins PN, Boteon APCS, Boteon YL reviewed the manuscript critically; and all authors contributed to editing and approved the final manuscript version.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

Country/Territory of origin: Brazil

ORCID number: Thais Mellato Loschi 0000-0001-9546-2272; Melline D T A Baccan 0000-0002-0269-7644; Bianca Della Guardia 0000-0002-0937-4063; Paulo N Martins 0000-0001-9333-0233; Amanda P C S Boteon 0000-0001-7029-4153; Yuri L Boteon 0000-0002-1709-9284.

S-Editor: Wang JJ
L-Editor: A
P-Editor: Cai YX

REFERENCES

1 **Fried LP**, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, Seeman T, Tracy R, Kop WJ, Burke G, McBurnie MA; Cardiovascular Health Study Collaborative Research Group. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001; **56**: M146-M156 [PMID: 11253156 DOI: 10.1093/gerona/56.3.m146]

2 **Williams FR**, Milliken D, Lai JC, Armstrong MJ. Assessment of the Frail Patient With End-Stage Liver Disease: A Practical Overview of Sarcopenia, Physical Function, and Disability. *Hepatol Commun* 2021; **5**: 923-937 [PMID: 34141980 DOI: 10.1002/hep4.1688]

3 **Gordon AL**, Masud T, Gladman JR. Now that we have a definition for physical frailty, what shape should frailty medicine take? *Age Ageing* 2014; **43**: 8-9 [PMID: 24148267 DOI: 10.1093/ageing/aft161]

4 **Rodríguez-Mañas L**, Féart C, Mann G, Viña J, Chatterji S, Chodzko-Zajko W, Gonzalez-Colaço Harmand M, Bergman H, Carcaillon L, Nicholson C, Scuteri A, Sinclair A, Pelaez M, Van der Cammen T, Beland F, Bickenbach J, Delamarche P, Ferrucci L, Fried LP, Gutiérrez-Robledo LM, Rockwood K, Rodríguez Artalejo F, Serviddio G, Vega E; FOD-CC group (Appendix 1). Searching for an operational definition of frailty: a Delphi method based consensus statement: the frailty operative definition-consensus conference project. *J Gerontol A Biol Sci Med Sci* 2013; **68**: 62-67 [PMID: 22511289 DOI: 10.1093/gerona/gls119]

5 **Tandon P**, Tangri N, Thomas L, Zenith L, Shaikh T, Carbonneau M, Ma M, Bailey RJ, Jayakumar S, Burak KW, Abraldes JG, Brisebois A, Ferguson T, Majumdar SR. A Rapid Bedside Screen to Predict Unplanned Hospitalization and Death in Outpatients With Cirrhosis: A Prospective Evaluation of the Clinical Frailty Scale. *Am J Gastroenterol* 2016; **111**: 1759-1767 [PMID: 27481305 DOI: 10.1038/ajg.2016.303]

6 **Cron DC**, Friedman JF, Winder GS, Thelen AE, Derck JE, Fakhoury JW, Gerebics AD, Englesbe MJ, Sonnenday CJ. Depression and Frailty in Patients With End-Stage Liver Disease Referred for Transplant Evaluation. *Am J Transplant* 2016; **16**: 1805-1811 [PMID: 26613640 DOI: 10.1111/ajt.13639]

7 **Tandon P**, Montano-Loza AJ, Lai JC, Dasarathy S, Merli M. Sarcopenia and frailty in decompensated cirrhosis. *J Hepatol* 2021; **75** Suppl 1: S147-S162 [PMID: 34039486 DOI: 10.1016/j.jhep.2021.01.025]

8 **Tandon P**, Raman M, Mourtzakis M, Merli M. A practical approach to nutritional screening and assessment in cirrhosis. *Hepatology* 2017; **65**:

