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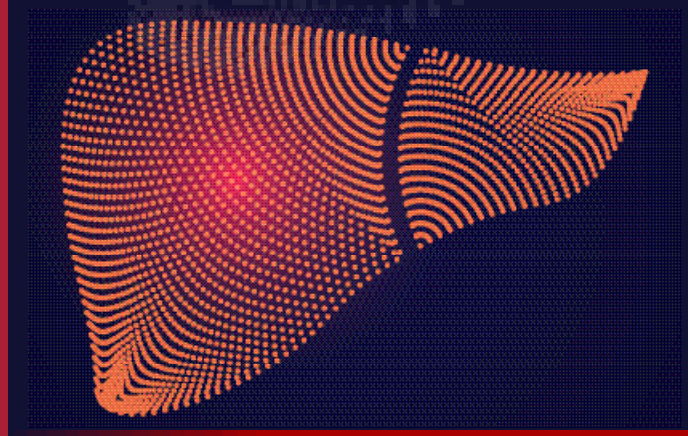

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The Hepatology Forum aims to publish original articles, review articles, editorials, case reports and correspondence (letters to the editor) on clinical and basic research in the field of hepatology.

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Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. Manuscripts submitted to the Hepatology Forum will be evaluated through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers. The Editor in Chief is the final authority to decide for publication for all submissions. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal.

An approval report from the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for drug studies, clinical and experimental studies and for some case reports. Ethics committee report or a similar official document may be asked from the authors if required. Materials and Methods section of the manuscript should include the information on patient consent, the name of the ethics committee, and the ethics committee approval number. For the experimental research studies conducted on humans, a statement declaring that written informed consent from the patients and volunteers was obtained before the procedures that they may undergo.

Manuscripts reporting experiments using animals must include a statement giving assurance that all animals received human care and that study protocols comply with the institution's guidelines. Studies involving animal experiments should conform to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (http://www.nc3rs.org.uk/arrive-guidelines), developed by the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) to improve standards and reporting of animal research. Please review the ARRIVE checklist and disclose all relevant animal research information as directed. For the studies that was performed on animals; a statement such as "all measures were taken to prevent pain and suffering of the animals" should be declared.

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All material to be considered for publication in Hepatology Forum must be submitted in electronic form via the Journal's online submission system. Once you have prepared your manuscript according to the Instructions below, please visit the online submission system here or instructions on how to submit your manuscript online please click here.

Preparing your Manuscript

I- Types of Manuscripts

Original manuscripts

They should not exceed 6000 words, including the abstract, references, tables, and figure legends. A maximum of 8 tables and/or figures is allowed. References should not exceed a maximum of 100.

Review articles

The maximum length is 5000 words. The inclusion of a maximum of 8 high quality tables and/or colored figures to summarize critical points is highly desirable. Reviews should include 5 to 10 key points that briefly summarize or highlight the main content of the article. References should not exceed a maximum of 150.

Editorials

This section consists of invited editorial comments on articles published in the Hepatology Forum. The length of an editorial should not exceed 1500 words and 1 table or 1 figure is allowed. References should not exceed a maximum of 20.

Case reports

The length of a case report should not exceed 3000 words. A total number of 2 tables or figures is allowed. References should not exceed a maximum of 10.

Correspondence (Letters to the Editor)

Letters to the Editor will be considered for publication if they are related to articles published in recent issues of the Hepatology Forum. The length of a Letter to the Editor should not exceed 800 words. A total number of 1 table or figure is allowed. References should not exceed a maximum of 10.

II- Article Structure

The manuscript must be arranged as follows

- Title page
- Abstract in the Hepatology Forum format, including a lay summary
- Introduction
- Materials and methods (or Patients and methods)
- Results
- Discussion
- Acknowledgements
- References
- Tables
- Figure legends
- Figures

Title Page

A title page must be provided for all submissions The title page should consist of all the following headings:

- Title: no more than 130 characters. Please refrain from using abbreviations in the title that may not be possible for the wide readership of the Hepatology Forum. The title of an accepted article may be modified by the editors.
- Authors: a list of all authors with first and surnames. Author names should be spelled out.
- Affiliations: names of department(s) and institution(s) of all authors
- Corresponding author: name, address, telephone and fax numbers, and electronic mail address.
- Keywords: a minimum of three and maximum of 6 keywords. These keywords will be used for indexing purposes. Please refer to https://www.nlm.nih.gov/mesh/MeSHonDemand.html to compile a comprehensive list of keywords
- Electronic word count
- Number of tables and figures
- Conflict of interest statement: a statement to declare any conflict of interest. For further information see our Conflict of interest section
- Financial support statement: a statement of all authors' financial support given in order to complete the study or write the manuscript. See the Financial disclosure section
- Authors contributions: a list of the authors' contributions to the study; concept and design, experiments and procedures; writing of article etc.
- Clinical trial number (if available)

Abstract

Abstracts should be no longer than 200 words. Non-standard abbreviations, footnotes or references should not be used in the abstract. An electronic word count of the abstract must be included at the end of the abstract section.

Case reports, Reviews, Special section articles, and Letters to the Editor do not require a structured abstract.

A structured abstract, should have the following layout:

- Background & Aims: Should state the main aim or objective of the study
- Materials and Methods: Essential information on the methods used, including details of the study design (e.g. randomized controlled trial, cross sectional study, cohort study, case series, etc.); study location (primary or tertiary care setting, hospital, general community, etc.); number of participants and the way they were selected; intervention, the method of administration and the duration
- Results: The key findings of the study (such as absolute values, confidence intervals, p values etc.) should be mentioned in this section
- Conclusions: Concise summary and the important finding of the study should be mentioned with a maximum of 2 sentences.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results. This section should also include a study objective and hypotheses paragraph.

Materials/Patients and Methods

All original manuscripts must provide a methods section that highlights the method the study was performed. Methods that has previously been published should be indicated by a reference. The manuscripts should include a description of the design, measurement and collection of data, type and source of subjects, inclusion and exclusion criteria and measures of outcome, number of subjects studied and why this number was chosen. The baseline characteristics of any compared groups should be described in detail and, if necessary, adjusted for in the analysis of the outcome. Please refer to our Statistics section for statistical methods required for publication and our Editorial policies section below for providing details on statistics and relation to animal and human trials, drugs and chemicals.

For all manuscripts reporting animal experimentation the authors must state a statement referring to the above guidelines mentioned in the editorial policy section and with a statement on institutional approval and where applicable:

- The strain and sex should be reported, if both male and female animals that were used
- Genetic background of the animals used
- In relation to cell cultures and tissue samples the sex of the animals
- State the transgenic or genetic mouse model used, and what control mice were used
- Housing of animals, cage system, enriched environment, diet, food, light or dark cycle.

Results

Results should be concise, explained and illustrated by using Tables and Figures. There is a maximum of 8 tables and/or figures per original article. Please refer to tables and figures formatting section.

Discussion

The discussion section should provide a summary of the key results and discuss the scientific importance of the findings of the original work. It should also include supportive knowledge and comparison of the new findings to the previously published literature.

Acknowledgements

Acknowledgements should be in a separate section at the end of the article before the references. People who provided help during the research should be listed in this section.

Formatting of Text

The submitted manuscript must be typed double-spaced throughout and pages numbered (including references, tables and figure legends). Preferably using a "standard" font (we prefer Times/Arial 12). For mathematical symbols, Greek letters, and other special characters, use normal text.

Language

Please write your text in good English. Authors who feel their English language manuscript may require editing could inform the editorial office for further editing.

Formatting of Tables

Tables should be provided as Word files (*.doc) compatible files. No TIFF, JPG, PDF or PowerPoint files are acceptable. When submitting tables in Microsoft Word use the table function, no tab, space or colors should be used. Tables should contain a maximum of 10 columns. Tables should include a table number, title (in bold), table legend, and if necessary, footnotes (including any abbreviations). Include tables in the submitted manuscript as a separate section at the end of the manuscript.

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All graphics submitted to the Hepatology Forum should be sent at their actual size, which is 100% of their print dimension and in portrait orientation.

- Figures should be supplied in the following preferred file formats: PDF (*.pdf), PowerPoint (*.ppt), Adobe Illustrator (*.ai, *.eps), Photoshop (*.psd) files in grayscales or in RGB color mode. Figures should be sent in an editable format, and not compressed into a .ppt or .pdf file. Figures should not be sent in JPG (*.jpg) format.
- Photographs (scans, immunofluorescences, EM, and histology images) should be submitted as: 1) TIFF (*.tif) with a resolution of at least 300 pixels per inch; or 2) Illustrator compatible EPS files with RGB color management (*.eps); or 3) Photoshop (*.psd) or editable PDF (*.pdf) files (grayscales or RGB) at the appropriate resolution which is: 300 dpi for color figures, 600 dpi for black and white figures and 1200 dpi for line-art figures.

Photographs of identifiable patients should be accompanied by written permission to publish from patient(s).

Panel lettering should be in Arial bold 14 pt, capitalized and no full stop (A, B) while lettering in figures (axes, conditions), should be in Arial 8 pt, lower case type with the first letter capitalized and no full stop. No type should be smaller than 6 pt.

If after acceptance the quality of the figures does not match the standards of Hepatology Forum, the authors will be asked to resubmit the figures at the required quality.

Figure Legends

Figure legends should be listed one after the other, as part of the text document, separate from the figure files. Please do not write a legend below each figure.

Each figure legend should have a brief overarching title (in bold with figure number) that does not exceed 100 words and describes the entire figure.

The statistical test used as well as the values of statistical significance (whether significant or not) should always be included in the figure legends.

The abbreviated word for figure "Fig." should be typed and bolded, followed by the figure number and a period (i.e., "Fig. 1."). Every figure legend should have a title written in bold. If a figure contains multiple sections (i.e., a, b, c, d) the letter for these subsections should be in minuscule letters, and should be surrounded by parenthesis [i.e., (a)…(b)…(c)…(d)]. Figures should be numbered according to the order which they were cited.

An example of how a figure caption should look:

Figure 1. Serum ALT levels of patient who were HCV+, HBV+, or controls. (a) Mean serum levels (bars represent SD and bold lines inside the box plot median levels). Levels of significance: *p = 0.032; ˆ p = 0.003. (b)….

References

Responsibility for the accuracy of bibliographic citations lies entirely with the authors. If an ahead-of-print publication is cited, the DOI number should be provided. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first three authors should be listed followed by "et al."

An example of how references should look within the text:

HVPG was measured by hepatic vein catheterization using a balloon catheter according to a procedure described elsewhere[14,15] and used as an index of portal hypertension.[16]

An example of how the reference list should look:

10. Lok AS, Zoulim F, Dusheiko G, Ghany MG. Hepatitis B cure: From discovery to regulatory approval. Hepatology 2017;66(4):1296-1313.
11. Lok AS, McMahon BJ, Brown RS Jr, Wong JB, Ahmed AT, Farah W, et al. Antiviral therapy for chronic hepatitis B viral infection in adults: A systematic review and meta-analysis. Hepatology 2016;63(1):284-306.

Citation in Text

References are ordered as they appear in the text. All articles in the list of references should be cited in the text and, conversely, all references cited in the text must be included in the list. Personal communications and unpublished data should be cited directly in the text by the first author, without being numbered. For revised manuscripts, the authors need to check all the citations in the Reference Section.

Web References

The full URL of the material should be given with the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) or can be included in the reference list.

The Statistical Methods

Special or complex statistical methods should be explained and referenced in the text. Meanwhile complex analyses should be performed with the assistance of a qualified statistician. The actual p values – whether significant or not – should always be presented (not n.s.). For small data sets and if variable distributions are non-normal, distribution free (non-parametric) statistical methods should be used. Continuous variables can always be summarized using the median and range which are therefore preferred. Only in the infrequent case of a normal distribution are the mean and standard deviation (SD) useful. Confidence intervals convey more information than p values and should be presented whenever possible. Complex analyses (including Cox and logistic regression analysis) should be presented in sufficient detail: i.e., variable scoring, regression coefficients, standard errors and any constants. Odds-ratios or relative risks are not sufficient documentation of such analyses. The handling of any missing values in the data should be clearly specified. Figures showing individual observations e.g., scatter plots and histograms are encouraged. An independent statistician is a part of the editorial board of the journal and statistical review of the paper will be sought when necessary.

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III- Review Process

Manuscripts will be screened upon submission and only the manuscripts, which fully comply with the submission requirements outlined will enter the peer review process.

First Submission

After a successful submission of the manuscript, an acknowledgement will be sent by e-mail to the corresponding author. All further correspondence will be with the corresponding author. The number of the manuscript should be used for further communications with the Editorial Office.

The manuscripts will be reviewed by the Editors and papers that are not considered by the editors to be strong candidates for publication or outside the scope of the Journal will be returned to the authors without detailed review, typically within 3-5 days. Otherwise, manuscripts will be sent to reviewers. After review, the corresponding author will be informed via e-mail. The author will be notified by letter of the decision taken by the Editor(s). This letter will be accompanied in most, but not all, cases by the comments of the reviewers.

Resubmission

In some cases, authors will be invited to submit a revised version of the manuscript for further review. In general, revised manuscripts must be received in the Editorial Office within four months of the date of the first decision. Authors should submit the resubmitted manuscript with all changes underlined. The resubmitted manuscript should be accompanied by a cover letter stating that the manuscript has been revised according to the comments made by the Editor and the Reviewers. Figures and tables must be uploaded. Please ensure that a separate point by point response to the reviewers is included with the covering letter. Revised manuscripts should be uploaded in the Editorial System website.

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Research Article

Potent antiviral agent in cirrhotic patients

HEPATOLOGY FORUM

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The impact of long-term potent antiviral therapy on the natural course of disease in patients with hepatitis B virus-related cirrhosis

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Abstract

Background and Aim: The present study compared the long-term efficacy of weak and potent antiviral treatments in patients with hepatitis B virus (HBV)-related cirrhosis.

Materials and Methods: A total of 120 patients with HBV-related cirrhosis were enrolled. The primary outcome measure was viral suppression. A secondary outcome measure was to determine the development of decompensation or hepatocellular carcinoma (HCC).

Results: The virological response (VR) was significantly better in patients treated with potent antiviral agents than in those treated with weaker antiviral agents over time ($p<0.001$). With intention-to-treat, the VR after 1 year, 2 years, 3 years, and 4 years of potent antiviral treatment was 69.7%, 77.0%, 82.2%, and 81.2%, respectively, while the VR with weak antiviral therapies was 50.0%, 41.6%, 37.5%, and 37.5%. HBeAg (Hepatitis B e-Antigen) loss was achieved in 30.4% of HBeAg-positive patients. None of the patients had experienced HBsAg loss while on antiviral treatment. New HCC developed in 10 patients. The cumulative probability of the development of HCC was 2.6% at 1 year, 6.8% at 2 years, and 8.7% at 3 and 5 years of antiviral therapy. MELD scores among patients treated with potent antiviral treatment significantly improved from baseline to week 60 ($p=0.006$). Antiviral therapies were well tolerated.

Conclusion: Potent antiviral treatment effectively maintained VR in the long-term follow-up of patients with HBV-related cirrhosis. HCC may still develop, albeit at a lower rate in these patients.

Keywords: Chronic hepatitis B; cirrhosis; entecavir; lamivudine; tenofovir disoproxil fumarate.

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Introduction

Hepatitis B virus (HBV)-related chronic liver disease (CLD) and cirrhosis remain a major cause of liver Turkiye -related morbidity and mortality in Turkiye.[1,2] HBV-related cirrhosis with/without hepatocellular carcinoma (HCC) accounted for approximately half of cases of liver transplantation.[1–4] Etiological trends of cirrhosis are also changing in Turkiye.

Morbidity and mortality in patients with chronic viral hepatitis are linked to the persistence of viral replication. Viral suppression with oral antiviral therapy against HBV has achieved clinical benefits due to the prevention of disease progression, reduction in hepatic decompensation, and HCC development.[5–8] Lamivudine (LMV), adefovir dipivoxil (ADV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF) are inhibitors of HBV polymerase/reverse transcriptase.[7] Currently, LMV and ADV are no longer used in the treatment of chronic hepatitis B (CHB).[7] The aims of the present study were to compare the long-term efficacy of weak antiviral therapies, such as LMV or ADV, and potent antiviral treatments, such as ETV or TDF, in patients with HBV-related cirrhosis, and to investigate whether virological response (VR) with these antiviral treatments results in a lower probability of disease progression and the development of HCC in such patients.

Materials and Methods

Patients

This was a retrospective single-center HBV cohort study. A total of 120 consecutive patients with HBV-related cirrhosis were enrolled in the study between January 2005 and January 2014. The diagnosis of CHB was made based on the biochemical, serological, virological, and histological data, when available.[7] ICD-10 codes were used to identify CHB, cirrhosis, and its complications. Among the 120 patients, 24 were treated with LMV, 100 mg daily; 35 were treated with ETV, 0.5 mg daily; while 61 were treated with TDF, 245 mg daily, at the investigators' discretion. All cirrhotic patients were followed for at least 6 months. Decompensation of cirrhosis, including ascites, variceal bleeding (VB), hepatic encephalopathy (HE), acute kidney injury (AKI), and HCC, were evaluated. Data were collected from outpatient visit charts. The Ankara Universtiy School of Medicine Ethics Committee of our faculty approved this study (Ethics Decision Number: 01-04-14, 13.01.2014). The Declaration of Helsinki conducted our study.