1044-1057 [PMID: 28027577 DOI: 10.1002/hep.29003]
9 **Chen HW**, Dunn MA. Arresting frailty and sarcopenia in cirrhosis: Future prospects. *Clin Liver Dis (Hoboken)* 2018; **11**: 52-57 [PMID: 30992788 DOI: 10.1002/cld.691]
10 **Kim HY**, Jang JW. Sarcopenia in the prognosis of cirrhosis: Going beyond the MELD score. *World J Gastroenterol* 2015; **21**: 7637-7647 [PMID: 26167066 DOI: 10.3748/wjg.v21.i25.7637]
11 **Chang KV**, Chen JD, Wu WT, Huang KC, Lin HY, Han DS. Is sarcopenia associated with hepatic encephalopathy in liver cirrhosis? A systematic review and meta-analysis. *J Formos Med Assoc* 2019; **118**: 833-842 [PMID: 30279030 DOI: 10.1016/j.jfma.2018.09.011]
12 **Kremer WM**, Nagel M, Reuter M, Hilscher M, Michel M, Kaps L, Labenz J, Galle PR, Sprinzl MF, Wörns MA, Labenz C. Validation of the Clinical Frailty Scale for the Prediction of Mortality in Patients With Liver Cirrhosis. *Clin Transl Gastroenterol* 2020; **11**: e00211 [PMID: 32764204 DOI: 10.14309/ctg.0000000000000211]
13 **Lai JC**, Covinsky KE, Dodge JL, Boscardin WJ, Segev DL, Roberts JP, Feng S. Development of a novel frailty index to predict mortality in patients with end-stage liver disease. *Hepatology* 2017; **66**: 564-574 [PMID: 28422306 DOI: 10.1002/hep.29219]
14 **Treacy D**, Hassett L. The Short Physical Performance Battery. *J Physiother* 2018; **64**: 61 [PMID: 28645532 DOI: 10.1016/j.jphys.2017.04.002]
15 **Alameri HF**, Sanai FM, Al Dukhayil M, Azzam NA, Al-Swat KA, Hersi AS, Abdo AA. Six Minute Walk Test to assess functional capacity in chronic liver disease patients. *World J Gastroenterol* 2007; **13**: 3996-4001 [PMID: 17663517 DOI: 10.3748/wjg.v13.i29.3996]
16 **Dunn MA**, Josbeno DA, Tevar AD, Rachakonda V, Ganesh SR, Schmotzer AR, Kallenborn EA, Behari J, Landsittel DP, DiMartini AF, Delitto A. Frailty as Tested by Gait Speed is an Independent Risk Factor for Cirrhosis Complications that Require Hospitalization. *Am J Gastroenterol* 2016; **111**: 1768-1775 [PMID: 27575708 DOI: 10.1038/ajg.2016.336]
17 **Hanai T**, Shiraki M, Nishimura K, Ohnishi S, Imai K, Suetsugu A, Takai K, Shimizu M, Moriwaki H. Sarcopenia impairs prognosis of patients with liver cirrhosis. *Nutrition* 2015; **31**: 193-199 [PMID: 25441595 DOI: 10.1016/j.nut.2014.07.005]
18 **Gilbert T**, Neuburger J, Kraindler J, Keeble E, Smith P, Ariti C, Arora S, Street A, Parker S, Roberts HC, Bardsley M, Conroy S. Development and validation of a Hospital Frailty Risk Score focusing on older people in acute care settings using electronic hospital records: an observational study. *Lancet* 2018; **391**: 1775-1782 [PMID: 29706364 DOI: 10.1016/S0140-6736(18)30668-8]
19 **Woods NF**, LaCroix AZ, Gray SL, Aragaki A, Cochrane BB, Brunner RL, Masaki K, Murray A, Newman AB; Women's Health Initiative. Frailty: emergence and consequences in women aged 65 and older in the Women's Health Initiative Observational Study. *J Am Geriatr Soc* 2005; **53**: 1321-1330 [PMID: 16078957 DOI: 10.1111/j.1532-5415.2005.53405.x]
20 **Tapper EB**, Finkelstein D, Mittleman MA, Piatkowski G, Lai M. Standard assessments of frailty are validated predictors of mortality in hospitalized patients with cirrhosis. *Hepatology* 2015; **62**: 584-590 [PMID: 25846824 DOI: 10.1002/hep.27830]
21 **Charette RS**, Sarpong NO, Weiner TR, Shah RP, Cooper HJ. Registration of Bony Landmarks and Soft Tissue Laxity during Robotic Total Knee Arthroplasty is Highly Reproducible. *Surg Technol Int* 2022; **41** [PMID: 36108169 DOI: 10.52198/22.sti.41.os1633]
22 **Lai JC**, Dodge JL, Kappus MR, Dunn MA, Volk ML, Duarte-Rojo A, Ganger DR, Rahimi RS, McCulloch CE, Haugen CE, McAdams-DeMarco M, Ladner DP, Segev DL, Verna EC; Multi-Center Functional Assessment in Liver Transplantation (FrAILT) Study. Changes in frailty are associated with waitlist mortality in patients with cirrhosis. *J Hepatol* 2020; **73**: 575-581 [PMID: 32240717 DOI: 10.1016/j.jhep.2020.03.029]
23 **Lai JC**, Feng S, Terrault NA, Lizaola B, Hayssen H, Covinsky K. Frailty predicts waitlist mortality in liver transplant candidates. *Am J Transplant* 2014; **14**: 1870-1879 [PMID: 24935609 DOI: 10.1111/ajt.12762]
24 **Carey EJ**, Steidley DE, Aqel BA, Byrne TJ, Mekeel KL, Rakela J, Vargas HE, Douglas DD. Six-minute walk distance predicts mortality in liver transplant candidates. *Liver Transpl* 2010; **16**: 1373-1378 [PMID: 21117246 DOI: 10.1002/lt.22167]
25 **Mathur S**, Janaudis-Ferreira T, Wickerson L, Singer LG, Patcai J, Rozenberg D, Blydt-Hansen T, Hartmann EL, Haykowsky M, Helm D, High K, Howes N, Kamath BM, Lands L, Marzolini S, Sonnenday C. Meeting report: consensus recommendations for a research agenda in exercise in solid organ transplantation. *Am J Transplant* 2014; **14**: 2235-2245 [PMID: 25135579 DOI: 10.1111/ajt.12874]
26 **Webborn ADJ**. ACSM's exercise management for persons with chronic diseases and disabilities. *Br J Sports Med* 1997; **31**: 35427
27 **García-Pagàn JC**, Santos C, Barberá JA, Luca A, Roca J, Rodriguez-Roisin R, Bosch J, Rodés J. Physical exercise increases portal pressure in patients with cirrhosis and portal hypertension. *Gastroenterology* 1996; **111**: 1300-1306 [PMID: 8898644 DOI: 10.1053/gast.1996.v111.pm8898644]
28 **Moher D**, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol* 2009; **62**: 1006-1012 [PMID: 19631508 DOI: 10.1016/j.jclinepi.2009.06.005]
29 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]
30 **Al-Judaibi B**, Alqalami I, Sey M, Qumosani K, Howes N, Sinclair L, Chandok N, Eddin AH, Hernandez-Alejandro R, Marotta P, Teriaky A. Exercise Training for Liver Transplant Candidates. *Transplant Proc* 2019; **51**: 3330-3337 [PMID: 31732200 DOI: 10.1016/j.transproceed.2019.08.045]
31 **Lin FP**, Visina JM, Bloomer PM, Dunn MA, Josbeno DA, Zhang X, Clemente-Sanchez A, Tevar AD, Hughes CB, Jakicic JM, Duarte-Rojo A. Prehabilitation-Driven Changes in Frailty Metrics Predict Mortality in Patients With Advanced Liver Disease. *Am J Gastroenterol* 2021; **116**: 2105-2117 [PMID: 34313620 DOI: 10.14309/ajg.0000000000001376]
32 **Williams FR**, Vallance A, Faulkner T, Towey J, Durman S, Kyte D, Elsharkawy AM, Perera T, Holt A, Ferguson J, Lord JM, Armstrong MJ. Home-Based Exercise in Patients Awaiting Liver Transplantation: A Feasibility Study. *Liver Transpl* 2019; **25**: 995-1006 [PMID: 30859755 DOI: 10.1002/lt.25442]
33 **Chen HW**, Ferrando A, White MG, Dennis RA, Xie J, Pauly M, Park S, Bartter T, Dunn MA, Ruiz-Margain A, Kim WR, Duarte-Rojo A. Home-Based Physical Activity and Diet Intervention to Improve Physical Function in Advanced Liver Disease: A Randomized Pilot Trial. *Dig Dis Sci* 2020; **65**: 3350-3359 [PMID: 31907774 DOI: 10.1007/s10620-019-06034-2]
34 **Lin FP**, Bloomer PM, Grubbs RK, Rockette-Wagner B, Tevar AD, Dunn MA, Duarte-Rojo A. Low Daily Step Count Is Associated With a High Risk of Hospital Admission and Death in Community-Dwelling Patients With Cirrhosis. *Clin Gastroenterol Hepatol* 2022; **20**: 1813-1820.e2 [PMID: 35331941 DOI: 10.1016/j.cgh.2022.03.012]
35 **Dunn MA**, Josbeno DA, Schmotzer AR, Tevar AD, DiMartini AF, Landsittel DP, Delitto A. The gap between clinically assessed physical performance and objective physical activity in liver transplant candidates. *Liver Transpl* 2016; **22**: 1324-1332 [PMID: 27348200 DOI: 10.1002/lt.24506]

36 **Ney M**, Gramlich L, Mathiesen V, Bailey RJ, Haykowsky M, Ma M, Abraldes JG, Tandon P. Patient-perceived barriers to lifestyle interventions in cirrhosis. *Saudi J Gastroenterol* 2017; **23**: 97-104 [PMID: 28361840 DOI: 10.4103/1319-3767.203357]

37 **Lai JC**, Volk ML, Strasburg D, Alexander N. Performance-Based Measures Associate With Frailty in Patients With End-Stage Liver Disease. *Transplantation* 2016; **100**: 2656-2660 [PMID: 27495749 DOI: 10.1097/TP.0000000000001433]

38 **Oikonomou IM**, Sinakos E, Antoniadis N, Goulis I, Giouleme O, Anifanti M, Katsanos G, Karakasi KE, Tsoulfas G, Kouidi E. Effects of an active lifestyle on the physical frailty of liver transplant candidates. *World J Transplant* 2022; **12**: 365-377 [PMID: 36437844 DOI: 10.5500/wjt.v12.i11.365]

39 **Lai JC**, Rahimi RS, Verna EC, Kappus MR, Dunn MA, McAdams-DeMarco M, Haugen CE, Volk ML, Duarte-Rojo A, Ganger DR, O'Leary JG, Dodge JL, Ladner D, Segev DL. Frailty Associated With Waitlist Mortality Independent of Ascites and Hepatic Encephalopathy in a Multicenter Study. *Gastroenterology* 2019; **156**: 1675-1682 [PMID: 30668935 DOI: 10.1053/j.gastro.2019.01.028]

40 **Tandon P**, Ismond KP, Riess K, Duarte-Rojo A, Al-Judaibi B, Dunn MA, Holman J, Howes N, Haykowsky MJF, Josbeno DA, McNeely M. Exercise in cirrhosis: Translating evidence and experience to practice. *J Hepatol* 2018; **69**: 1164-1177 [PMID: 29964066 DOI: 10.1016/j.jhep.2018.06.017]