Table 1. Characteristics of patients with HBV-related cirrhosis

	Overall (n=120)	HBeAg positive (n=23)	HBeAg negative (n=97)	p
Age (year)	55.9±10.4 (57)	52.7±11.5 (54)	56.7±10.0 (58)	0.101
Gender (M/F)	96/24	18/5	78/19	0.778
Baseline ALT (U/L)	67.2±71.0 (43.5)	72.2±52.4 (48)	66.0±75.0 (40)	0.158
Baseline HBV-DNA Log 10 IU/ml	4.9±1.2 (3.45)	8.7±1.8 (2.9)	4.0±1.0 (2.9)	0.243
Baseline total bilirubin (mg/dl)	1.48±1.8 (1.18)	1.34±1.3 (1.1)	1.51±1.9 (1.2)	0.33
Baseline GGT (U/L)	75.8±96.8 (45)	35.7±15.5 (34)	85.2±105.3 (50)	**0.014**
Baseline albumin (g/dl)	3.75±0.57 (3.8)	3.8±0.56 (3.9)	3.74±0.57 (3.8)	0.59
Baseline INR	1.12±0.1 (1.1)	1.09±0.1 (1.08)	1.13±0.1 (1.1)	0.30
Baseline creatinine (mg/dl)	0.85±0.2 (0.82)	0.87±0.2 (0.82)	0.85±0.2 (0.82)	0.53
Thrombocyte count X 10^9 /lt	146±67.3 (142)	161±63.1 (172)	142±68.1 (138)	0.23
Baseline MELD score	9.16±2.7 (8)	8.65±2.9 (8)	9.28±2.7 (9)	0.13

Data were given Mean±SD (median). SD: Standart deviation; HBeAg: Hepatitis B e-Antigen; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; GGT: Gamma-glutamyl transpeptidase; INR: International normalised ratio; MELD: Model for end-stage liver disease.

Methods

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), bilirubin, and complete blood cell counts were measured by our central laboratory. Serological markers for viral infections (anti-HAV IgM, HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc IgG, anti-HCV, anti-HDV, anti-HIV, anti-HEV, anti-cytomegalovirus [CMV], anti-herpes simplex virus [HSV], and anti-Epstein-Barr virus [EBV]), serum iron, ferritin, ceruloplasmin, and alpha-1 antitrypsin levels were measured. Serological studies were performed for anti-nuclear antibodies, anti-smooth muscle antibodies, anti-liver kidney membrane-1, and anti-mitochondrial antibodies. All patients underwent abdominal sonography confirming the presence of cirrhosis, its complications, and HCC.

HBV DNA levels were measured using the Roche COBAS TaqMan assay. HBsAg and HBeAg loss, seroconversion, and drug resistance were monitored.

Definitions

The primary outcome measure was VR, as defined by serum HBV DNA negativity. Intention-to-treat (ITT) analysis was used to evaluate VR under antiviral treatment. A secondary outcome measure was to determine the development of decompensation or HCC. A virological breakthrough was defined as a >1 log10 increase in serum HBV DNA level above nadir or confirmed detectability of HBV DNA after having an undetectable result.

Follow-up

Patients were seen at 3-month intervals in the outpatient clinic during the follow-up period. A physical examination and biochemical, serological, and virological tests were performed at each visit. The Model for End-Stage Liver Disease (MELD) score was used to assess the severity of chronic liver disease. Further investigations included surveillance for HCC with radiological imaging and alpha-fetoprotein determinations every 6–12 months. If necessary, dynamic computed tomography or magnetic resonance imaging was performed. Possible adverse events (AE) of the antiviral agents were assessed.

Statistical Analyses

Data analysis was performed using Statistical Package for the Social Sciences version 22.0 (SPSS, Inc., Chicago, IL, USA). Mean, standard deviation, median, and percent were used for descriptive statistics. The conformity of the data to the normal distribution was assessed with a histogram and the Kolmogorov-Smirnov test. Comparisons were made using the Paired Samples t-test and the Independent Samples t-test, as appropriate. Nominal variables were evaluated using the Chi-Square test. Kaplan-Meier analysis was used to estimate the cumulative risk of HCC, cumulative HBeAg seroconversion, and emergence rate of LMV resistance. A p-value less than 0.05 was considered significant.

Results

A total of 120 cirrhotic patients were enrolled in the analysis. The median age was 57.0 years (range, 29–86 years), 80% were men, and 19.2% were HBeAg-positive. The median baseline serum alanine aminotransferase (ALT) and HBV DNA levels were 43.5 U/L and 3.45 log10 copies/mL, respectively. No significant differences in baseline characteristics among patients with e-antigen positive or negative were observed, except baseline serum GGT levels were higher in patients with HBeAg negative (85.2±105.3 U/L vs 35.7±15.5 U/L, p=0.014). The baseline characteristics of the patients are given in Table 1.

Among 120 cirrhotic patients, 77.5% of them were compensated, and 22.5% were decompensated: 75% of the patients were classified as having Child-Pugh class A, 21.7% as having Child-Pugh class B, and 3.3% as having Child-Pugh class C. Ascites (66.7%) was the most common finding of decompensation, followed by VB (22.2%) and AKI (11.1%). Median Child-Pugh and MELD scores were 5 (range: 5–10) and 8

Table 2. Baseline characteristics of patients treated with potent and weak antiviral agents

	Patients on Potent Antivirals (TDF/ETV)	Patients on Weak Antivirals (LMV)	p
Age (year)	54.8±9.8 (57)	60.5±11.57 (59.5)	**0.015**
Gender (M/F)	75/21	21/3	0.400
Baseline ALT (U/L)	72.7±76.9 (46.5)	44.7±31.4 (38.5)	0.055
Baseline HBV-DNA Log 10 IU/ml	4.94±1.2 (3.0)	4.78±1.28 (5.6)	0.995
Baseline total bilirubin (mg/dl)	1.47±1.97 (1.10)	1.51±0.89 (1.27)	0.21
Baseline GGT (U/L)	69.6±95.0 (42.5)	100.3±102.3 (65)	**0.021**
Baseline albumin (g/dl)	3.8±0.6 (3.9)	3.5±0.6 (3.5)	**0.044**
Baseline INR	1.11±0.2 (1.1)	1.15±0.2 (1.15)	0.258
Baseline creatinine (mg/dl)	0.83±0.2 (0.81)	0.93±0.2 (0.85)	0.068
Thrombocyte count X 10^9 /lt	149±68 (147)	132±62 (127)	0.269
Baseline MELD score	8.9±2.7 (8)	9.8±2.5 (10)	0.05

TDF: Tenofovir disoproxil fumarate; ETV: Entecavir; LMV: Lamivudine; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; GGT: Gamma-glutamyl transpeptidase; INR: International normalised ratio; MELD: Model for end-stage liver disease.

Table 3. Virological response and ALT normalization during the antiviral treatment

Month	6		12		24		36		48	
	HBV DNA	ALT	HBV DNA	ALT	HBV DNA	ALT	HBV DNA	ALT	HBV DNA	ALT
Potent antiviral (TDF, ETV)	55/96 (57.3%)	34/55 (61.8%)	67/96 (69.7%)	36/55 (65.4%)	74/96 (77 %)	40/55 (72.7%)	79/96 (82.2%)	41/55 (74.5%)	78/96 (81.2%)	42/55 (76.3%)
Weak antiviral (LMV)	13/24 (54.1%)	6/9 (66.6%)	12/24 (50%)	7/9 (77.7%)	10/24 (41.6%)	7/9 (77.7%)	9/24 (37.5%)	4/9 (44.4%)	9/24 (37.5%)	4/9 (44.4%)

HBV: Hepatitis B virus; ALT: Alanine aminotransferase; TDF: Tenofovir disoproxil fumarate; ETV: Entecavir; LMV: Lamivudine.

(range: 6–23), respectively. When admitted, three patients (2.5%) were diagnosed with HCC.

Virological Response (VR)

The median treatment duration was 60 months (19–156 months). Patients treated with weak antiviral agents were older than patients with potent antiviral agents (60.5±11.6 years vs 54.8±9.8 years, p=0.015) (Table 2). Baseline serum HBV DNA levels, ALT levels, and disease severity did not significantly differ between patients receiving weak and potent antiviral treatments (Table 2).

With ITT, the VR after 6 months, 1 year, 2 years, 3 years, and 4 years of potent antiviral treatments was 57.3% (55/96), 69.7% (67/96), 77.0% (74/96), 82.2% (79/96), and 81.2% (78/96), respectively, while the VR with weak antiviral treatments was 54.1% (13/24), 50.0% (12/24), 41.6% (10/24), 37.5% (9/24), and 37.5% (9/24) (p<0.001 for 2, 3, and 4 years of therapy). ALT normalization after 6 months, 1 year, 2 years, 3 years, and 4 years of potent antiviral treatment was 61.8% (34/55), 65.4% (36/55), 72.7% (40/55), 74.5% (41/55), and 76.3% (42/55), respectively. In comparison, ALT normalization with weak antiviral treatment was 66.6% (6/9), 77.7% (7/9), 77.7% (7/9), 44.4% (4/9), and 44.4% (4/9) (Table 3).

Serological Response

HBeAg loss was achieved in 30.4% of 23 HBeAg-positive patients while on antiviral treatment. The cumulative probability of HBeAg loss increased from 4.3% at 1 year to 18.7% at 4 years and 31.2% at 5 years of antiviral therapy (Fig. 1). HBeAg loss was slightly higher in patients treated with potent antiviral treatment than those treated with weak antiviral treatment (6.3% vs 4.2%, p=0.697). None of the patients had experienced HBsAg loss during the antiviral therapy.

Development of HCC

New HCC developed in 10 patients (8.5%, 10/117). The cumulative probability of the development of HCC was 2.6% at 1 year, 6.8% at 2 years, and 8.7% at 3 and 5 years of antiviral therapy (Fig. 2). Four of the ten patients received weak antiviral treatment, while six were on potent antiviral treatment. Three patients were diagnosed with HCC in the first year of the antiviral treatment, five in the second year, and two in the third year. At the time of the HCC diagnosis, seven patients had a detectable serum HBV DNA level, whereas the remaining three had VR (Table 4).

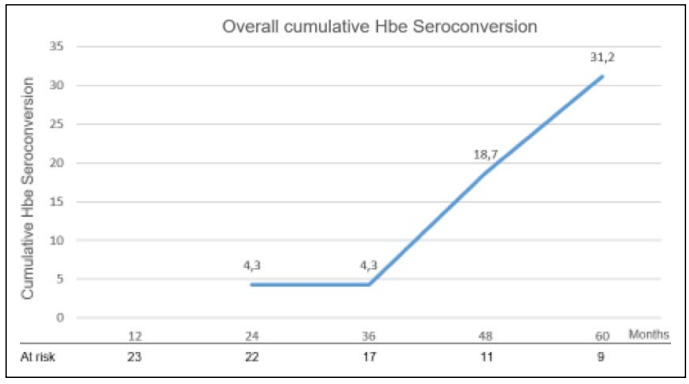

Figure 1. Cumulative probability of HBeAg seroconversion to antiHBe in patients with HBV-related cirrhosis while on antiviral treatment.

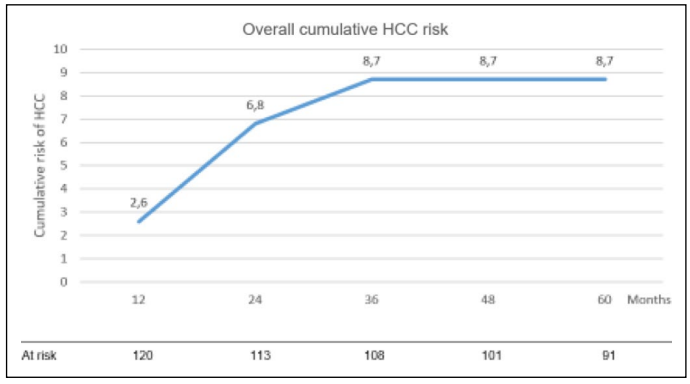

Figure 2. Cumulative probability of HCC development in patients with HBV-related cirrhosis over time.

Table 4. Characteristics of patients who developed HCC during the follow-up period

HCC development	Antiviral treatment	ALT level (U/L)	HBV-DNA level Log10 IU/ml	Liver disease
1. case	ETV	31	6.54	Decompensated
2. case	TDF	25	4.81	Compensated
3. case	TDF	34	4.11	Decompensated
4. case	TDF	25	–	Compensated
5. case	LMV	21	–	Decompensated
6. case	LMV	52	2.43	Decompensated
7. case	ETV	22	2.55	Decompensated
8. case	TDF	24	2.74	Decompensated
9. case	LMV	70	2.46	Decompensated
10. case	LMV	19	–	Decompensated

HCC: Hepatocellular carcinoma; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; TDF: Tenofovir disoproxil fumarate; ETV: Entecavir; LMV: Lamivudine.

MELD scores among patients treated with potent antiviral treatment significantly improved from baseline to week 60 (8.0±3.0 to 7.0±3.2, p=0.006, respectively).

Safety

Antiviral treatments were well tolerated. None of the patients discontinued antiviral therapy because of AEs. No significant difference in the mean baseline serum creatinine level between the two groups was observed (0.83±0.2 mg/dL vs 0.93±0.2 mg/dL, p=0.068). Serum creatinine levels among patients did not significantly change over time (0.83±0.20 mg/dL to 0.88±0.23 mg/dL, p=0.505 and 0.89±0.48 mg/dL to 0.85±0.22 mg/dL, p=0.575, respectively). Only four patients experienced a 0.5 mg/dL increase in serum creatinine levels at 5 years of the treatment.

The overall emergence rate of LMV resistance was observed in 15 of 24 patients treated with LMV. The cumulative probability of emergence of LMV resistance was 4.2% after 1 year, 8.3% at 2 years, 25.6% at 3 years, 34.9% at 4 years, and 49.9% at 5 years of the treatment. TDF treatment was initiated in patients with an emergence rate of LMV resistance.

Decompensation was developed in 6 compensated patients treated with weak antiviral agents and 10 compensated patients treated with potent antiviral agents.

Overall, 29 patients died of causes considered unrelated to antiviral treatment. Among them, 19 patients were on potent antiviral treatment, whereas 10 were on weak antiviral treatment.

Discussion

This study demonstrates that long-term potent antiviral treatment, ETV or TDF, effectively suppressed HBV replication in patients with HBV-related cirrhosis. VR was significantly higher in patients treated with potent antiviral treatments than those treated with weak antiviral treatments. These findings are consistent with previous studies[5,6,9,10] suggesting that ETV or TDF is effective in the long-term management of patients with HBV-related cirrhosis.

Previous studies have shown that the risk of progression to cirrhosis and the development of its complications strongly correlates with HBV viral load in CHB patients.[11,12] Moreover, HBV viral suppression with antiviral therapy reduced the incidence of disease progression and improved the clinical outcome.[7,8,13] Marcellin et al.[14] demonstrated that long-term HBV viral suppression with TDF improved clinical outcomes and led to the regression of cirrhosis. Zoutendijk et al.[15] reported that a VR with ETV reduces the probability of developing clinical events in patients with HBV-related cirrhosis. This study confirms that long-term ETV or TDF, as potent antiviral agents, effectively suppressed viral replication in patients with cirrhosis and significantly improved clinical outcomes, as indicated by an improvement in the baseline MELD score.

The goals of effective antiviral therapy in HBeAg-positive CHB patients are HBeAg seroconversion to anti-HBe and, ultimately, HBsAg seroconversion to hepatitis B surface antibody (anti-HBs).[7] HBsAg seroclearance predicts long-lasting viral suppression, diminishes disease progression, and improves clinical outcomes.[7,16,17] HBsAg seroclearance is suboptimal under oral antiviral treatment.[7,16–18] The present study achieved HBeAg loss in 30% of HBeAg-positive patients with cirrhosis. An increasing probability of HBeAg loss over time is observed. The results of this study are comparable to those of previous studies.[16,17,19] Unfortunately, none of the cirrhotic patients had expe-

rienced HBsAg loss while on antiviral treatment. These results indicate that serological response was maintained and steadily increased through antiviral treatment periods.

Several studies have mentioned the association of high HBV viral load with the development of HCC.[11,12,20–22] In the present study, the cumulative probability of the development of HCC increased from 2.6% at 1 year to 8.7% at 5 years of antiviral therapy. Ten patients were diagnosed with new HCC in the first 3 years of the antiviral treatment. HCC in these ten patients may have already developed before the antiviral therapy. HCC occurred more in nonresponding CHB patients or patients with viral breakthroughs than in those who experienced VR.[22] At the time of the new HCC diagnosis, seven of the ten patients on antiviral treatment had a detectable serum HBV DNA level.

Oral antiviral agents were well tolerated in cirrhotic patients in the present study. None of the patients discontinued antiviral therapy because of AE. Serum creatinine levels remained stable during the treatment period.

Conclusion

In conclusion, potent antiviral treatment effectively maintains the virological response, reduces the incidence of disease progression, and improves the clinical outcome during the long-term follow-up of patients with HBV-related cirrhosis. Although HCC may still develop, it occurs at a lower rate. Long-term antiviral treatment can be safely continued in patients with HBV-related cirrhosis.

Ethics Committee Approval: The Ankara Universtiy School of Medicine Ethics Committee granted approval for this study (date: 13.01.2014, number: 01-04-14).

Author Contributions: Concept – GK, TG, EB, ZME, HG, RI; Design – GK, TG, EB, ZME, HG, RI; Supervision – GK, TG, EB, ZME, HG, RI; Materials – GK, TG, EB, ZME; Data Collection and/or Processing – GK, TG, EB, ZME; Analysis and/or Interpretation – GK, HG, RI; Literature Search – GK, TG, EB, ZME, HG, RI; Writing – GK, RI; Critical Reviews – GK, TG, EB, ZME, HG, RI.

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Research Article

Infection in liver transplantation

HEPATOLOGY FORUM

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Liver transplantation without pneumocytsis jirovecii prophylaxis - Single center experience

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Abstract

Background and Aim: Pneumocystis jirovecii (PJ) can be seen in solid organ transplant (SOT) recipients. Despite guidelines recommending PJP prophylaxis for 6–12 months post-transplantation, the necessity for liver transplant patients remains controversial, with conflicting evidence on PJP rates. This study examined PJP occurrence in 242 liver transplant patients at a single center who received no PJP prophylaxis.

Materials and Methods: A retrospective study examined the clinical and microbiological data of 242 liver transplant (LTx) patients to evaluate PJP incidence within one year post-transplant. PJP was diagnosed microbiologically and/or radiologically in cases of clinical suspicion, without systematic screening. The study investigated PJP infection risk factors reported previously, including cytomegalovirus (CMV) infection, bolus steroid therapy, age >65, prolonged neutropenia, and anti-thymocyte globulin (ATG) usage.