41 **Duarte-Rojo A**, Ruiz-Margáin A, Montaño-Loza AJ, Macías-Rodríguez RU, Ferrando A, Kim WR. Exercise and physical activity for patients with end-stage liver disease: Improving functional status and sarcopenia while on the transplant waiting list. *Liver Transpl* 2018; **24**: 122-139 [PMID: 29024353 DOI: 10.1002/lt.24958]

42 **Brustia R**, Savier E, Scatton O. Physical exercise in cirrhotic patients: Towards prehabilitation on waiting list for liver transplantation. A systematic review and meta-analysis. *Clin Res Hepatol Gastroenterol* 2018; **42**: 205-215 [PMID: 29162460 DOI: 10.1016/j.clinre.2017.09.005]

43 **Guralnik JM**, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, Scherr PA, Wallace RB. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol* 1994; **49**: M85-M94 [PMID: 8126356 DOI: 10.1093/geronj/49.2.M85]

44 **ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories**. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111-117 [PMID: 12091180 DOI: 10.1164/ajrccm.166.1.at1102]

45 **Tandon P**, Zanetto A, Piano S, Heimbach JK, Dasarathy S. Liver transplantation in the patient with physical frailty. *J Hepatol* 2023; **78**: 1105-1117 [PMID: 37208097 DOI: 10.1016/j.jhep.2023.03.025]

46 **Chang KV**, Wu WT, Huang KC, Han DS. Effectiveness of early versus delayed exercise and nutritional intervention on segmental body composition of sarcopenic elders - A randomized controlled trial. *Clin Nutr* 2021; **40**: 1052-1059 [PMID: 32723507 DOI: 10.1016/j.clnu.2020.06.037]

World Journal of Hepatology

Submit a Manuscript: https://www.f6publishing.com

World J Hepatol 2023 October 27; 15(10): 1164-1169

DOI: 10.4254/wjh.v15.i10.1164

ISSN 1948-5182 (online)

CASE REPORT

Inflammatory pseudotumors in the liver associated with influenza: A case report

Ankoor Patel, Alexander Chen, Alexander T Lalos

Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): A
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): D
Grade E (Poor): 0

P-Reviewer: Ataei-Pirkooh A, Iran; Francica G, Italy; Liu K, China

Received: August 14, 2023
Peer-review started: August 14, 2023
First decision: September 11, 2023
Revised: September 18, 2023
Accepted: October 11, 2023
Article in press: October 11, 2023
Published online: October 27, 2023

Ankoor Patel, Alexander Chen, Department of Medicine, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ 08901, United States

Alexander T Lalos, Department of Gastroenterology and Hepatology, Rutgers-Robert Wood Johnson Medical School, New Brunswick, NJ 08901, United States

Corresponding author: Ankoor Patel, MD, Doctor, Department of Medicine, Rutgers-Robert Wood Johnson Medical School, No. 125 Paterson Street, New Brunswick, NJ 08901, United States. ahp60@rwjms.rutgers.edu

Abstract

BACKGROUND

Inflammatory pseudotumor (IPT) is a rare and benign lesion that mimics malignancy and can develop in any part of the body. The pathophysiology and etiology of these quasineoplastic lesions remain unclear.

CASE SUMMARY

We report a case of a 65-year-old male who presented with fevers, night sweats, and unintentional weight loss following an influenza infection and was found to have multiple hepatic IPT's following an extensive work up.

CONCLUSION

Our case highlights the importance of considering hepatic IPT's in the differential in a patient who presents with symptoms and imaging findings mimicking malignancy shortly following a viral infection.

Key Words: Inflammatory pseudotumor; Influenza; Malignancy; Liver mass; Case report

Core Tip: Inflammatory pseudotumor (IPT) is a rare and benign lesion that can develop in any part of the body. Although the pathophysiology remain unclear, it is thought to develop in the setting of infection, inflammation, autoimmunity, trauma, *etc.* In this case report, we are the first to highlight the development of IPT in the liver in a patient following a recent influenza infection. Our case report emphasizes the importance of including IPT in the differential in a patient who presents with symptoms and radiologic findings concerning for malignancy shortly after a viral infection, such as Influenza.

Citation: Patel A, Chen A, Lalos AT. Inflammatory pseudotumors in the liver associated with influenza: A case report. *World J Hepatol* 2023; 15(10): 1164-1169
URL: https://www.wjgnet.com/1948-5182/full/v15/i10/1164.htm
DOI: https://dx.doi.org/10.4254/wjh.v15.i10.1164

INTRODUCTION

Inflammatory pseudotumor (IPT) is a rare, mostly benign inflammatory solid tumor containing spindle cells, myofibroblasts, plasma cells, and histiocytes. Although IPT can occur in any age, it typically affects children and young adults. The pathophysiology and etiology of IPT is not fully understood and the diagnosis continues to remain one of exclusion. IPTs often mimic malignancy and can develop in various organs, including the gastrointestinal (GI) tract (*e.g.*, liver). Case reports of IPT involving the GI tract are scarce.