Results: The study involved 242 liver transplant recipients, with an average age of 56 years, predominantly male (71%), and a mean Model for End-Stage Liver Disease (MELD) score of 16. No PJP cases were reported. Among PJP risk factors, none had prolonged neutropenia, though two developed CMV infection. Empirical steroid bolus treatment for suspected acute cellular rejection was given to 62 patients (26%). The cohort included 22 (9%) individuals over 65 years old, and none received ATG.

Conclusion: This pioneering study examines a substantial living liver donor transplantation (LDLT) cohort without PJP prophylaxis, suggesting it may be unnecessary in centers with low immunosuppression and a low percentage of risk factors. Prospective studies are essential to establish targeted prophylactic approaches due to variations in PJP incidence across centers.

Keywords: Immunosupression; liver transplantation; prophylaxis; pneumocytis jirovecii pneumonia.

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Introduction

Pneumocystis jirovecii (PJ), formerly referred to as Pneumocystis carinii, is a widespread opportunistic organism that primarily affects immunocompromised individuals, such as those who have received solid organ transplants (SOT). The majority of opportunistic infections emerge within 1 to 6 months following orthotopic liver transplantation (OLT), coinciding with the period of higher-intensity immunosuppression.[1] According to established protocols, SOT recipients are typically recommended to undergo Pneumocystis jirovecii pneumonia (PJP) prophylaxis during the initial 6–12 months post-transplantation.[2–6]

Trimethoprim-sulfamethoxazole (TMP-SMX) is considered the optimal preventive treatment for SOT. Although PJP prophylaxis is advocated for SOT recipients in guidelines, reviews, and population-based studies, its necessity for liver transplant patients remains controversial.[7–9] While in the 1980s the incidence of PJP was high, recent studies have shown a low PJP incidence in LT recipients without prophylaxis.[10–14]

On the other hand, other single-center studies show an increased risk of PJP that warrants prophylaxis.[14–16] Although there is some evidence indicating no significant increase in the incidence of PJP without preventive measures, a comprehensive population-based study revealed an elevated risk of PJP among transplant recipients compared to the general population, persisting even after two years post-transplantation.[9]

The aim of this study is to examine the occurrence of PJP in a cohort of 242 liver transplant patients without PJP prophylaxis at a single center.

Material and Methods

Study Population

A retrospective study examined the clinical and microbiological data of 248 consecutive liver transplant (LTx) patients. Data were gathered retrospectively from patient records. Patients were monitored monthly for the first three months, and subsequently every three months for the first year. International patients who could not attend our centers had follow-up appointments via televisits with the same schedule. Any medical treatment outside our center was consulted with our team.

The analysis excluded six patients who did not survive beyond five days post-LTx. One died due to intraoperative cardiac arrest, two due to primary non-graft function, two due to multi-organ failure, and one due to intracranial hemorrhage. No postmortem analysis for PJ was conducted. Two patients who received second transplants at 150 and 1,071 days due to primary disease recurrence were evaluated as new index transplants. The study omitted two patients who underwent combined

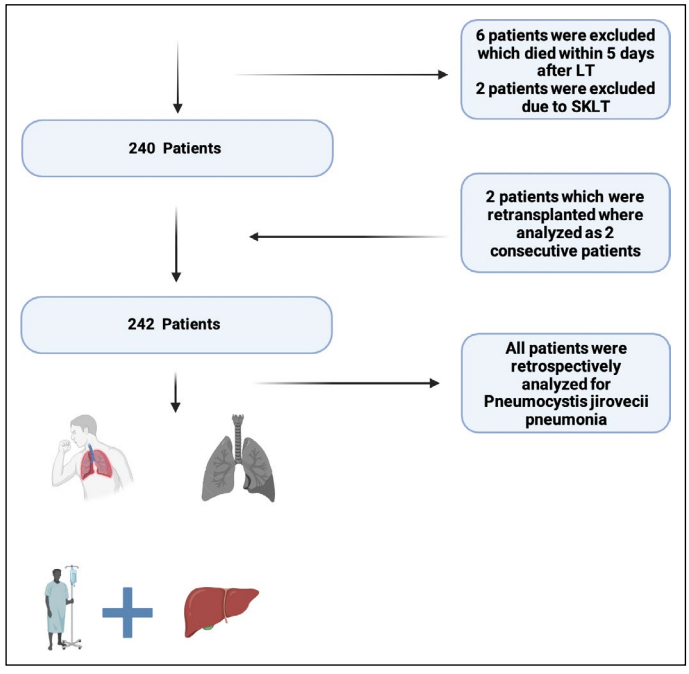

Figure 1. Flow chart of the study.

SKLT: Simultaneous kidney-liver transplantation.

kidney-liver transplantation, as they received PJP prophylaxis per protocol in our kidney transplant patients. Consequently, the final analysis included a total of 242 LTs (Fig. 1). Table 1 presents the recipients' demographic and perioperative data. All surviving patients were monitored for at least one year.

Living donors provided right liver grafts for 225 of the 242 LT patients (93%). The piggy-back technique was used in all cases, including cadaveric transplants. An infectious disease expert, collaborating with a multidisciplinary medical group, managed the treatment of liver transplant patients with suspected or confirmed infections. Patients received piperacillin-tazobactam as prophylaxis during the perioperative phase. Antifungal and anti-PJP prophylaxis were not administered. Instead, patients were prescribed nystatin 50,000 U thrice daily for the initial three months post-transplantation to prevent mucosal candidiasis.

When infections occurred, broad-spectrum antimicrobials were empirically administered after obtaining appropriate cultures. Treatment was adjusted based on the identified pathogens and antibiotic susceptibility. Patients underwent a three-month course of valganciclovir for anti-cytomegalovirus (CMV) prophylaxis, beginning within the first week after liver transplantation, and were monitored for potential infectious complications.

If a patient had pneumonia with suggestive radiographic findings, PJP was clinically suspected. Definitive diagnosis of PJP was established by identification of the organism in sputum samples (when accessible) or bronchoalveolar lavage (BAL) specimens either by Giemsa staining, fluorescent antibody staining, or polymerase chain reaction assays. Analysis of BAL was performed by a pathologist together with an infectious disease specialist. No lung biopsies were taken. When definitive diagnosis could not be made in patients with highly suggestive clinical and radiological findings, a presumptive diagnosis was made, and the existing empirical treatment was continued or newly started. No systematic screening for PJ was conducted using any tests.

Table 1. Patient basic characteristics

Characteristic	Evaluable subjects (n=242)
Age, mean (SD)	56±11*
Male, n (%)	171 (71%)
MELD†, mean (SD)	16 (6–40)**
Etiology, n (%)	
Non-tumor	164 (68%)
With tumor	78 (32%)
HCC	74 (31%)
Perioperative variables	
CIT, min	80 (40–632)**
Op time, min	501 (185–950)**
PRBC, units	3.1±3.1
ICU stay, days	1 (1–30)**
ICU stay >48 h, n (%)	66 (27%)
BL, n (%)	40 (17%)
LDLT, n (%)	223 (92%)

SD: Standard deviation; MELD: Model of end stage liver disease; †: Biologic MELD; HCC: Hepatocellular carcinoma; CIT: Cold ischemia time; PRBC: Packed red blood cells; ICU: Intensive care unit; BL: Biliary leak; LDLT: Living donor liver transplantations. *: Mean±SD; **: Median 25–75%.

Additional outcome measures included the frequency of severe acute rejection that did not resolve spontaneously and the incidence of active CMV infection or disease. Risk factors previously defined by guidelines from the American Society of Transplantation Infectious Diseases Community of Practice were evaluated in our cohort.[5] All protocols were performed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The Gazi University Ethical Commission approved this study (31.01.2025/E.1157653).

Immunosuppressive Protocol

The immunosuppression protocol employed a triple regimen of low-dose tacrolimus. Initially, tacrolimus was given at 0.05 mg/kg/day, divided into two doses, with target whole-blood trough levels of 8–10 ng/mL during the first three months post-transplantation, and 6–8 ng/mL subsequently. Mycophenolate mofetil was initiated within 24 hours of transplant at 1.5 g/day, administered in two doses.

The corticosteroid regimen began with 100 mg/day methylprednisolone on the first day, gradually tapered to a maintenance dose of 15 mg/day by the tenth day and discontinued after three months, except for cases with autoimmune liver disease as the etiology of cirrhosis. Acute cellular rejection episodes were managed with intravenous bolus corticosteroid therapy using 2 g methylprednisolone.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 26; IBM Corporation, Armonk, NY, USA). Categorical variables were presented as numbers and percentages, whereas normally dis-

Table 2. Number of patients having risk factors for PJP infection

Risk factors	n (%)
Prolonged neutropenia	0 (0)
CMV infection	2 (1)
Empiric bolus corticosteroid therapy	62 (26)
Advanced age >65	22 (9)
ATG	0 (0)

CMV: Cytomegalovirus infection; ATG: Antithymocyte globulin.

tributed variables were expressed as the mean±standard deviation. For non-parametrically distributed variables, the median (minimum–maximum) and interquartile range (25%–75%) were used.

Results

Demographic Variables

In our patient group, the average age was 56 years, with males comprising 71% of the participants. The mean MELD score was 16. Among the 242 liver transplant (LT) recipients, 164 (68%) had no underlying malignancy.

Incidence of Opportunistic Infections

Fifteen patients underwent BAL due to suspected PJP, while sputum samples were analyzed for 20 patients, and no lung biopsies were performed. There was not a single case with a definitive or presumptive diagnosis of PJP in the cohort.

Two cases of CMV infection were encountered. A total of 14 fungal infections were recorded, seven of which were classified as invasive fungal infections. Antifungal and antimicrobial therapy within 100 days post-transplant was documented. In the first 100 days following surgery, 42 patients (17%) were administered empirical antifungal treatment alongside antibiotics due to suspected fungal infections. Once a fungal infection was ruled out, antifungal medication was discontinued. Fifteen patients were treated solely with antimicrobial therapy due to bacterial infections.

Risk Factors for PJP

The American Society of Transplantation Infectious Diseases Community of Practice identifies several risk factors for PJP, including prolonged neutropenia, CMV infection, empirical bolus steroid therapy, age >65, and administration of ATG. Neutropenia was defined as $<0.5 \times 10^9$/L.

During the evaluation of defined PJP risk factors, no patients experienced prolonged neutropenia, although two individuals developed CMV infection. A total of 62 patients were administered empirical steroid bolus therapy due to suspected acute cellular rejection (ACR). Given that the majority of transplants were living donor liver transplants (LDLT), liver biopsies are frequently avoided in suspected ACR cases to minimize potential risks to the graft. Only two patients underwent liver biopsies, which confirmed ACR. All patients responded positively to empirical steroid treatment. The patient cohort included 22 (9%) individuals over 65 years old. No patient received ATG.

Discussion

PJP is a devastating condition with a high mortality rate among immunocompromised patients. Hence, guidelines recommend PJP prophylaxis in SOT patients.[5,17]

Despite these recommendations, various medical facilities worldwide implement total prophylaxis, selective prophylaxis, or none. A multicenter survey endorsed by the European Liver and Intestine Transplant Association found large variations in antibiotic and antifungal prophylaxis across transplant centers.[18]

According to the protocol at our center, no preventive treatment was administered to patients at risk for PJP. No cases of PJP infection were observed during the first year of follow-up. This finding aligns with other studies showing a low rate or no incidence of PJP infection without prophylaxis or with short-term prophylaxis for three months.[15–17,19,20]

A systematic review and statistical analysis of randomized controlled trials suggest that adult patients should be considered for PJP preventive measures when their risk exceeds 3.5%.[8] In contrast, a recent population-based study comparing 10,530 SOT recipients, including 4,281 LT recipients, with non-SOT individuals using propensity score matching found that SOT recipients had a greater risk of developing PJP, which could manifest at any time post-transplantation. SOT recipients with coexisting HIV infection, hematologic malignancies, or vasculitis have an increased risk of PJP.[9]

During the early years of liver transplantation, more intensive protocols adopted from kidney transplant practices necessitated PJP prophylaxis. Over the past ten years, less aggressive immunosuppressive strategies have been adopted, raising questions about the necessity of such preventive measures. Some centers still utilize rigorous immunosuppressive approaches, with ATG administered to 20% of patients and triple immunosuppressive therapy administered to 60%.[21]

The absence of PJP cases in our center may be attributed to our minimal immunosuppressive protocol. Additionally, PJP infection risk factors were scarce; only two patients had CMV infection, none experienced prolonged neutropenia, and none received ATG. Empirical bolus steroid therapy was administered to only 62/242 (25%) patients, while 22/242 (9%) were >65 years old (Table 2). The low prevalence or absence of PJP infection risk factors likely contributed to the low incidence of PJP in our cohort, consistent with previous studies showing a correlation between these risk factors and PJP development.[22,23]

Research and guidelines on invasive fungal infection risk identify biliary leaks and high MELD scores as contributing factors.[24,25] Most studies reporting low PJP incidence without prophylaxis did not include MELD scores in their analyses. Our center's median MELD score of 16 is comparatively low for many Western counterparts. Our experience with living donor liver transplantation (LDLT) and elective procedures could be the primary reason for fewer complications and reduced post-intensive care unit stay. In contrast, Western centers predominantly perform deceased donor liver transplantation (DDLT) with high MELD scores, which could explain the higher risk of PJP infection.

Following transplantation, drug toxicity often causes liver enzyme elevation, with antibiotics being the main culprit in most cases.[26] TMP-SMX is known to induce various side effects, including bone marrow suppression, elevated creatinine, hyperkalemia, rash, Stevens-Johnson syndrome, and liver enzyme elevation.[27] Consequently, it is advisable to minimize unnecessary antibiotic use in these patients.

Globally, conflicting results regarding anti-PJP prophylaxis exist owing to variations in immunosuppressive protocols, geographical differences

in PJP incidence, risk factor prevalence, LDLT versus DDLT, and potentially MELD scores. Therefore, prospective multicenter studies are essential to avoid unnecessary prophylaxis and to implement a targeted, selective approach.

The main drawbacks of our study include its retrospective nature, the limited one-year follow-up period, and the inability to define PJP risk factors due to the absence of PJP infection cases.

Conclusion

This study represents the first comprehensive analysis of a major LDLT cohort without PJP prophylaxis. Our findings indicate that anti-PJP prophylaxis may not be essential in centers incorporating low-intensity immunosuppressive protocols with a low incidence of PJP risk factors. The persistence of variations in PJP incidence among centers necessitates prospective studies to develop targeted prophylactic strategies globally.

Ethics Committee Approval: The Gazi University Clinical Research Ethics Committee granted approval for this study (date: 31.01.2025, number: E.1157653).

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Research Article

HEPATOLOGY FORUM

Results of liver transplantation due to celiac

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Long-term results of celiac disease patients who underwent liver transplantation

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Abstract

Background and Aim: Although there are a few studies reporting transplantation for celiac disease (CD), there are no studies reporting long-term outcomes after transplantation in CD patients. Therefore, we aimed to report the long-term outcomes of patients who underwent liver transplantation (LT) for CD in our high-volume liver transplantation center.

Materials and Methods: Our study was a single-center, retrospective study and included 28 CD patients who underwent LT at Inonu University. CD diagnosis was made based on anti-tissue transglutaminase or anti-endomysium antibody positivity and/or duodenal biopsy results.

Results: The 1-, 3-, 5-, and 10-year survival rates after transplantation were 92.9%, 92.9%, 84.4%, and 75%, respectively. The most striking finding in the study was the high frequency of biliary complications. Another important finding was the significant difference in body mass index (BMI) between pre-transplant and post-transplant ($p<0.001$). The incidence of rejection and recurrence was 39.1% and 25%, respectively. The number of patients with high anti-tissue transglutaminase (anti-TTG) levels after transplantation decreased significantly ($p<0.001$).

Conclusion: Our study suggests that the frequency of post-transplant biliary complications is very high in CD patients and that LT had positive effects on BMI and anti-tissue transglutaminase levels.

Keywords: Celiac disease; liver; transplantation.

Introduction

Celiac disease (CD) is a T-cell autoimmune disorder of the small intestine characterized by malabsorption resulting from the ingestion of gluten, the main protein fraction in wheat, rye, and barley, in genetically predisposed individuals. The prevalence in the general population varies between 0.5% and 2%, with an average of around 1%.[1] Although CD is defined as a disease with malabsorption in the small intestine, untreated cases also affect other organs, including the liver. Liver involvement may progress to end-stage liver failure in patients who do not adhere to a gluten-free diet.[2] There is also a close relationship between celiac disease and autoimmune liver disease. Studies have found the prevalence of celiac disease to be 3–7% in patients with primary biliary cirrhosis, 3–6% in patients with autoimmune hepatitis, and 2–3% in patients with primary sclerosing cholangitis.[3–5]

In liver transplant patients with end-stage liver disease from different causes, the prevalence of CD varies between 3% and 4.3%.[2] Although there are a few studies reporting transplantation for CD,[6] there are no studies reporting long-term outcomes after transplantation in CD patients. Therefore, we aimed to report the long-term outcomes of CD patients who underwent liver transplantation in our high-volume liver transplantation center.

Materials and Methods

Our study was a single-center, retrospective study and included 28 CD patients who underwent liver transplantation at Inonu University between January 2004 and December 2023. Celiac disease diagnosis was made based on anti-tissue transglutaminase or anti-endomysium antibody positivity and/or duodenal biopsy results. Among patients who underwent transplantation due to cryptogenic cirrhosis, patients with positive anti-tissue transglutaminase or anti-endomysium antibody were also considered as celiac patients. Clinical and laboratory data of patients were obtained by reviewing the patients' electronic files. This study was carried out with the permission of the Inonu University Ethics Committee with the decision number 2024/5649. The study was conducted in accordance with the Declaration of Helsinki.

Pre-Transplant Parameter

Patients' age, gender, blood group, concomitant diseases, transplantation type (living donor-cadaveric), Model End Stage Liver Disease-Na

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(MELD-Na) scores at the time of transplantation, anti-tissue transglutaminase level before and after transplantation, presence of iron deficiency anemia and osteoporosis, body mass index, transplantation indication, donor's age, gender, blood group, consanguinity between the donor and the recipient, presence of Rh-incompatible transplantation, and presence of hepatocellular carcinoma were recorded.

Post-Transplant Parameters

Presence of hepatocellular carcinoma after transplantation, immunosuppressive treatment used after transplantation, use of ursodeoxycholic acid or steroids after transplantation, survival time, causes of death, presence of diarrhea after transplantation, presence of portal vein thrombosis, hepatic vein thrombosis, hepatic artery thrombosis after transplantation (if any), duration, development of recurrence and rejection, relationship with other malignancies, presence of second and third transplantation, cytomegalovirus infection, biliary complication frequency and feature, type of bile duct anastomosis (duct-to-duct anastomosis/hepaticojejunal anastomosis), treatment of post-transplant biliary complications, and liver biopsy results (acute rejection, chronic rejection, antibody-mediated rejection, disease recurrence, if any) were recorded.

Statistical Analyses

The analyses were evaluated using SPSS (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) version 22. In the study, descriptive data were shown as n, % values for categorical data and as mean ± standard deviation (mean±SD) values for continuous data. Chi-square analysis (Pearson Chi-square) was applied to compare categorical variables between groups. The Kolmogorov-Smirnov test was used to evaluate the compliance of continuous variables with normal distribution. Student t-test was used to compare binary groups. In the analyses, the statistical significance level was accepted as $p<0.05$.

Results

A total of 28 patients were included in the study: 12 (42.9%) male and 16 (57.1%) female. The mean age of the patients was 21.3±14.1 years. Eight patients were younger than 18 years old. Two (7.1%) of the transplants were deceased donor, and 26 (92.9%) were living donor liver transplantation. The mean follow-up period of the patients was 73.3±46.1 months. Table 1 shows demographic characteristics of patients undergoing liver transplantation due to CD.

The results of all patients after liver transplantation are summarized in Table 2. One of the six patients who died had a deceased donor transplant. Half of the deaths were biliary and the other half were secondary to rejection. The 12-month and 36-month survival rates were 92.9%; the 60-month survival rate was 84.4%, and the 120-month survival rate was 75% (Fig. 1). Factors affecting mortality were analyzed, and only age was found to be a significant factor. The average age of those who died was significantly lower than the average age of those who did not die ($p=0.041$). Other parameters had no significant effect on mortality ($p>0.05$) (Table 3).

The most striking finding in the study was the high frequency of biliary complications. Biliary complications were found to develop in one of two deceased donor transplants (50%) and in 73% of living donor transplants. Biliary complications occurred in 3 of 4 patients (75%) with HJ and 17 of 24 patients (70.8%) with duct-to-duct anastomosis. Of the

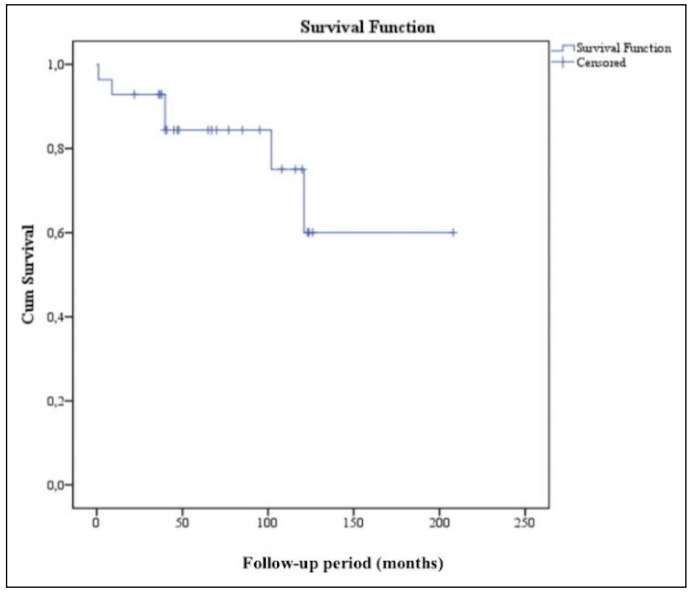

Figure 1. Kaplan–Meier survival curve of celiac disease patients after liver transplantation.

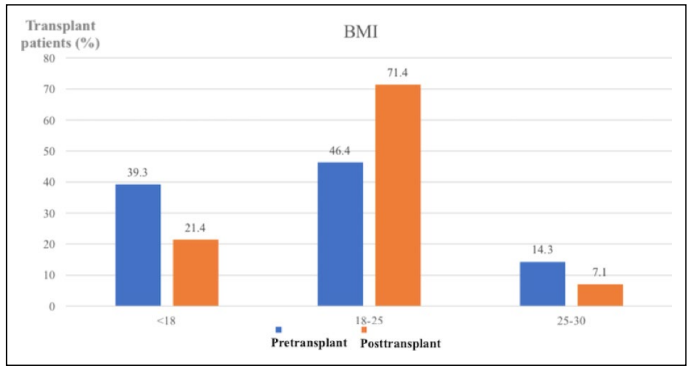

Figure 2. Comparison of body mass index (BMI) before and after liver transplantation in celiac disease patients.

patients with duct-to-duct anastomosis, 11 had biliary stricture, 3 had stricture and leakage, and 3 had stricture and stones. Of the patients with HJ, 2 had stricture and 1 had leakage. All patients with HJ and biliary complications (100%) were successfully treated with percutaneous transhepatic biliary interventions (PTBI). ERCP was performed in 16 patients with duct-to-duct anastomosis and biliary complications, and biliary problems were resolved in 9 patients with ERCP. The endoscopic success rate was 56.2%. PTBI was performed in 7 patients with duct-to-duct anastomosis. One patient with duct-to-duct anastomosis and biliary complications recovered with medical treatment without the need for any interventional treatment. All patients with duct-to-duct anastomosis and biliary complications (100%) were successfully treated with ERCP + PTBI. Surgery was not performed in any patient with biliary complications.

Another important finding was the significant difference in BMI between pre-transplant and post-transplant ($p<0.001$, Fig. 2). While 39.3% of the patients had a BMI of <18 before transplantation, 46.4% had a BMI of 18–25 and 14.3% had a BMI of 25–30. After transplantation, 21.4% of the patients had a BMI of <18, 71.4% had a BMI of 18–25, and 7.1% had a BMI of 25–30.

Table 1. Demographic characteristics of patients undergoing liver transplantation due to Celiac disease

	Cadaveric (n=2)		Living donor (n=26)		Total (n=28)	
	n	%	n	%	n	%
Gender						
Male	0	0	12	46.2	12	42.9
Female	2	100	14	53.8	16	57.1
Age, Mean±SD (median)	18.0±17.0 (18.0)		21.6±14.2 (19.50)		21.3±14.1 (19.50)	
Follow-up period, Mean±SD (months)	58.5±26.2		74.4±47.5		73.3±46.1	
Dietary compliance before transplantation						
Yes	1	50	4	15.4	5	17.9
No	1	50	22	84.6	23	82.1
HCC before transplantation						
Yes	1	50	2	7.7	3	10.7
No	1	50	24	92.3	25	89.3
Associated disease						
Yes	0	0	18	69.2	18	64.3
No	2	100	8	30.8	10	35.7
Associated disease						
Type 1 diabetes mellitus	0	0	1	5.6	1	5.6
Primary biliary cholangitis+Crohn	0	0	1	5.6	1	5.6
Irritable bowel syndrome	0	0	1	5.6	1	5.6
IgA nephropathy	0	0	1	5.6	1	5.6
Autoimmune hepatitis	0	0	13	72.2	13	72.2
Fibrocystic liver disease	0	0	1	5.6	1	5.6
Transplant indication						
High MELD score	1	50	19	73.1	20	71.4
Persistent pruritus	0	0	1	3.8	1	3.6
Portal Hypertension-related complications	0	0	6	23.1	6	21.4
HCC	1	50	0	0	1	3.6
Bile duct anastomosis						
HJ	1	50	3	11.6	4	14.3
Duct-to duct	1	50	23	88.4	24	85.7

SD: Standard deviation; MELD: The model for end-stage liver disease; HCC: Hepatocellular carcinoma; HJ: hepaticojejunostomy.

Celiac recurrence developed in 7 patients, and these patients had living donor transplants except for one patient. Six of these patients had no dietary compliance after transplantation. Among the patients who underwent living donor transplantation, 2 developed acute rejection, 4 developed chronic rejection, and 3 developed antibody-mediated rejection. It was found that half of deceased transplant patients (1/2, 50%) developed chronic rejection. A total of 11 patients (39.3%) developed rejection.

While 77.8% of the patients had high anti-tissue transglutaminase levels before transplantation, this rate became 10.5% after transplantation, and the rate of normality increased significantly ($p<0.001$). Iron deficiency anemia, osteoporosis, and duodenal biopsy results of the patients before and after transplantation did not change significantly ($p>0.05$). The frequency of iron deficiency anemia was 35.7% (10/28) before transplantation and 46.4% (13/28) after transplantation. The frequency of osteoporosis was 10.7% (3/28) before transplantation and 17.9% (5/28) after transplantation. Findings compatible with celiac disease in duodenal biopsy were 32.1% (9/28) before transplantation and 25% (7/28) after transplantation.

Table 2. The results of all patients after liver transplantation

	Cadaveric (n=2) n (%)	Living donor (n=26) n (%)	Total (n=28) n (%)
Drugs after transplantation			
Tacrolimus	0 (0)	2 (7.7)	2 (7.1)
Everolimus+Tacrolimus+MMF	1 (50)	8 (30.8)	9 (32.1)
Tacrolimus+MMF	1 (50)	7 (26.9)	8 (28.6)
Tacrolimus+Cyclosporine+Everilumus+MMF	0 (0)	7 (26.9)	7 (25.0)
Unknown	0 (0)	1 (3.8)	1 (3.6)
Tacrolimus+Everilumus+Cyclosporine	0 (0)	1 (3.8)	1 (3.6)
Corticosteroid	0 (0)	4 (15.4)	4 (14.3)
UDCA	1 (50)	3 (11.5)	4 (14.3)
Corticosteroid+UDCA	1 (50)	18 (69.2)	19 (67.9)
Death			
Yes	1 (50)	5 (19.2)	6 (21.4)
No	1 (50)	21 (80.8)	22 (78.6)
Cause of death			
Biliary sepsis	1 (50)	2 (7.6)	3 (10.7)
Rejection	0 (0)	3 (11.5)	3 (10.7)
Recurrence			
Yes	1 (50)	6 (23.1)	7 (25)
No	1 (50)	20 (76.9)	21 (75)
Rejection			
Var	1 (50)	10 (38.5)	11 (39.3)
Yok	1 (50)	16 (61.5)	17 (60.7)
Biliary complication			
Yes	1 (50)	19 (73)	20 (71.4)
No	1 (50)	7 (27)	8 (28.6)
Second transplantation			
Yes	0 (0)	3 (11.5)	3 (10.7)
No	2 (100)	23 (88.5)	25 (89.3)
Portal vein thrombosis			
Yes	0 (0)	2 (7.7)	2 (7.1)
No	2 (100)	24 (92.3)	26 (92.9)
Hepatic arthery thrombosis			
Yes	0 (0)	1 (3.8)	1 (3.6)
No	2 (100)	25 (96.2)	27 (96.4)
Hepatic vein thrombosis			
Yes	0 (0)	1 (3.8)	1 (3.6)
No	2 (100)	25 (96.2)	27 (96.4)

Table 2 (cont). The results of all patients after liver transplantation

	Cadaveric (n=2) n (%)	Living donor (n=26) n (%)	Total (n=28) n (%)
Surgery for biliary complication			
Yes	0 (0)	0 (0)	0 (0)
No	2 (100)	26 (100)	28 (100)
ERCP			
Yes	1 (50)	15 (57.7)	16 (57.1)
No	1 (50)	11 (42.3)	12 (42.9)
PTBI			
Yes	1 (50)	9 (34.6)	10 (35.7)
No	1 (50)	17 (65.3)	18 (64.3)
CMV			
Yes	1 (50)	2 (7.7)	3 (10.7)
No	1 (50)	24 (92.3)	25 (89.3)
HCC after transplantation			
Yes	0 (0)	0 (0)	0 (0)
No	2 (100)	26 (100)	28 (100)
Diarrhea after transplantation			
Yes	1 (50)	6 (23.1)	7 (25.0)
No	1 (50)	20 (76.9)	21(75.0)

MMF: Mycophenolate mofetil; UDCA: Ursodeoxycholic acid; ERCP: Endoscopic cholangiopancretaography; PTBI: Percutaneous transhepatic biliary intervention; CMV: Cytomegalovirus; HCC: Hepatocellular carcinoma.

Discussion

In our study, we found the 1-, 3-, 5-, and 10-year survival rates in CD patients who underwent transplantation to be 92.9%, 92.9%, 84.4%, and 75%, respectively. The mean age of patients who died after transplantation was significantly lower than that of survivors. We also found that liver transplantation had positive effects on BMI and anti-tissue transglutaminase levels. One of the most striking findings in our study was the high frequency of biliary complications.

Since there are very few publications in the literature on liver transplantation outcomes due to CD, we had to compare our results, especially regarding survival, with the results after liver transplantation due to autoimmune diseases. In a study conducted by Mottershead et al.[7] in 2008 on patients who underwent liver transplantation due to autoimmune hepatitis, 1- and 5-year survival rates were found to be 87% and 80–90%, respectively. In two different studies, post-transplant survival rates in patients with autoimmune hepatitis were also reported as 85–97% and 78.4%, respectively.[8,9] In the European Liver Transplant Registry, the 5- and 10-year survival rates for PSC were 80% and 83% after transplantation.[10] In a study conducted by Egawa et al.[11] on 444 patients who underwent transplantation due to PBC, 5-year survival was found to be 76.6%. The survival rate of CD patients who underwent liver transplantation in our center is similar to liver transplantations performed due to other autoimmune diseases worldwide. Our results suggest that survival rates after liver transplantation are quite satisfactory in patients with end-stage liver disease due to CD, for which no other treatment options are available.

In our study, recurrence was observed in 7 (28%) patients who underwent liver transplantation due to CD. In a study conducted by Alabraba et al.,[12] recurrence was observed in 259 (18.5%) of 1,399 patients who underwent liver transplantation due to PSC. In two studies, the recurrence rate after liver transplantation in patients with AIH was 17.9% and 41%, respectively.[9,13] Khettry et al.[14] reported that 18.6% of 43 PBC-related transplant patients developed recurrence. Our results suggest that the recurrence rate after liver transplantation due to CD is similar to other autoimmune diseases.

Since CD is an autoimmune disease, it may be accompanied by other autoimmune diseases. The prevalence of autoimmune hepatitis in celiac disease is 1.6%.[15] Lawson et al.[16] reported the prevalence of PBC as 0.1% in a study of 4,732 CD patients. In a study including a very large number (136,735) of CD patients, the prevalences of AIH, PBC, and PSC were 0.32%, 0.15%, and 0.004%, respectively.[17] Kaukinen et al.[18] reported that CD was detected in 8 of 185 liver transplant patients. Among the patients with CD, 3 had primary biliary cirrhosis, 1 had autoimmune hepatitis, 1 had primary sclerosing cholangitis, 1 had congenital liver fibrosis, and 1 had secondary sclerosing cholangitis.

In our study, in patients who underwent transplantation due to CD, 46% had autoimmune hepatitis, 3.5% had PBC, 3.5% had IgA nephropathy, and 3.5% had Type 1 DM. All these data and our results suggest that the frequency of AIH and PBC is higher in CD patients with liver transplantation and that autoimmune liver diseases may coexist in CD patients with liver transplantation.

Table 3. Factors affecting mortality

	Death				p*
	Yes		No		
	n	%	n	%	
Gender					0.673
Male	2	16.7	10	83.3	
Female	4	25	12	75.0	
Biliary complication					0.871
Yes	5	21.1	15	78.9	
No	1	22	7	78	
Type of biliary complications					0.247
Stricture	3	23	10	76.9	
Leak	1	100	0	0	
Stricture + leak	0	0	3	100	
Stricture + stone	1	33	2	66	
MELD before LT					0.640
0–15	1	12.5	7	87.5	
>15	5	25.0	15	75.0	
Bloood group					0.880
A	3	30.0	7	70.0	
B	1	12.5	7	87.5	
0	2	22.2	7	77.8	
AB	0	0	1	100	
BMI before LT					0.696
<18	3	27.3	8	72.7	
18–25	3	23.1	10	76.9	
25–30	0	0	4	100	
BMI after LT					0.130
<18	3	50.0	3	50	
18–25	3	15.0	17	85.0	
25–30	0	0	2	100.0	
Dietary compliance before LT					0.932
Yes	1	20.0	4	80.0	
No	5	21.7	18	78.3	
Dietary compliance after LT					0.549
Yes	0	0	4	100	
No	6	25.0	18	75.0	
Donor gender					0.653
Male	3	27.3	8	72.7	
Female	3	17.6	14	82.4	
Age, Mean±SD	29.5±10.5		41.2±16.1		**0.041**

Table 3 (cont). Factors affecting mortality

	Death				p*
	Yes		No		
	n	%	n	%	
Donor blood group					0.826
A	2	20.0	8	80.0	
B	0	0	4	100.0	
0	4	28.6	10	71.4	
Donor degree of kinship (1st degree)					0.064
Yes	2	10.5	17	89.5	
No	4	44.4	5	55.6	
Rh incompatibility					0.443
Yes	0	0	2	100.0	
No	6	23.1	20	76.9	
HCC before LT					0.107
Yes	2	66.7	1	33.3	
No	4	16.0	21	84.0	
HCC after LT					–
Yes	0	0	0	0	
No	6	21.4	22	78.6	
Associated disease					0.891
Yes	4	22.2	14	77.8	
No	2	20.0	8	80.0	
PVT after LT					0.443
Yes	0	0	2	100.0	
No	6	23.1	20	76.9	
HAT after LT					0.214
Yes	1	100.0	0	0	
No	5	18.5	22	81.5	
HVT after LT					0.595
Yes	0	0	1	100.0	
No	6	22.2	21	77.8	
CMV					0.338
Yes	0	0	3	100.0	
No	6	24.0	19	76.0	

MELD: The model for end-stage liver disease; BMI: Body mass index; HCC: Hepatocellular carcinoma; PVT: Portal vein thrombosis; HAT: Hepatic artery thrombosis; HVT: Hepaitc vein thrombosis; CMV: Cytomegalovirus; LT: Liver transplantation.