Several hypotheses suggest systemic inflammatory conditions, infection, autoimmune conditions, and trauma/surgical inflammation to be causes of IPT. One case series reported three patients with hepatic IPTs that occurred following either biliary drainage and stent placement or hepatic abscess[1]. Viral infections may contribute to the development of IPTs[2]. A case report of IPT following coronavirus disease 2019 vaccination has been reported[3]. However, there are no cases reporting the development of IPT in the GI tract following influenza. We report a case of a 65-year-old male who we believe developed hepatic IPTs following influenza.

CASE PRESENTATION

Chief complaints

A 65 year old male was referred to our hospital after multiple liver masses were detected on computed tomography (CT) of the abdomen and pelvis.

History of present illness

In March 2023, a 65-year-old male with a history of microcytic anemia due to erosive gastritis, diverticulosis, and diabetes mellitus presented for evaluation of multiple liver masses. He reported high-grade fevers, night sweats, confusion, and unintentional weight loss of 40 pounds weight in a 3-wk timespan that occurred five months prior to presentation in our hepatology clinic.

History of past illness

Patient with a past medical history of microcytic anemia due to erosive gastritis, diverticulosis, and diabetes mellitus.

Personal and family history

Personal and family history is noncontributory.

Physical examination

During the general examination, no abnormalities were detected.

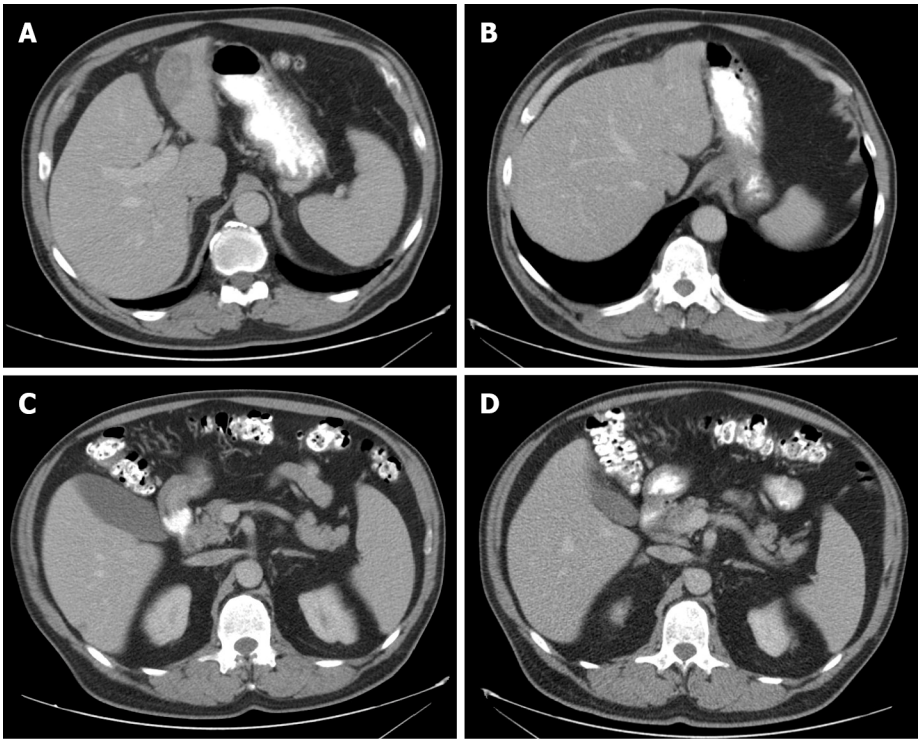
Laboratory examinations

Bloodwork at that time revealed a microcytic anemia of hemoglobin 10.2 g/dL and elevated C-reactive protein (CRP) and erythrocyte sedimentation rate of 191.0 mg/L and > 130 mm/h, respectively. He was initially diagnosed with influenza A *via* PCR of nasal swab.

Alpha-fetoprotein, hepatitis B (core and surface antibody) and C, antinuclear antibody, mitochondrial antibody, actin (smooth muscle) antibody and carcinoembryonic antigen were all negative. IgG antibody titers to hepatitis B surface antibody were low/non-immune. Cancer antigen 19-9 was measured at 42 U/mL (reference range: 0-35 U/mL).

Imaging examinations

A CT scan was performed, which showed heterogeneous left hepatic lobe masses measuring 5.0 cm × 3.0 cm, 4.0 cm × 3.1 cm and 4.1 cm × 3.7 cm as well as a hypodense right hepatic lobe mass measuring 1.1 cm × 1.1 cm (Figure 1). Adenopathy of the periportal, paraceliac, and multiple mediastinal lymph nodes were noted. The patient was referred to oncology for



DOI: 10.4254/wjh.v15.i10.1164

Figure 1 Computed tomography of the Abdomen and Pelvis illustrating the interval changes of the hepatic inflammatory pseudotumor in a span of 3 mo. A: A 5.0 cm × 3.0 cm mildly hypodense hepatic mass on computed tomography (CT) A/P dual phase liver; B: A 1.9 cm × 1.3 cm mildly hypodense hepatic mass (previously 5.0 cm × 3.0 cm) on CT A/P dual phase liver; C: A 1.1 cm × 1.1 cm right hepatic lobe mass on CT A/P dual phase liver; D: Resolution of the 1.1 cm × 1.1 cm right hepatic lobe mass (visualized in C).

presumed metastatic malignancy and underwent a positron emission tomography (PET) scan which showed small focal areas of uptake. However, the overall uptake of the left lobe hepatic masses appeared similar to the remainder of the liver (Figure 2).

Biopsy of the liver masses showed inflammatory cells but no malignant cells, and subsequent core biopsies revealed replacement of multiacinar liver parenchyma by fibrous and myxoid stroma with dense lymphoplasmacellular infiltrate (Figure 3). Trichrome stain highlighted dense fibrosis, CK7 immunostain showed preserved bile ducts, and smooth muscle actin immunostain showed extensive reactivity in the fibrous stroma. Immunostains for IgG4 and ALK1 were negative.

The patient was subsequently referred to hepatology and underwent a repeat CT scan, which showed a decrease in size of the left hepatic lobe masses and resolution of the right hepatic lobe mass. Previously enlarged lymph nodes either decreased in size or remained stable except for a 1.1 cm × 0.9 cm soft tissue density anterior to the left hepatic lobe which was suspicious for a new lymph node. An esophagogastroduodenoscopy and colonoscopy were performed to investigate his microcytic anemia and were significant for erosive gastritis.

FINAL DIAGNOSIS

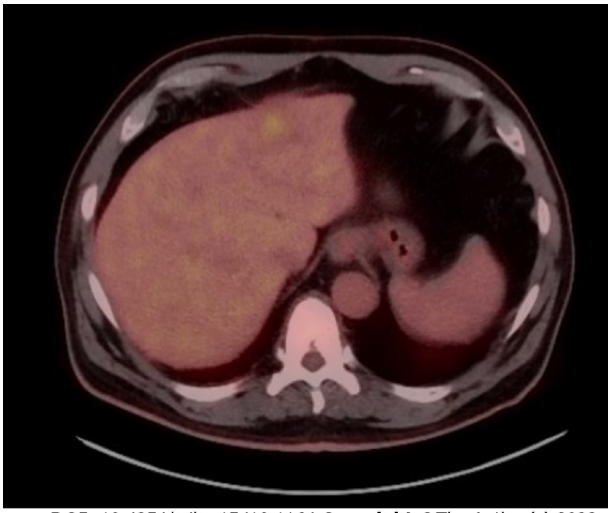
Based on radiologic and histologic examination of the liver masses, the patient was diagnosed with inflammatory pseudotumors of the liver.

TREATMENT

The treatment of hepatic IPTs in our patient was conservative management with supportive care and serial imaging to ensure resolution.