In our study, it was observed that the number of patients with high anti-TTG levels after transplantation decreased significantly. In a study conducted by Rubio-Tapia et al.[2] on patients who underwent liver transplantation due to CD, it was reported that the rate of patients with pre-transplant anti-TTG positivity decreased significantly after transplantation and even became negative. One study suggested that there may be two reasons for the decrease in anti-TTG levels after transplantation in CD patients. The first reason is that removal of the diseased liver reduces the level of anti-TTG antibodies, as it may be a target organ. The second reason is that immunosuppressive drugs used

after transplantation affect the production of autoantibodies.[19] In addition, correction of intestinal barrier dysfunction caused by cirrhosis after transplantation may also reduce the antigenic environment and B-cell activation and lead to normalization of anti-TTG.[2] Our results are similar to the study by Rubio-Tapia et al.,[2] suggesting that liver transplantation has a positive effect on anti-TTG levels in CD patients who underwent liver transplantation.

In our study, post-transplant acute and chronic rejection rates in CD patients were 7% and 21.4%, respectively. In the study conducted by Rubio-Tapia et al.,[2] it was reported that 3 (30%) of 10 double (anti-TTG and EMA) positive patients developed rejection. In a study conducted by Chouik et al.[20] on a large number of patients who underwent liver transplantation due to autoimmune hepatitis, acute rejection was detected in an average of 23.5%, and this rate decreased from 45.7% in the early years of liver transplantation to 13.4% over the years. The AASLD AIH guideline reported the incidence of chronic rejection as 16% for AIH, 5.2% for PSC, and 8.2% for PBC.[21] In a study conducted by our group at our center on patients who underwent liver transplantation due to PSC, the incidence of acute and chronic rejection was 13.3% and 10%, respectively.[22] Our results suggest that the rate of post-transplant rejection in patients with CD in our center is higher than liver transplants performed for other autoimmune diseases worldwide. In addition, although the number of studies reporting the frequency of post-transplant rejection in CD patients is very small, the results of Rubio-Tapia et al.[2] and ours suggest that post-transplant rejection is common in CD patients.

In our study, the frequency of post-transplant biliary complications was found to be quite high. Biliary complications occurred in 50% of deceased donor liver transplants and 73% of living donor liver transplants. There are studies reporting the frequency of biliary complications after transplantation in AIH and PSC patients as 25.3% and 36.1%, respectively.[20,23] In a study including a large number of patients, biliary complications were reported in 11.1% of 6,471 deceased donor liver transplant patients and in 20.8% of 389 living donor liver transplant patients.[24] It has been reported that ERCP was applied to 283 (18.7%) of 1,506 LDLT patients with duct-to-duct anastomosis in our institute between 2015 and 2021.[25] The majority of the patients in our study had living donor LT. Therefore, it is possible that our results are related to technical reasons associated with living donors. However, because of the small number of patients in our study, it is not easy to interpret whether the high frequency of biliary complications is related to celiac disease or technical reasons. Although our case number is small, our results suggest that the frequency of post-transplant biliary complications in CD is higher than in other autoimmune diseases.

Another important finding in our study was the significant difference in BMI before and after transplantation. Our results suggest that liver transplantation provides significant benefits on weight gain in CD. In this study, no significant change was detected in the iron deficiency anemia (IDA), osteoporosis, and duodenal biopsy results of the patients before and after transplantation. The lack of change in the frequency of osteoporosis may be related to the side effects of immunosuppressive drugs used after transplantation on the bones. Our result regarding iron deficiency anemia can also be explained by the lack of positive change in small intestine histology after transplantation.

Conclusion

Our results suggest that the frequency of post-transplant biliary complications is very high in CD patients and that LT had positive effects on BMI and anti-tissue transglutaminase levels.

Ethics Committee Approval: The Inonu University Clinical Research Ethics Committee granted approval for this study (date: 05.03.2024, number: 2024/5649).

Informed Consent: The requirement for individual informed consent was waived due to the retrospective nature of the study.

Conflict of Interest: The authors have no conflict of interest to declare.

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Research Article

HEPATOLOGY FORUM

Effects of rifaximin on fructose-induced steatohepatitis

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Effects of rifaximin in fructose-induced steatohepatitis in rats

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Abstract

Background and Aim: Metabolic-dysfunction-associated steatotic liver disease and its related mortality are increasing worldwide. This study evaluated the potential of rifaximin in preventing and treating steatohepatitis induced by a high-fructose diet by modulating intestinal pathology.

Materials and Methods: Forty-two rats were randomly divided into six groups: one group received a normal diet, another was fed a fructose diet, two groups received rifaximin (once or three times weekly) along with a fructose diet, and the remaining two groups were given rifaximin (once or three times weekly) with a normal diet. After eight weeks, liver tissues were examined for malondialdehyde, tumor necrosis factor-α, nuclear factor-κB, and nuclear factor erythroid 2–related factor 2 using Western blot analysis, while blood samples were analyzed for uric acid, liver enzymes, triglycerides, and cholesterol; plasma tumor necrosis factor-α was measured by ELISA.

Results: The fructose diet group showed significant increases in body and liver weights, ballooning degeneration, lobular inflammation, and macrovesicular steatosis. Metabolic dysfunction-associated steatotic liver disease developed in 21 rats, yet steatohepatitis was observed only in the fructose-only group. Biochemical markers, including liver enzymes, triglycerides, and cholesterol, were significantly elevated in the fructose group. Moreover, plasma and tissue tumor necrosis factor-α and nuclear factor-κB levels were higher in the fructose group (p=0.03), while Nrf-2 levels were elevated in the rifaximin-treated groups (p=0.043). Additionally, MDA levels were markedly increased in the fructose-only group (p=0.033) and decreased dose-dependently with rifaximin treatment (p=0.029).

Conclusion: These findings suggest that rifaximin's anti-inflammatory and antioxidant effects may alleviate fructose-induced steatohepatitis, although further clinical studies are warranted.

Keywords: Fructose; non-alcoholic fatty liver disease; rifaximin.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), one of the most common liver diseases in the world, can begin as simple steatosis and progress to cirrhosis.[1,2] It has been suggested that increased dietary fructose consumption is causing a parallel increase in diabetes, obesity, and MASLD in industrialized societies.[3,4] In daily clinical practice in Turkiye, it has been observed that MAFLD has a very high prevalence.[5]

Endotoxin-dependent cytokine production has been shown to play a role in the pathogenesis of MASLD. Increased bacterial overgrowth has been found in the small intestine of patients with metabolic dysfunction-associated steatohepatitis (MASH) compared to healthy controls.[6] In MASH, cytokines, and especially tumor necrosis factor (TNF)-α, are released from Kupffer cells in response to endotoxin released from intestinal flora, hepatocytes, and adipose tissue macrophages, and free fatty acids increase. In addition, oxidative stress increases Kupffer cell activation and free fatty acid oxidation in hepatocyte mitochondria, peroxisomes, and microsomes. Nuclear factor erythroid 2–related factor (Nrf-2), an antioxidant, decreases in oxidative stress. Malondialdehyde (MDA) is an essential product of membrane lipid peroxidation.[7]

Fructose is absorbed by active transport in the intestines; insulin is required to enter the cells, thus generating a glycemic response. Long-term fructose administration causes hepatic macro- and microvesicular steatosis, a 98% increase in hepatic triglyceride, and an 89% increase in hepatic cholesterol content.[8] Endotoxin levels were high in portal plasma in fructose-induced MASLD in mice. Increased endotoxin levels may activate proinflammatory cytokines, leading to pathologies ranging from simple steatosis to the development of MASH.[9] Rifaximin (RFX) is a non-absorbable antibiotic in the gastrointestinal tract and has minimal systemic effects. Rifaximin has effectively treated hepatic encephalopathy and irritable bowel syndrome in recent years. RFX acts by inhibiting bacterial translocation and through bacterial decontamination.[10] This study aims to investigate the inhibitory role of RFX in an experimental model of fructose-induced MASLD. In our study, the effects of a fructose-rich diet on various physiological and biochemical parameters and the potential reducing effects of RFX supplementation were investigated in rats.

Materials and Methods

The study was carried out at the Firat University Research Center after obtaining approval from the Firat University Animal Experiments Ethics Committee, following the standard ethical rules for experimental animal studies. The study was conducted in accordance with the

Declaration of Helsinki. A total of 42 male Sprague-Dawley rats (average weight 220 g) were housed at 22±1°C with a 12-hour light/dark cycle and fed standard pellet feed and water. The experiment lasted eight weeks. Standard pellet feed and tap water were used for feeding the animals. The body weights of the rats were monitored throughout the experiment. The experiment lasted eight weeks. Rats in rifaximin groups received 15 mg/kg, and those on a fructose diet had 50% fructose in their drinking water. The study groups (n=7) were: Control (normal diet), Fructose (fructose diet), F+Rif1 (fructose diet + rifaximin once a week), F+Rif3 (fructose diet + rifaximin three times a week), ND+Rif1 (normal diet + rifaximin once a week), and ND+Rif3 (normal diet + rifaximin three times a week).

Bottles were regularly cleaned, and solutions were refreshed to prevent pathogen development. After eight weeks, rats were fasted overnight, decapitated under anesthesia, and blood samples were taken and centrifuged. Livers were removed and weighed, and tissue samples were fixed in 10% formalin for further analysis.

Biochemical Analysis

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), cholesterol, triglyceride, glucose, and uric acid levels were measured in serum (Olympus AU600). Serum TNF-α levels were studied by the Enzyme-Linked ImmunoSorbent Assay (ELISA) method using an appropriate commercial kit.

Measurement of Protein Expression by Western Blot Analysis

Tissue TNF-α (Anti-TNF-α antibody, Abcam, Cambridge, UK), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Anti-NFκBp65 antibody, Abcam, Cambridge, UK), and Nrf-2 (Nuclear factor (erythroid-derived 2)-like, Abcam, Cambridge, UK) were studied by the Western blot method using kits.

Liver tissue was homogenized in a 1:10 (w/v) solution of 5 µM soy (soluble powder; Sigma, St. Louis, MO, USA) as a trypsin inhibitor and 10 mM Tris-HCl (pH 7.4, 0.1 mM NaCl). Protein concentration was measured using the Lowry method (Protein Kit, Sigma). Proteins were transferred to nitrocellulose membranes (Schleicher and Schuell Inc., Keene, NH, USA) and incubated for 1 hour. After washing, protein loading was verified using a β-actin monoclonal antibody (A5316; Sigma, St. Louis, MO, USA). Protein levels were analyzed densitometrically using ImageJ (National Institutes of Health, Bethesda, USA).

Analysis in High-Performance Liquid Chromatography (HPLC)

Lipid peroxidation was measured in terms of malondialdehyde (MDA) production, using the high-performance liquid chromatography (HPLC, Shimadzu, Tokyo, Japan) method with a Shimadzu UV–Vis SPD-10 AVP detector and C18-ODS-3.5 µm, 4.6×250 mm column.

Histopathological Evaluation

After opening the abdomen, hepatic lobes were removed, weighed, and placed in a 10% formalin and standard saline solution. Tissue from the right lobe was stained with hematoxylin-eosin (HE) and examined under an Olympus BX-50 light microscope. Scoring was performed for hepatic inflammation, macrovesicular steatosis, and ballooning degeneration based on images from 10 random fields.

Mallory's bodies were assessed as present/absent, and inflammation was scored as a percentage using a 4-point scale: grade 0 (no inflammation at ×200), grade 1 (less than 2 foci), grade 2 (2–4 foci), grade 3 (more than 4 foci). Ballooning degeneration was scored as: 0 (none), 1 (mild), 2 (diffuse). Fibrosis was scored as follows: 0 (none), 1 (perisinusoidal or periportal fibrosis), 2 (perisinusoidal or portal or periportal), 3 (cirrhosis and severe inflammation in the portal). Steatosis was evaluated as follows: 0 (none), 1 (steatosis in 5% of hepatocytes), 2 (5–33%), 3 (33–66%), 4 (>66%). In addition, MASLD activity scores were collected, and a score of >5 was accepted as MASH.[1,11]

Statistical Analysis

Paired t-test was used to evaluate the between-group parameters of the data obtained in the study. The Mann-Whitney U test was preferred in dual evaluations and was given as mean ± standard deviation. In addition, Pearson and Spearman correlation tests were used for some parameters. Statistical evaluations were made using the SPSS 12.0 package program. A p-value of <0.05 was considered statistically significant.

Results

In this study, MASLD developed in 21 rats in 3 groups, 7 rats in each group, fed with a fructose diet: Fructose (fructose diet), F+Rif1 (fructose diet + rifaximin once a week), and F+Rif3 (fructose diet + rifaximin three times a week). However, steatohepatitis findings were evident only in the group receiving the fructose diet. Significant weight gain and liver weight gain were found in the fructose diet group compared to the control group ($p<0.05$). No significant difference was found in weight gain and liver weight between the fructose diet group and the group receiving different doses of RFX in addition to the fructose diet ($p>0.05$). Again, no significant difference was found between the groups receiving RFX in addition to the normal diet and the control group in terms of weight measurements and liver weights (Table 1).

There was a significant increase in uric acid, ALT, ALP, GGT, triglyceride, and cholesterol levels in rats fed with fructose compared to the control group ($p<0.05$). There was a decrease in the biochemical parameters measured in the groups' blood given different doses of RFX ($p<0.05$). No significant difference was observed when the fasting glucose level measurements were examined. Glucose measurements between groups were analyzed, and similar values were found (Table 2).

Plasma TNF-α levels were significantly highest in rats on a fructose diet compared to the control group ($p=0.03$). However, RFX treatment significantly decreased TNF-α levels compared to the non-rifaximin group ($p=0.04$, $p=0.02$), with no significant difference between different RFX doses ($p=0.06$). In our study, tissue TNF-α levels were highest in the fructose group ($p=0.037$). TNF-α levels were also lower in the two groups given different doses of RFX with the fructose diet. However, the results of TNF-α levels were the same for the rifaximin groups receiving different amounts ($p=0.07$). In other words, TNF-α levels measured in plasma and TNF-α levels measured in tissue yielded parallel results. RFX given for therapeutic purposes confirmed our hypothesis; that is, levels were found to be low in the treatment group (Fig. 1).

NF-κB levels were significantly higher in the fructose diet group compared to the control group ($p<0.001$). They were significantly lower in the groups receiving RFX in addition to the fructose diet compared to those receiving only fructose ($p=0.026$). The NF-κB value was found to be lower in the RFX in addition to the normal diet and control groups ($p<0.05$), but this result was not statistically significant ($p=0.088$) (Fig. 2).

Table 1. Rat and liver weights in the groups

Groups	Basal weight Mean±SE	Final weight Mean±SE	Liver weight Mean±SE
Group 1 (control)	219±20.4[a]	280±13.2[b]	8.84±1.19[b]
Group 2 (fructose)	219±44.5[a]	355±6.8[a]	12.23±1.30[a]
Group 3 (fructose+R1)	220±20.1[a]	352.14±13.1[a]	10.7±1.40[a]
Group 4 (fructose+R3)	220±30.1[a]	350.86±6.81[a]	10.4±1.49[a]
Group 5 (normal diet+ R1)	220±26.0[a]	265±6.8[b]	9.1±1.09[b]
Group 6 (normal diet+ R3)	220±30.5[a]	273±6.8[b]	8.88±1.01[b]

SE: Standard error; a–d: The difference between the groups with different letters in the sam eline is statistically significant (p<0.05).

Table 2. Biochemical parameter results of the groups

	Glucose Mean±SE	Uric acid Mean±SE	ALT Mean±SE	ALP Mean±SE	GGT Mean±SE	Triglyceride Mean±SE	Cholesterol Mean±SE
Control	110.43±3.7[a]	1.29±0.54[c]	60±6.8[b]	306.7±6.4[b]	0.43±0.1[c]	120±6.8[c]	53±2.9[b]
Fructose	109.86±2.4[a]	4.54±1.59[a]	75.2±5.0[a]	463.1±60.9[a]	1.5±0.2[a]	180±41[a]	68.4±3.78[a]
F+Rif 1	112.29±10.3[a]	3.07±0.30[b]	66.7±1.6[b]	395.5±77.3[b]	1.0±0.2[b]	150±40.8[b]	58±7.3[b]
F+Rif 3	112.57±2.7[a]	2.21±0.62[b]	61±10.6[b]	350.8±49.1[b]	0.74±0.22[b]	130±26.7[c]	55.7±10.7[b]
ND+R1	111.43±6.29[a]	1.36±0.34[c]	62.8±2.19[b]	314.4±25.7[b]	0.46±0.21[c]	125±68.8[c]	54.7±3.3[b]
ND+R3	110.14±8.1[a]	1.50±0.25[c]	62.7±6.6[b]	310.8±20.8[b]	0.44±0.17[c]	125±35.5[c]	56.5±4.9[b]

SE: Standard error; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase GGT: Glutamyltransferase. a–d: The difference between the groups with different letters in the same line is statistically significant p<0.05.

Table 3. Histopathological findings

	Control	Fructose	F+Rif 1	F+Rif 3	ND+Rif 1	ND+Rif 3	p*
Inflammatory focus	0.14±0.378	1.29±0.488	0.43±0.535	0.43±0.535	0.14±0.378	0.14±0.378	0.003
Macrovesicular adipositiy	0.00±0.00	1±0.00	0.575±0.535	0.57±0.535	0.00±0.00	0.00±0.00	<0.001
Ballooning degeneration	0.14±0.378	2.71±0.488	2±0.577	1.86±0.690	0.14±0.378	0.29±0.488	<0.001
Pericellular fibrosis	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	>0.005

*: Kruskal-Wallis test value.