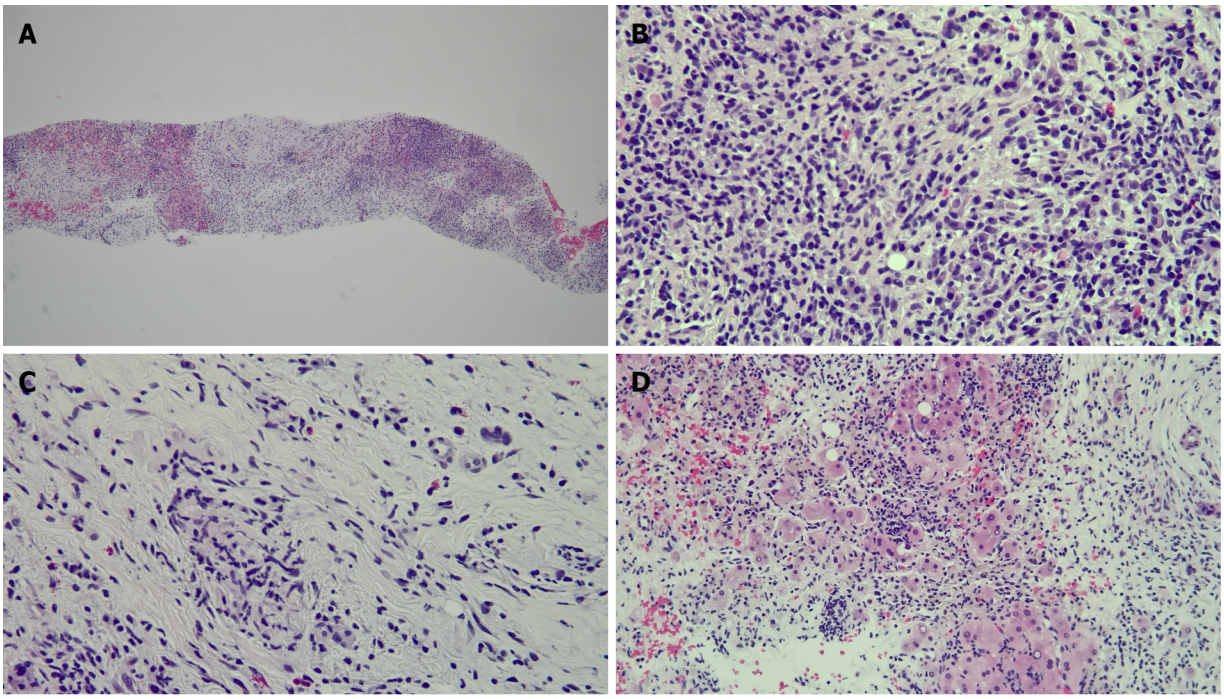
OUTCOME AND FOLLOW-UP

Serial CT imaging showed significant improvement in lymphadenopathy, significant decrease in size of the left hepatic lobe masses, and resolution of the right hepatic lobe mass. Normalization of inflammatory marker (CRP). Patient has



DOI: 10.4254/wjh.v15.i10.1164 **Copyright** ©The Author(s) 2023.

Figure 2 Whole body fluorodeoxyglucose-positron emission tomography/computed tomography illustrating overall uptake of left lobe hepatic masses similar to the remainder of the liver.



DOI: 10.4254/wjh.v15.i10.1164 **Copyright** ©The Author(s) 2023.

Figure 3 Ultrasound-guided fine-needle aspiration of the liver mass visualized on computed tomography. A: Core biopsy of the liver mass; B: Core biopsy showing lymphoplasmacytic infiltrate and absence of malignant cells; C: Core biopsy with replacement of multiacinar liver parenchyma by fibrous and myxoid stroma with dense lymphoplasmacellular infiltrate; D: Core biopsy visualizing benign hepatocytes.

returned to his baseline health and weight.

DISCUSSION

IPT is a benign, rare disease process which mimics malignancy on both clinical and imaging findings. It can present as a solitary mass or multiple masses composed of polymorphous inflammatory cell infiltrate. Although it can occur anywhere in the body and at any age, it is more typically found in the lungs and occurs more commonly in children and young adults. Hepatic IPT is a rare phenomenon first described by Pack and Baker[4] in 1953 and is most frequently seen in men[5,6].

Although the pathophysiology and etiology of hepatic IPT remains unclear, infection and autoimmune disorders are thought to be a few of the causes. Various infectious agents have been hypothesized to play a role in the development of IPTs, such as Mycoplasma and Nocardia in lung, Epstein-Barr virus in the spleen and lymph nodes, mycobacteria in spindle cell tumors, and Actinomycetes in liver pseudotumors[5]. Recently, a patient with human immunodeficiency virus was diagnosed with biopsy-proven, herpes simplex virus-positive pseudotumor in the gastroesophageal junction [7]. Otherwise, IPT's have been associated with autoimmune conditions such as IgG4-related disease. Interleukin 1, a cytokine produced by monocytes and macrophages, contributes to the local and systemic effects of IPT. Although treatment of GI IPT's with surgical resection is usually curative, other reports have shown response to steroids, nonsteroidal anti-inflammatory drugs, and thalidomide. The reported recurrence rate of GI IPT's is between 18% and 40% [8]. If there is recurrence following initial therapy, surgical resection is highly advised. Spontaneous regression and malignant transformation have also been rarely reported.

While diagnosis and differentiation of IPT from other etiologies requires histologic examination of the tissue, radiographic characteristics may be helpful in ascertaining whether there is potential of the tumor to be malignant[9]. CT and magnetic resonance imaging findings are variable which is attributed to the variability in histologic composition[10]. Similarly, contrast enhanced ultrasound was unable to solely differentiate IPT from other hepatic malignancies[11,12]. However, several imaging characteristics have been studied and may help guide diagnostic workup[13,14].

We believe our case is likely a consequence of the inflammatory state caused by influenza. The temporal association with contracting influenza may be coincidental but the patient does not have any other risk factors including recent infections, malignancy, immunosuppression, trauma, or autoimmune conditions. Our diagnosis of IPT is supported by the lack of uptake on PET-CT, the absence of malignant cells on biopsy, the presence of mixed inflammatory infiltrate and fibrosis on cytopathology, and the resolution with anti-viral treatment directed against influenza.

CONCLUSION

Due to the rarity of hepatic IPT's and their features mirroring that of malignancy, our case highlights the importance of including pseudotumor in the differential diagnoses of a new liver mass(es) associated with regional lymphadenopathy following a viral infection, such as Influenza. To date, there have been no reported cases of IPT following influenza and therefore would add to current gaps in knowledge when evaluating patients with masses concerning for malignancy.

FOOTNOTES

Author contributions: Patel A, Chen A, and Lalos A had substantial contributions to the conception and study design, analysis and interpretation of the data, creating figure/tables, drafting manuscript, final approval of manuscript.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report and any accompanying images.

Conflict-of-interest statement: The authors declare that they have no conflict of interest to disclose.

CARE Checklist (2016) statement: The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).

Country/Territory of origin: United States

ORCID number: Ankoor Patel 0000-0002-6557-0624; Alexander Chen 0000-0001-9614-6562; Alexander T Lalos 0000-0002-0414-4664.

Corresponding Author's Membership in Professional Societies: American College of Gastroenterology; American Gastroenterological Association; American Association for the Study of Liver Diseases.

S-Editor: Lin C
L-Editor: A
P-Editor: Lin C

REFERENCES

1 **Zhao J**, Olino K, Low LE, Qiu S, Stevenson HL. Hepatic Inflammatory Pseudotumor: An Important Differential Diagnosis in Patients With a History of Previous Biliary Procedures. *ACG Case Rep J* 2019; **6**: e00015 [PMID: 31598534 DOI: 10.14309/crj.0000000000000015]

2 **Zhang Z**, Jin F, Sun L, Wu H, Chen B, Cui Y. Inflammatory pseudotumor of the thymus: A case report and review of the literature. *Oncol Lett* 2014; **7**: 1414-1418 [PMID: 24765147 DOI: 10.3892/ol.2014.1895]

3 **Yucel Gencoglu A**, Mangan MS. Orbital Inflammatory Pseudotumor following mRNA COVID-19 Vaccination. *Ocul Immunol Inflamm* 2023; **31**: 1141-1144 [PMID: 35914301 DOI: 10.1080/09273948.2022.2093757]

4 **Pack GT**, Baker HW. Total right hepatic lobectomy; report of a case. *Ann Surg* 1953; **138**: 253-258 [PMID: 13066016 DOI: 10.1097/00000658-195308000-00012]

5 **Patnana M**, Sevrukov AB, Elsayes KM, Viswanathan C, Lubner M, Menias CO. Inflammatory pseudotumor: the great mimicker. *AJR Am J Roentgenol* 2012; **198**: W217-W227 [PMID: 22358018 DOI: 10.2214/AJR.11.7288]

6 **Park JY**, Choi MS, Lim YS, Park JW, Kim SU, Min YW, Gwak GY, Paik YH, Lee JH, Koh KC, Paik SW, Yoo BC. Clinical features, image findings, and prognosis of inflammatory pseudotumor of the liver: a multicenter experience of 45 cases. *Gut Liver* 2014; **8**: 58-63 [PMID: 24516702 DOI: 10.5009/gnl.2014.8.1.58]

7 **Kripalani S**, Williams J, Joneja U, Bansal P, Spitz F. Herpes Simplex Virus Pseudotumor Masking as Gastric Malignancy. *ACG Case Rep J* 2023; **10**: e00985 [PMID: 36911758 DOI: 10.14309/crj.0000000000000985]

8 **Sanders BM**, West KW, Gingalewski C, Engum S, Davis M, Grosfeld JL. Inflammatory pseudotumor of the alimentary tract: clinical and surgical experience. *J Pediatr Surg* 2001; **36**: 169-173 [PMID: 11150459 DOI: 10.1053/jpsu.2001.20045]

9 **Bian Y**, Jiang H, Zheng J, Shao C, Lu J. Basic Pancreatic Lesions: Radiologic-pathologic Correlation. *J Transl Int Med* 2022; **10**: 18-27 [PMID: 35702187 DOI: 10.2478/jtim-2022-0003]

10 **Kawaguchi T**, Mochizuki K, Kizu T, Miyazaki M, Yakushijin T, Tsutsui S, Morii E, Takehara T. Inflammatory pseudotumor of the liver and spleen diagnosed by percutaneous needle biopsy. *World J Gastroenterol* 2012; **18**: 90-95 [PMID: 22228976 DOI: 10.3748/wjg.v18.i1.90]

11 **Liao M**, Wang C, Zhang B, Jiang Q, Liu J, Liao J. Distinguishing Hepatocellular Carcinoma From Hepatic Inflammatory Pseudotumor Using a Nomogram Based on Contrast-Enhanced Ultrasound. *Front Oncol* 2021; **11**: 737099 [PMID: 34692513 DOI: 10.3389/fonc.2021.737099]

12 **Kong WT**, Wang WP, Shen HY, Xue HY, Liu CR, Huang DQ, Wu M. Hepatic inflammatory pseudotumor mimicking malignancy: the value of differential diagnosis on contrast enhanced ultrasound. *Med Ultrason* 2021; **23**: 15-21 [PMID: 32905565 DOI: 10.11152/mu-2542]

13 **Kong WT**, Wang WP, Cai H, Huang BJ, Ding H, Mao F. The analysis of enhancement pattern of hepatic inflammatory pseudotumor on contrast-enhanced ultrasound. *Abdom Imaging* 2014; **39**: 168-174 [PMID: 24327255 DOI: 10.1007/s00261-013-0051-3]

14 **Möller K**, Stock B, Ignee A, Zadeh ES, De Molo C, Serra C, Jenssen C, Lim A, Görg C, Dong Y, Klinger C, Tana C, Meloni MF, Sparchez Z, Francica G, Dirks K, Hollerweger A, Kinkel H, Weskott HP, Montagut NE, Srivastava D, Dietrich CF. Comments and illustrations of the WFUMB CEUS liver guidelines: Rare focal liver lesions - non-infectious, non-neoplastic. *Med Ultrason* 2023 [PMID: 37369029 DOI: 10.11152/mu-4192]

Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com