Nrf-2 was lower in the fructose-only group (p<0.001). A significant difference was found in the groups receiving RFX in addition to the fructose diet compared to the group receiving only fructose (p=0.043). However, the protective role of RFX was independent of the dose. When the fructose diet group was compared with the ND+Rif1 and ND+Rif3 groups, Nrf-2 values were found to be higher in the ND+Rif1 and ND+Rif3 groups (p=0.022) (Fig. 3).

MDA was significantly higher in the group using only fructose compared to the other groups (p=0.033). At the same time, there was a statistically significant decrease in MDA levels in all groups receiving different doses of RFX compared to the control group (p=0.029). While the level of MDA, one of the inflammation markers, was low in all groups receiving RFX and in the control group, the level of MDA was high in the group receiving fructose (Fig. 4). Accordingly, it was interpreted that fructose exposure increases inflammation, while rifaximin reduces inflammation.

In the histopathological examination, steatohepatitis developed with a fructose diet. Ballooning degeneration, lobular inflammation, and macrovesicular adiposity were significantly observed in the fructose diet group. However, it was observed that steatohepatitis decreased in the RFX-only groups when compared to the groups given RFX at different doses in addition to the fructose diet. Steatohepatitis findings were similar in the control and normal diet groups. While fibrosis was not observed in any group, other histopathological findings are shown in Table 3 and Figure 5.

Discussion

In this study, we investigated the effects of a fructose-rich diet on physiological and biochemical parameters in rats, as well as the potential mitigating effects of rifaximin supplementation. Our findings revealed significant weight gain in the fructose diet group compared to the con-

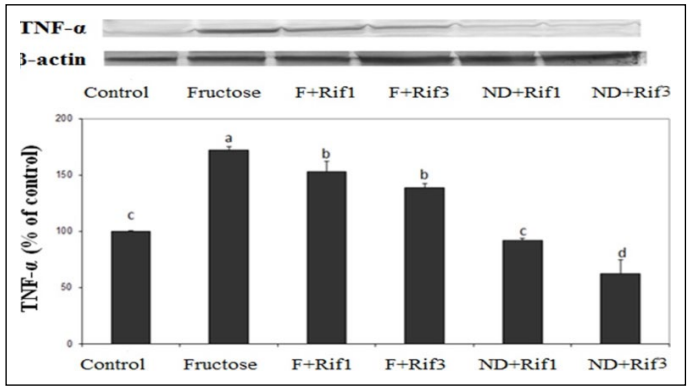

Figure 1. Hepatic tissue TNF-a levels.

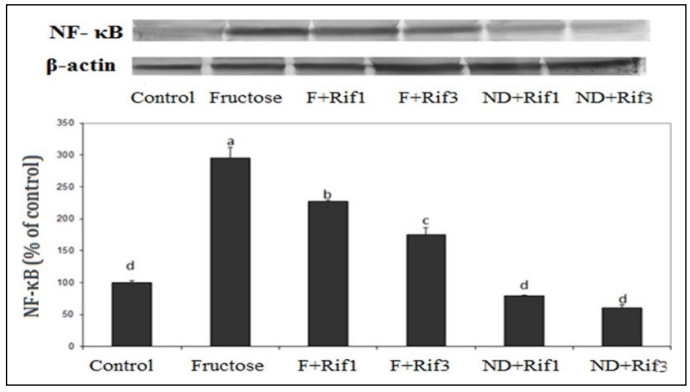

Figure 2. Hepatic tissue NF- κB levels.

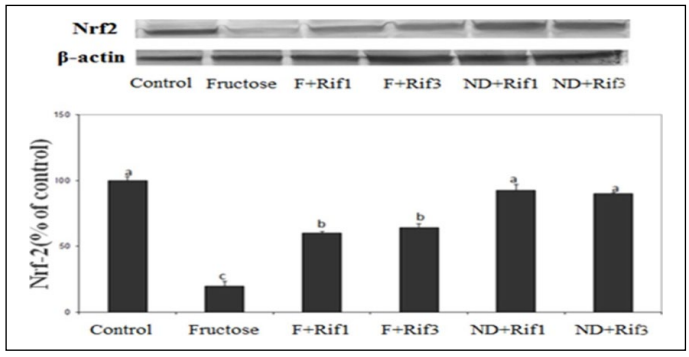

Figure 3. MDA levels of the groups.

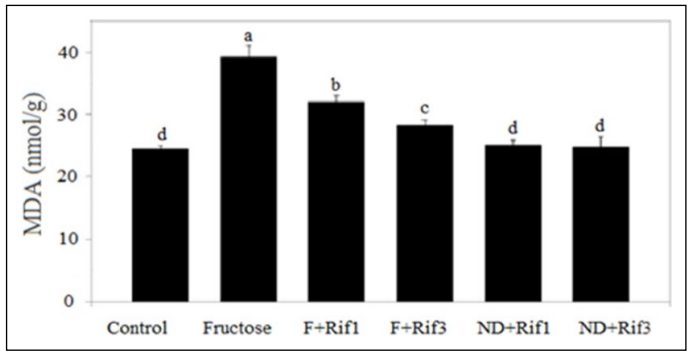

Figure 4. Hepatic tissue Nrf-2 levels.

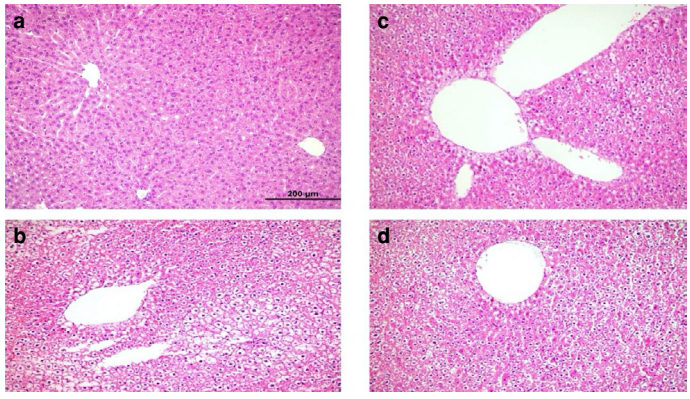

Figure 5. (a) Histopathological appearance of the hepatic tissue in the rats in the control group. **(b)** Severe ballooning degeneration and sporadic hepatic inflammation were found in the livers of the rats who were fed with 50% fructose solution. In this group, steatohepatitis findings were observed most extensively. **(c)** Regression in ballooning degeneration was found in the group who received RFX once a week in addition to 50% fructose solution compared to the group who received a fructose diet alone. **(d)** Macrovesicular adiposity, severe ballooning degeneration, or pericellular fibrosis were not found in the livers of the rats who were given RFX three times a week in addition to 50% fructose solution. The mildest findings were observed in this group compared to those who received a fructose diet.

trol group, with no substantial difference in weight gain between the fructose diet group and those that received varying doses of rifaximin. Liver weights were also significantly elevated in the fructose group; however, no significant differences were found between the fructose diet group and those receiving rifaximin. Biochemically, we noted increased levels of uric acid, ALT, ALP, GGT, triglycerides, and cholesterol in the fructose-fed rats, while rifaximin supplementation resulted in a significant decrease in these parameters. Moreover, TNF-α levels were significantly higher in the fructose group and were notably lower in the rifaximin-treated groups, indicating rifaximin's protective potential against steatohepatitis. The study also documented significantly elevated NF-κB levels in the fructose group and a marked reduction in both NF-κB and Nrf-2 levels in the rifaximin groups, suggesting that rifaximin may inhibit the inflammatory response caused by a fructose-rich diet. Histopathological assessments confirmed the development of steatohepatitis associated with the fructose diet and showed improvement in histological findings in rifaximin-treated groups. Overall, these results highlight the detrimental effects of a fructose-rich diet on metabolic health and the potential therapeutic benefits of rifaximin in mitigating steatohepatitis and associated inflammatory responses.

Markers such as TNF and NF-κB, which are indicators of oxidative stress in the blood, were elevated, and pathological indicators of steatohepatitis such as an increase in inflammatory cells, ballooning degeneration, and fibrosis were increased in the liver tissues evaluated in histopathological examination.

In animal and human studies, it has been suggested that endotoxins secreted from the intestine play a critical role in the development of MASLD. The liver is in constant communication with products derived from the intestine. Many studies have observed and proven that the intestinal microflora LPS-TLR4 signaling pathway may play a critical role in the pathogenesis of MASLD.[12,13] RFX is part of most treatments in the gastroenterological field. It is a gut-selective, oral antimicrobial agent that specifically reduces the recurrence of hepatic encephalopathy (HE).[14,15]

Most animal studies have concluded that chronic fructose intake induces high reactive oxygen species (ROS) formation in the liver of rodents.[11,12] Bergheim et al.[16] reported that feeding 30% fructose solution to mice for eight weeks significantly increased their markers for ROS formation. In our study, we found that rats fed a 50% fructose solution for eight weeks developed steatohepatitis. We found a significant reduction in histopathological findings of steatohepatitis, such as steatosis, inflammation, and ballooning degeneration, with RFX treatment. Our study is a rare study showing that different doses of RFX prevent early signs of fructose-induced steatohepatitis in rats. In one study, increased portal endotoxin levels and ROS formation were associated with induction of intrahepatic TNF-α expression.[17] Sapp et al.[18] observed that rapamycin reduced fructose in a zebrafish MASLD model. In mice fed a diet rich in fructose and cholesterol, endotoxin levels and lipid peroxidation increased TNF-α expression in portal blood. They noted a decrease in inflammatory markers when polymyxin B and neomycin were used simultaneously.[16] RFX has been shown to exert an anti-inflammatory effect through NF-κB. In steatohepatitis models using RFX, intestinal permeability increases and circulating NF-κB decreases.[19,20] Our data were in line with other studies. NF-κB, a proinflammatory cytokine, was similar between the control group and the groups receiving RFX in addition to the normal diet, suggesting that RFX is hepatoprotective against steatohepatitis.

Treatment with rifaximin is known to lead to a decrease in lipid peroxidation, thus reducing the levels of ROS and MDA.[21] In studies in patients with non-alcoholic steatohepatitis proven by liver biopsy, improvements in liver enzymes (ALT, AST, GGT), circulating endotoxins, TNF-α levels, and metabolic homeostasis were observed when six months of RFX was applied.[19] It has been proven in the literature that rifaximin regresses liver inflammation markers involved in the pathogenesis of steatohepatitis. As seen in the study by Longo et al.,[22] in our study, it was determined histopathologically and biochemically that steatohepatitis regressed in rat liver samples in the rifaximin group. In our study, tissue MDA and Nrf-2, markers of oxidative stress, were analyzed. We found that these two markers increased in rats fed a high-fructose diet and decreased with RFX. In addition, ALT levels, cholesterol, triglyceride levels, and other basic biochemical and metabolic features of steatohepatitis increased in rats fed a high-fructose diet and decreased with RFX.

In our study, we detected early signs of steatohepatitis histopathologically. However, we did not observe fibrosis development in any of the rats. Therefore, we thought that the rats should be fed a high-fructose diet for a longer period of time. In addition, this situation was identified as a limitation of the study.

Conclusion

In conclusion, our study shows that RFX alleviates early signs of fructose-induced steatohepatitis, drawing attention to RFX treatment in mitigating NAFLD/MASLD. The modulation of oxidative stress markers and improvement in histopathological findings highlight the need for further research to elucidate the mechanisms underlying the gut-liver axis in MASLD and to investigate the long-term effects of interventions such as RFX.

Ethics Committee Approval: The Firat University Animal Experiments Ethics Committee granted approval for this study (date: 16.02.2012, number: 13).

Conflict of Interest: The authors have no conflict of interest to declare.

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Case Report

Isolated IgG4-related sclerosing cholangitis

HEPATOLOGY FORUM

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Isolated IgG4-related sclerosing cholangitis mimicking hilar cholangiocarcinoma: A case report and review

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Abstract

Immunoglobulin G4-related sclerosing cholangitis (IgG4-SC) is a rare autoimmune disease characterized by fibroinflammatory lesions and bile duct strictures, often associated with type 1 autoimmune pancreatitis (AIP). Isolated IgG4-SC, occurring without AIP, is particularly uncommon and can clinically and radiologically mimic hilar cholangiocarcinoma, presenting with jaundice and bile duct strictures. Accurate differentiation between these conditions is essential, as surgical resection is the standard treatment for cholangiocarcinoma, whereas steroid therapy is the first-line treatment for IgG4-SC. This case report discusses a 55-year-old female patient who underwent left hepatectomy due to a hilar bile duct stricture initially suspected to be cholangiocarcinoma but was ultimately diagnosed as isolated IgG4-SC based on postoperative histopathological and immunohistochemical findings. The report highlights the diagnostic challenges of isolated IgG4-SC and emphasizes the importance of integrating histology, imaging, and serology to prevent unnecessary surgical interventions.

Keywords: Cholangiocarcinoma; cholangitis; IgG4.

Introduction

Immunoglobulin G4-associated sclerosing cholangitis (IgG4-SC) is a rare autoimmune disorder that manifests with fibroinflammatory lesions and strictures in the bile ducts.[1] It is frequently associated with type 1 autoimmune pancreatitis (AIP), forming part of the spectrum of IgG4-related disease (IgG4-RD). However, in the absence of AIP, the condition is classified as isolated IgG4-SC. Isolated IgG4-SC is a clinically uncommon entity and poses a diagnostic challenge due to its resemblance to hilar cholangiocarcinoma. Both conditions present radiologically as bile duct strictures and clinically with symptoms such as jaundice, making differentiation difficult. While hilar cholangiocarcinoma typically requires surgical resection, the primary treatment for IgG4-SC is corticosteroid therapy, as recommended by international consensus.[2] Accurate diagnosis is therefore essential to avoid unnecessary surgical interventions.

This case report presents a patient with isolated IgG4-SC who underwent surgical treatment due to diagnostic uncertainty with hilar cholangiocarcinoma. The report also reviews current diagnostic and therapeutic strategies, emphasizing the importance of distinguishing isolated IgG4-SC from malignant conditions.

Case Report

A 55-year-old female with no significant medical or surgical history presented with progressive right upper quadrant pain and jaundice. On clinical examination, she exhibited scleral icterus, but no palpable masses or signs of hepatosplenomegaly were observed. Laboratory investigations revealed the following: CRP 27 mg/L (reference range: 0.0–5 mg/L), sedimentation rate 81 mm/h, total bilirubin 2.24 mg/dL (reference range: 0.1–1.2 mg/dL), direct bilirubin 1.84 mg/dL (reference range: 0–0.3 mg/dL), alkaline phosphatase (ALP) 118 U/L (reference range: 45–125 U/L), gamma-glutamyl transferase (GGT) 221 U/L (reference range: 10–60 U/L), alanine aminotransferase (ALT) 7 U/L (reference range: 9–50 U/L), and aspartate aminotransferase (AST) 12 U/L (reference range: 15–40 U/L). Tumor markers, including alpha-fetoprotein (AFP) at 1.58 ng/mL (reference range: 0–7 ng/mL), carcinoembryonic antigen (CEA) at 1.96 U/mL (reference range: 0–20 U/mL), and CA 19-9 at 38.5 U/mL (reference range: 0–40 U/mL), were within normal limits. Tests for hepatitis A, B, C, and E were negative. Serum IgG4 levels were measured at 4.41 mg/dL (reference range: 3.92–86.4 mg/dL).

Abdominal ultrasonography showed intrahepatic bile duct dilatation without evidence of mass lesions. Contrast-enhanced magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) identified significant narrowing in the left hepatic duct at the bifurcation and soft tissue density with malignant characteristics, resulting in intrahepatic bile duct dilatation (Fig. 1). No abnormalities were observed in the extrahepatic bile ducts or pancreas. To further evaluate the biliary stricture, endoscopic ultrasonography (EUS) and endoscopic retrograde cholangiopancreatography (ERCP) were performed. EUS revealed bile duct wall thickening, while ERCP confirmed luminal narrowing at the left hepatic duct bifurcation. Biopsies obtained during ERCP were nondiagnostic due to insufficient tissue.

Given the diagnostic uncertainty and the high suspicion for hilar cholangiocarcinoma based on imaging and clinical presentation, surgical intervention was deemed necessary. The patient underwent an exploratory laparotomy, which revealed thickened, edematous walls of the

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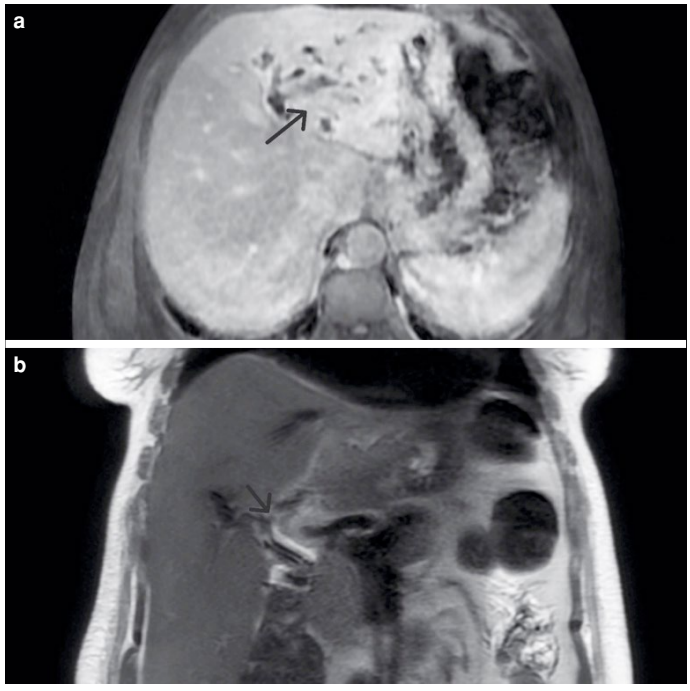

Figure 1. Contrast-enhanced magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) examinations revealed dilated bile ducts in the left lobe **(a)**, significant stenosis at the bifurcation of the left hepatic duct, and malignant soft tissue density **(b)**.

gallbladder and a fibrotic stricture involving the hepatic bifurcation and the common bile duct. A left hepatectomy was performed, with intraoperative frozen section analysis confirming benign surgical margins. Postoperative recovery was uneventful, and the patient was discharged on postoperative day 7.

Macroscopic examination of the resected specimen revealed fibrotic thickening of the perihilar bile ducts (Fig. 2). Histopathological analysis confirmed extensive fibrosis and inflammation, consistent with IgG4-SC. Immunohistochemical staining showed an elevated IgG4+ plasma cell count (>10 cells per high-power field) and a high IgG4/IgG ratio (>40%), leading to a definitive diagnosis of isolated IgG4-SC (Fig. 3).

Discussion

IgG4-SC is a chronic fibroinflammatory disease associated with IgG4-RD. It predominantly affects middle-aged to elderly males, with a male-to-female ratio of approximately 4:1.[3] Isolated IgG4-SC, defined as IgG4-SC occurring in the absence of AIP or other organ involvement, is extremely rare, accounting for about 8% of IgG4-SC cases.[3] The condition's clinical presentation and imaging findings often mimic those of hilar cholangiocarcinoma, especially in Type 4 IgG4-SC, where strictures are confined to the hilar bile ducts.[4] This diagnostic overlap frequently leads to unnecessary surgical resections.

IgG4-SC is categorized based on its association with AIP and the location of biliary strictures.[5] When associated with AIP, it often responds well to corticosteroid therapy. However, in isolated IgG4-SC, the absence of systemic or pancreatic involvement complicates the diagnosis.

The disease is further classified into four types based on the location of strictures: Type 1 involves the distal bile ducts; Type 2a includes

Figure 2. Left hepatectomy specimen showing fibrotic wall thickening around perihilar bile ducts.

intrahepatic bile duct strictures with dilation; Type 2b features strictures without dilation and reduced bile duct branches; and Type 3 involves strictures in both the hilar and distal bile ducts. Type 4, as observed in this patient, is limited to hilar bile ducts and is the least common subtype, accounting for approximately 10% of cases.[6]

The differentiation of IgG4-SC from malignant conditions such as hilar cholangiocarcinoma is critical to avoid overtreatment. Imaging modalities, including MRI, MRCP, and EUS, play essential roles in the evaluation of biliary strictures. On MRI and MRCP, IgG4-SC typically demonstrates bile duct wall thickening, homogeneous enhancement, and long-segment strictures without the mass effect seen in cholangiocarcinoma.[7] However, these findings are not always conclusive. EUS and intraductal ultrasound provide high-resolution images and enable fine-needle aspiration, though biopsies may yield nondiagnostic results due to sampling limitations, as occurred in this case.

Serological markers such as serum IgG4 levels are an integral part of the diagnostic criteria. According to the Japanese Biliary Association (JBA) guidelines, an IgG4 level ≥135 mg/dL is diagnostic, although levels below this threshold can occur in isolated cases.[8] In this patient, serum IgG4 levels were within normal limits, underscoring the importance of integrating clinical, radiological, and histopathological findings for accurate diagnosis.

Histopathology remains the gold standard for diagnosing IgG4-SC. Key features include dense lymphoplasmacytic infiltrates, storiform fibrosis, and obliterative phlebitis. Immunohistochemical staining for

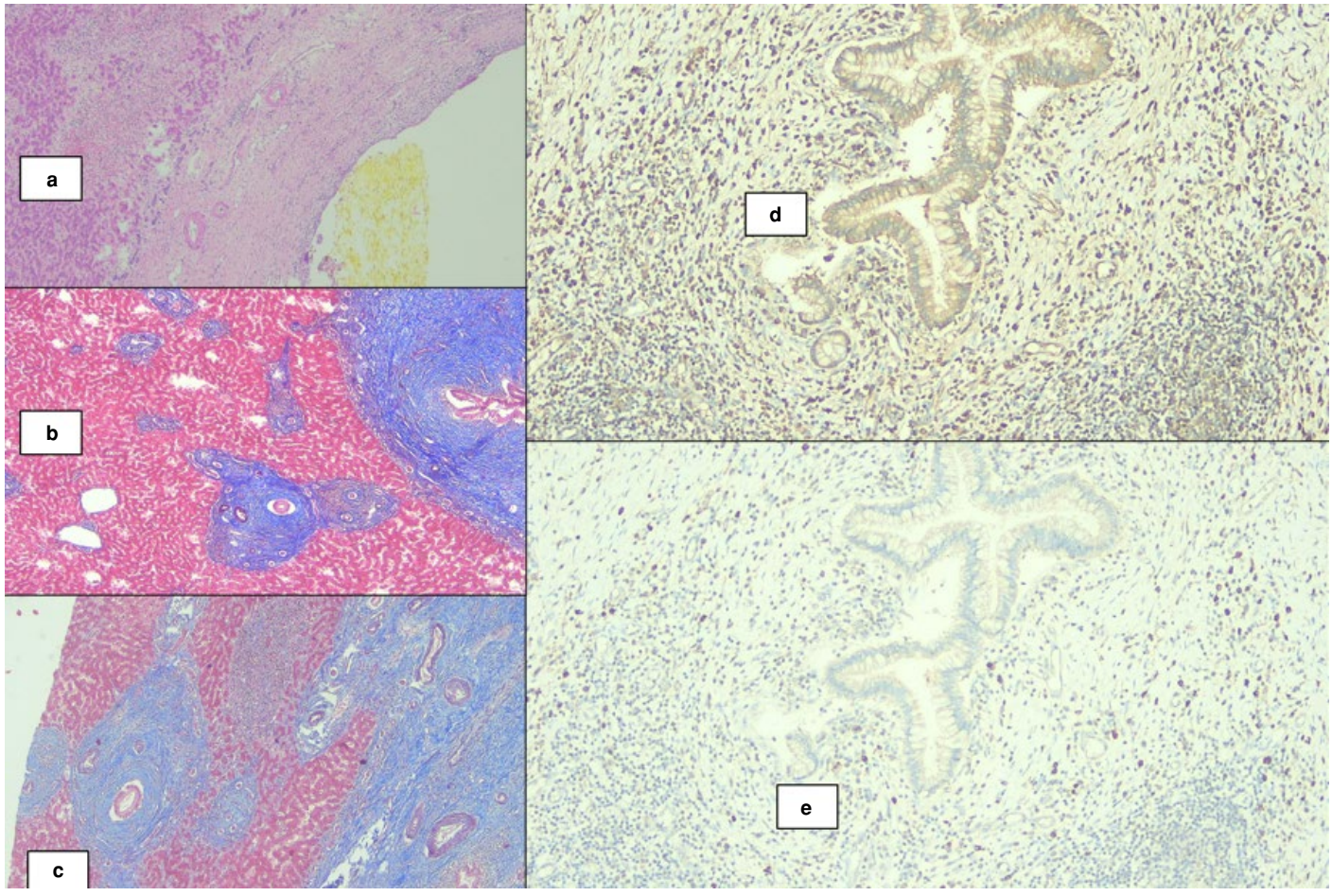

Figure 3. Lithiasis findings are observed within the lumen of perihilar large bile ducts (**a**; H&E; x40). Portal fibrosis and occasional onion skin fibrosis around bile ducts are seen (**b–c**; Masson trichrome; x40). Lymphoplasmacytic infiltration around bile ducts, showing IgG(+) plasma cells component (**d**; x100). Lymphoplasmacytic infiltration around bile ducts, showing IgG4(+) cell component (**e**; x100).

IgG4+ plasma cells and the IgG4/IgG ratio are essential for confirmation.[9] In this case, the resected specimen demonstrated all these findings, establishing the diagnosis of isolated IgG4-SC. Notably, the patient's advanced disease with significant fibrosis likely contributed to the absence of a marked IgG4 elevation in serum.

The literature includes cases of patients diagnosed with IgG4-related sclerosing cholangitis (IgG4-SC) incidentally who subsequently remained asymptomatic without requiring treatment.[10–13] However, corticosteroid therapy is the first-line treatment for IgG4-SC, achieving remission in the majority of patients during the inflammatory phase of the disease.[14,15] Early initiation of corticosteroids can mitigate inflammation and prevent progression to fibrosis. In patients who are unresponsive to steroids or who have already developed fibrosis, alternative therapeutic strategies, such as rituximab and azathioprine, are necessary.[16,17] Rituximab, a CD20 monoclonal antibody, effectively induces remission by depleting B cells and is particularly beneficial in cases of steroid resistance.

In this patient, surgical treatment was performed due to the high suspicion of malignancy. Although surgery is not the standard treatment for IgG4-SC, it provided definitive diagnosis and symptom relief in this case. Moving forward, the patient will require close follow-up to monitor for disease recurrence or progression in other organs.

Conclusion

This case highlights the diagnostic challenges associated with isolated IgG4-SC, particularly Type 4, which closely mimics hilar cholangiocarcinoma. Accurate diagnosis requires a multidisciplinary approach, integrating clinical, radiological, serological, and histopathological findings. While imaging and serology are valuable, histopathological confirmation remains the cornerstone of diagnosis. Early recognition of IgG4-SC is critical to initiating appropriate medical therapy and avoiding unnecessary surgical interventions. Clinicians should maintain a high index of suspicion for IgG4-SC in patients with biliary strictures to optimize outcomes.

Author Contributions: Concept – BGO, GO; Design – GO, HY; Supervision – SMD; Materials – HY, MAU; Data Collection and/or Processing – MAU, HY, BGO; Analysis and/or Interpretation – SMD, GO; Literature Search – MAU, HY, BGO; Writing – GO, MAU; Critical Reviews – SMD, GO.

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Peer-review: Externally peer-reviewed.

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Case Report

HEPATOLOGY FORUM

Biliary complications from chronic HCV infection

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Challenging biliary pain: An unusual extrahepatic manifestation of chronic hepatitis C

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Abstract

Hepatitis C virus (HCV) infection can cause various manifestations, including rare biliary complications. This case details a 44-year-old Thai woman with severe biliary pain but normal blood counts, liver function, and amylase levels. Abdominal MRI, MRCP, and endoscopic ultrasound ruled out mechanical obstruction but revealed diffuse thickening of the intrahepatic and common hepatic bile duct walls, and soft tissue thickening surrounding the left portal vein branch, suggestive of an inflammatory process. Further investigation confirmed positive HCV RNA. Serology revealed low complement levels, suggesting immune-mediated inflammation, though ANA, ANCA, and cryoglobulin were negative. Serum IgG4 levels were also normal. This led to a diagnosis of small vessel vasculitis of the biliary tract secondary to chronic HCV infection. Treatment with antiviral therapy and a short course of prednisolone resulted in significant symptom improvement. This case underscores the need for increased awareness of biliary complications associated with chronic HCV infection.

Keywords: Biliary pain; cholangitis; chronic hepatitis C; vasculitis.

Introduction

Hepatitis C virus (HCV) infection causes various extrahepatic manifestations (EHMs). This case highlights an unusual presentation of biliary pain related to HCV, contributing to the understanding of its extrahepatic effects.

Case Report

A 44-year-old Thai woman presented with severe dull, aching epigastric and right upper quadrant pain radiating to the back, worsened after meals, that had persisted for two days. The intensity was severe, ranging from 8 to 10 out of 10, and did not respond to proton pump inhibitors or antispasmodic medications. Partial relief was achieved with intravenous pethidine or morphine. No fever or jaundice was reported. One year prior, she had pruritic rashes on both legs, with a biopsy confirming small vessel vasculitis, but she was lost to follow-up. Six weeks ago, during a routine health check-up, a chest X-ray revealed a lung nodule; a computed tomography (CT) scan demonstrated consolidation in the right middle lung, and a bronchoscopy was scheduled.

Two days before this admission, the patient was admitted due to severe epigastric pain. Laboratory tests, including CBC and amylase, were unremarkable, as was the abdominal CT scan. An esophagogastroduodenoscopy found mild gastritis with Helicobacter pylori infection, treated with a quadruple regimen. However, her symptoms persisted.

On admission, the patient's vital signs were normal, and her abdomen was soft and non-tender, without hepatosplenomegaly. Blood samples collected during abdominal pain, including CBC, creatinine, liver function tests, and serum amylase, were within normal limits. Magnetic resonance imaging (MRI) and magnetic resonance cholangiopancreatography (MRCP) demonstrated long-segment circumferential wall thickening and enhancement of the intrahepatic and common hepatic ducts, without filling defects or stones (Fig. 1). No signs of pancreatitis or sphincter of Oddi dysfunction were observed. Endoscopic ultrasonography (EUS) revealed thickening of the left intrahepatic bile duct wall, but no definite lesion was identified (Fig. 2). A homogeneous hyperechoic lesion surrounding the left portal vein branch suggested thickened soft tissue. Other structures appeared normal.

The initial diagnosis included acute segmental cholangitis and active periportal inflammation. After multidisciplinary discussion, systemic inflammation was considered the most likely etiology. The patient was treated with intravenous dexamethasone 4 mg every 6 hours for one day, leading to dramatic improvement in symptoms. After the first dose of steroids, her abdominal pain completely resolved, prompting a switch to prednisolone at 0.5 mg/kg/day. The patient remained symptom-free thereafter.

Further investigation revealed positive anti-HCV and HCV RNA results (HCV RNA: log 6.46 IU/mL), but negative HBsAg. Serum autoantibodies, including ANA and cryoglobulin, were negative. P-ANCA was positive at a low titer of 1:10; however, anti-MPO and anti-PR3 results were both negative. Complement levels were low, with C3 measured at 0.52 g/L and C4 at <0.03 g/L. IgG4 levels were within the normal range at 19.40 mg/dL (reference range: 11–330 mg/dL). Bronchoscopy with biopsy of the lung consolidation revealed dense proliferation of small lymphoid cells, with immunohistochemical staining consistent with marginal zone lymphoma (MALT lymphoma).

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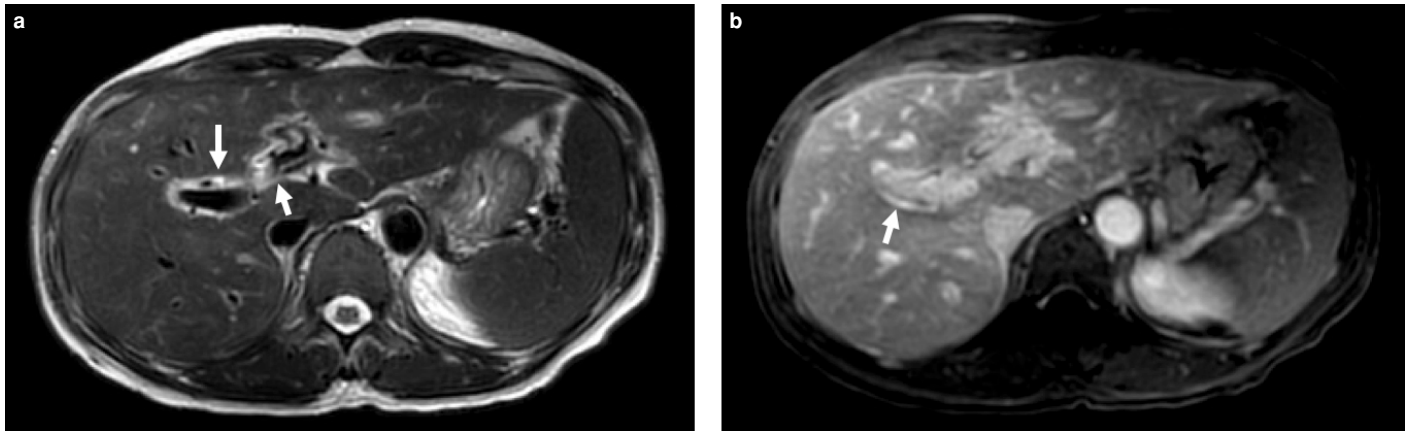

Figure 1. Abdominal MRI shows thickening and enhancement of the intrahepatic and common hepatic bile duct walls. **(a)** An axial T2-weighted image shows wall thickening of the intrahepatic bile duct (arrows). **(b)** An axial T2-weight image taken 5 minutes after Gadolinium injection shows enhancement of the wall thickening in the intrahepatic bile duct (arrow).

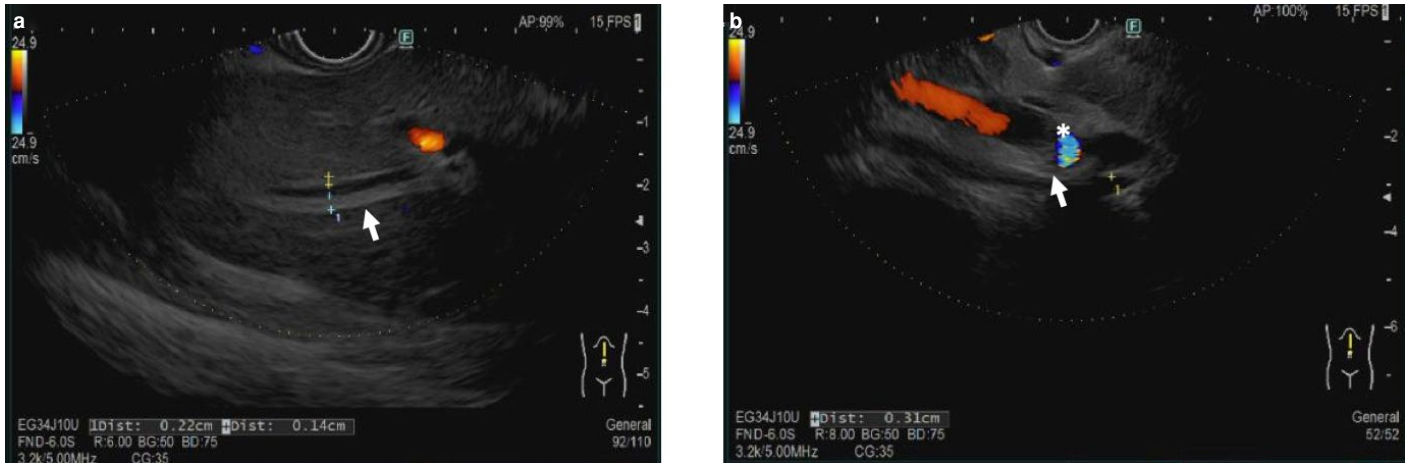

Figure 2. Linear endoscopic ultrasound shows thickening of the intrahepatic bile duct wall and periportal soft tissue. **(a)** Thickening of the intrahepatic bile duct wall (arrow). **(a)** Thickened whitish soft tissue (*) surrounding the left portal vein branch (arrow).

The final diagnosis was an extrahepatic manifestation (EHM) of chronic HCV infection, characterized by small vessel vasculitis (periportal inflammation and acute focal cholangitis and history of cutaneous vasculitis) and HCV-associated MALT lymphoma. The patient was treated with prednisolone 0.5 mg/kg/day for one week, with and tapering dose over the course of one month for small vessel vasculitis. HCV infection was managed with a 12-week course of Sofosbuvir/Velpatasvir (400/100 mg daily). One month after initiating steroid therapy, the patient reported significant improvement, with no abdominal pain. Continued monitoring and follow-up are planned to assess treatment response and address any residual issues.

Discussion

We present a challenging case of severe biliary pain attributed to an unusual complication of HCV infection. Initial tests (MRCP and EUS) ruled out mechanical obstruction and showed diffuse thickening of the intrahepatic bile duct wall and soft tissue surrounding the left portal vein, suggestive of an inflammatory or infiltrative process. Given the clinical presentation of sudden onset and intermittent pain, an inflammatory process seemed more plausible than an infiltrative or infectious process, which typically presents with a more gradual onset and persistent pain.

In considering the differential diagnosis, the patient's history of biopsy-confirmed small vessel vasculitis, coupled with low complement levels, indicates possible immune abnormalities and supports the diagnosis of immune complex-mediated vessel inflammation. With negative serology for primary autoimmune diseases, the most likely diagnosis is immune complex-mediated vasculitis, possibly secondary to chronic HCV infection or lymphoma. The significantly low C4 levels in this case suggest that the immune complexes may be influenced by both etiologies. Cryoglobulinemic vasculitis was also evaluated, as it can be associated with both malignancy and chronic HCV infection; however, this diagnosis was deemed less likely due to a negative cryoglobulin test in this case. The dramatic response to steroid treatment provides additional support for the diagnosis of vasculitis.

Previous studies have identified various pathophysiologic mechanisms linked to HCV complications. In terms of immune-related mechanisms, HCV-induced cryoglobulinemia and immune complex-mediated vasculitis are particularly relevant.[1] The chronic presence of the HCV antigen can drive polyclonal B-cell activation, leading to the formation of immune complexes that deposit in small- to medium-sized blood vessels. This deposition triggers an inflammatory response, activating complement pathways and recruiting immune cells, resulting in vascu-

lar damage and inflammation. Our patient exhibited low complement levels (C3 and C4), consistent with immune complex-mediated vasculitis described in the literature. Although cryoglobulinemia was negative, it is essential to note that only 40–60% of HCV-infected individuals test positive for cryoglobulinemia.[2] This highlights the variability of immune responses in chronic HCV infections.

The biliary complications observed in this case may be attributed to two potential mechanisms. First, HCV-induced immune complex vasculitis could lead to biliary damage through the formation of immune complexes and subsequent inflammatory responses affecting bile duct structures. Second, direct injury to bile duct cells by HCV cannot be ruled out. Previous studies indicate that intrahepatic bile duct cells are susceptible to HCV infection,[3,4] and the virus can be excreted into bile.[5] Chronic exposure to HCV may induce immune cross-reactivity with bile duct structures, possibly due to mimicry between epitopes on HCV viral polyproteins and human proteins, such as nitrogen oxide synthases, tyrosine kinase-Lck, and proto-oncogenes, leading to cross-reactivity to the bile duct[6] and further production of autoantibodies contributing to the observed complications. The association of HCV with other biliary conditions, such as primary biliary cholangitis, primary sclerosing cholangitis, and cholangiocarcinoma, remains inconclusive, with epidemiological studies suggesting links but lacking robust data elucidating the oncogenic mechanisms involved.[7,8] Further research is warranted to explore the underlying pathophysiological processes that might contribute to these associations.

In our case, EUS revealed a periportal soft tissue abnormality, raising the differential diagnosis of perivascular inflammation—potentially a complication of HCV—or infiltration by MALT lymphoma. Despite the lack of a confirmed pathological diagnosis, perivascular inflammation was deemed more likely due to the characteristic imaging patterns and absence of systemic evidence of malignancy. Perivascular inflammation typically exhibits a hyperechoic pattern in 91% of cases on ultrasound, with hematologic malignancies that commonly present as hypoechoic lesions.[9] While contrasting chronic inflammatory diseases causing perivascular soft tissue changes are sometimes observed in IgG4-related diseases, such phenomena have not been documented in other rheumatologic conditions. Histopathological features of perivascular inflammation, including vessel wall invasion, thrombosis, fibrinoid necrosis, and scar formation, have been noted in small- to medium-vessel vasculitides such as ANCA-associated vasculitis, cryoglobulinemic vasculitis, and drug-induced immune complex vasculitis.[10] In this case, periportal inflammation detected by EUS but not MRI is likely due to EUS's superior sensitivity for identifying abnormalities. This raises the possibility that perivascular inflammation in rheumatologic disease may be more common than the literature suggests, potentially under-recognized due to limitations in conventional imaging.

Treatment approaches for HCV-related vasculitis vary based on the severity of systemic involvement, ranging from antiviral therapy alone to combined regimens with immunosuppressive agents. In this case, given the patient's severe vasculitic symptoms but involvement of non-vital organs, a combination of antiviral therapy and a short course of prednisolone (0.5 mg/kg/day) was chosen, which effectively controlled symptoms. Due to the rarity of such cases and the lack of data on relapse rates, the decision was made to monitor the patient for clinical relapse without long-term immunosuppressive therapy. Should persistent HCV-associated MALT lymphoma arise, chemotherapy would be considered following viral eradication.

Conclusion

In conclusion, this case underscores the importance of awareness of biliary complications associated with chronic HCV infection. It contributes to the limited literature on this association and emphasizes the need for further research to elucidate the underlying pathophysiology that may contribute to these HCV complications.

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from participants.

Conflict of Interest: The authors have no conflict of interest to declare.

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Use of AI for Writing Assistance: Authors used ChatGPT4.0 for assistance with grammar corrections and to make to text more concise. The AI played no role in the creation of the content itself.

Author Contributions: Concept – TT; Data Collection and/or Processing – TK; Literature Search – TT; Writing – TT, TK; Critical Reviews – TT, TK.

Peer-review: Externally peer-reviewed.

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Case Report

Re-transplant with liver paired exchange donor

HEPATOLOGY FORUM

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Lifesaving re-transplantation with liver paired exchange donor

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Abstract

Liver re-transplantations (re-LTx) have been documented as high-risk operations considering technical and immunological challenges. However, improvements over the last two decades have increased success rates, bringing them closer to those of primary liver transplantations (LTx). At present, deceased organ shortage is a critical issue, and even potential live donors may not be suitable regarding vascular and biliary challenges, volume discrepancies, and ABO incompatibility for both primary and re-LTx. The hospital records of a patient who underwent two liver transplantations in our institution were evaluated retrospectively. A twelve-year-old girl with Progressive Familial Intrahepatic Cholestasis Type 3 underwent live-donor LTx with a graft from her mother. The patient required emergency re-LTx due to primary non-function of the graft, and there were no suitable deceased or live donors during that critical period. The patient was introduced to the liver paired exchange system and underwent a lifesaving re-LTx from an altruistic paired exchange donor. As a developing strategy, liver paired exchange transplantation is a reasonable solution to achieve the most suitable liver graft when it is most needed, especially in populations with very low deceased organ donation rates. There is a need for large studies to analyze the role and success of liver paired exchange transplantation in pediatric patients in urgent and elective situations.

Keywords: Children; liver paired exchange transplantation; liver re-transplantation.

Introduction

Liver transplantation (LTx) is a life-saving procedure for children with end-stage liver diseases and some inherited metabolic disorders. Outcomes of pediatric LTx have improved dramatically over the last two decades. However, 3–29% of the pediatric LTx population required re-transplantation (re-LTx) due to graft failure according to the literature. The major indications for re-LTx include vascular complications (especially hepatic artery thrombosis), primary graft non-function (PNF)/severe dysfunction, biliary complications, hyperacute, acute, and chronic rejection, and recurrence of the primary disease.[1–6]

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Liver re-transplantations have been documented as high-risk operations considering technical and immunological challenges. Ng et al. compared patient survival for primary transplants and re-transplants, revealing 88% and 67% one-year patient survival rates, respectively, in the Studies of Pediatric LTx (SPLIT) study among 246 pediatric re-LTx patients.[1] Moreover, patients who required early re-LTx showed 59% survival compared to 74% survival beyond 30 days, presenting the severity of early graft loss and the urgent requirement for a new graft to keep the patients alive. Vascular complications and PNF comprise more than 90% of early re-transplantation indications.[1–3]

Organ shortage becomes a critical issue when urgent transplantation is needed. Western studies reported that more than 90% of re-LTx cases were from deceased donors.[1,3] Unfortunately, countries on the eastern side of the world, including Turkiye, have very low organ donation rates and are mainly dependent on living donor LTx (LDLT). Additionally, some patients' potential live donors may not be suitable regarding vascular and biliary challenges, volume discrepancies, and ABO incompatibility. ABO-incompatible LTx and the use of technical variant grafts have been introduced with improving success rates, especially in Asian LTx centers. Furthermore, liver paired exchange transplantation (LPE-LTx) is an emerging approach to overcome difficulties in achieving the most suitable living liver allografts in the pediatric population.[7,8]

Materials and Methods

The hospital records of a patient who underwent emergency re-LTx from an LPE donor were evaluated retrospectively in terms of patient and graft characteristics and post-transplant outcomes. This study was approved by the Institutional Review Board of Inonu University (Approval no. 2025/8266).

Case Report

A twelve-year-old girl with Progressive Familial Intrahepatic Cholestasis Type 3 underwent left lobe LDLT with a graft from her mother. The patient's blood group was B Rh (−) and her mother's was B Rh (+). LTx was technically successful with normal reperfusion and Doppler ultrasonography (DUS) findings, and the patient was extubated within a couple of hours in the intensive care unit.

However, starting from the first post-transplant hours, transaminases, INR, bilirubin levels, and ammonia were observed at high levels, with profound acidosis. There was no thrombosis, and arterial, portal, and hepatic flows were normal as proven by DUS and triphasic computed tomography. Peak values were: ALT 3447 U/L, AST 3976 U/L, INR 7.59, lactate 7.5 mmol/L, total and direct bilirubin 30 and 12 mg/dL, and ammonia 1401 µg/dL. Continuous renal replacement therapy and plasmapheresis were started, and the patient was intubated with grade 3 encephalopathy.

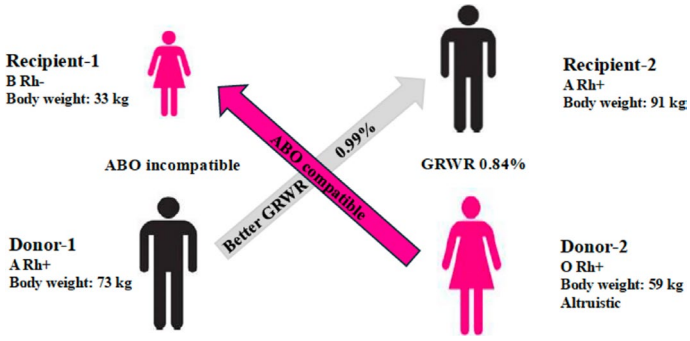

Figure 1. The 2-way LPE transplantation scheme with recipient and donor characteristics.

She was listed nationwide for emergency re-transplantation due to PNF with deceased donor allograft need. None of her family members nor volunteers were appropriate donors due to ABO incompatibility. Unfortunately, there was no available deceased donation at that time, and she was included in the LPE Program in the Liver Transplantation Institute at Inonu University. A suitable donor was designated by the system within 24 hours.

A forty-year-old female (Donor-2), who was originally accepted to be a right lobe liver donor for her father (Recipient-2), agreed to be an altruistic donor for our pediatric patient (Recipient-1) with the exchange of the child's older brother (Donor-1) as her father's donor. Donor-2 with a blood group O Rh (+) was an ABO-compatible fit for Recipient-1. Besides altruism as being the starter for this two-way LPE transplantation, Donor-1 with A Rh (+) blood group was a superior donor for Recipient-2 with identical blood group and a better graft-to-recipient weight ratio (GRWR) of 0.99% compared to his daughter's graft with a 0.84% GRWR value (Fig. 1).

Donor and recipient operations were all successful with no major complications. The re-transplanted patient was extubated on the first postoperative day and was fully recovered following two liver transplantations two days apart. Histopathological examination of the explanted graft revealed PNF (Fig. 2).

Discussion

Pediatric LTx outcomes have been improving over the decades as a result of advances in surgical techniques, immunotherapy modalities, and perioperative management. More than 90% patient and graft survival ratios have been reported in the current era for pediatric LTx. Graft failure and re-LTx rates have varied from 3% to 29% in both Eastern and Western pediatric LTx centers in the last 10 years.

Re-LTx has been recognized as a riskier operation than primary LTx because of technical and immunological challenges. However, many articles have shown improvements in re-LTx outcomes, proving that it is stringent but commendable for long-term survival of pediatric patients.[1–6] Dreyzin et al.[3] reported better success rates for re-LTx cases at 86% one-year patient survival after 2002 compared to 73% prior to 2002. Two landmark pediatric re-LTx studies from the SPLIT registry and Vock et al.[7] with Scientific Registry of Transplant Recipients (SRTR) data presented 43% and 49% early re-LTx ratios, which occurred in emergency settings due to graft vascular complications and PNF.

Additionally, chronic biliary strictures, chronic rejection, and recurrence of the primary disease were the main indications for late re-LTx cases. Unfortunately, early graft loss has been associated with worse

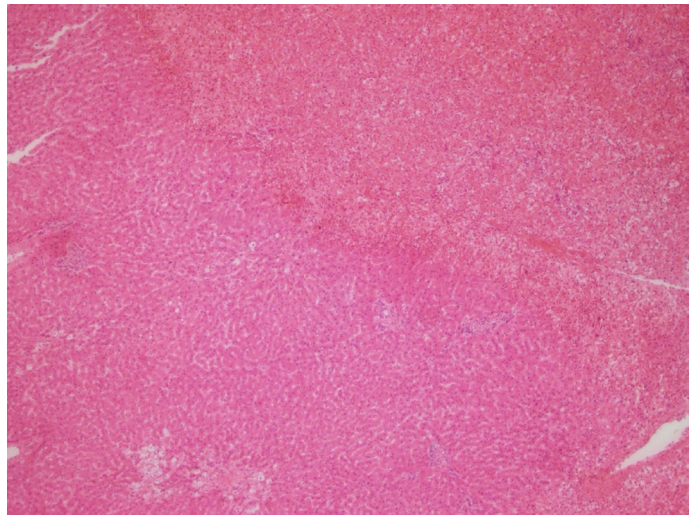

Figure 2. A well-demarcated zone of extensive hepatocyte necrosis in the upper right and liver parenchyma with viable hepatocytes below (HE, 40X).

patient survival due to factors including preoperative critical condition, ventilator support, intensive care unit stay, and long waiting times to find a proper graft, which may result in the patient being too sick to transplant.[1,4] However, Vock et al.[7] reported better survival for early re-LTx patients, contrary to most of the literature, stating that an early enough re-LTx before the patients became too sick and before they developed sepsis explained the improved outcomes.

PNF is one of the most challenging indications for urgent liver re-LTx in pediatric patients. PNF is defined as a transplanted graft with inadequate function in the absence of surgical problems, including vascular thrombosis. Patients rapidly develop liver failure with coagulopathy, profound acidosis, and multiorgan dysfunction, which is almost fatal without immediate intervention. PNF is relatively uncommon in the pediatric population and in live donor grafts due to careful young and healthy donor selection and advances in organ preservation. However, it still occurs in approximately 20% of cases. Survival chance for a pediatric PNF patient has been reported as up to 70% with a well-timed re-LTx.[1–7]

Living or deceased donor allografts with variations have been utilized for re-LTx depending on regional organ donation rates. North American and European countries have the opportunity to use more than 80% deceased donor grafts, in contrast to most Asian transplant centers that must rely mainly on LDLT.[1–10]

Identification of a suitable live donor can be challenging in urgent re-LTx even with many volunteers from the family circle. Unfortunately, only 30–55% of potential live donors can donate to their intended recipients, and alternative strategies are needed to overcome ABO incompatibility, disproportionate graft-residual volumes, and technical challenges involving vascular and biliary structures. ABO-incompatible LDLT has been introduced in this context, but especially in Western populations there is reluctance regarding results of antibody-mediated rejection and lack of consensus on pre- and post-transplant treatment modalities globally.[8–10]

LPE transplantation has been developed to overcome barriers to achieving the best allograft for the benefit of all participants in the exchange group. A milestone study by Yılmaz et al. from the Inonu LTx Institute recently reported the largest LPE experience with 85 adult and pediatric

cases consisting of 2–6-way exchanges as of 2024. The authors stated that none of the patients received a "less favorable" graft than a direct donation. The series included 45 incompatible pairs and 19 altruistic compatible pairs, which led to a 24.2% increase in LDLT volume of the center in 2023. Altruistic donors enabled patients with incompatible donors to achieve lifesaving LTx with the benefit of their original recipients (better GRWR, ABO-identical graft).[8–10]

Our patient was one of the survivors in Inonu University LTx Institute with an altruistic LPE donor in an emergency re-LTx, despite having a family member who wanted to donate but was not compatible.

Conclusion

Liver re-LTx is a complex but improving intervention that provides hope for pediatric patients with graft failure. Availability of a proper allograft remains the most critical problem when urgent liver re-LTx is required. As a developing strategy, LPE transplantation is a reasonable solution to achieve the most suitable live donor liver allograft when it is most needed, with good ethical practices. Large studies are needed to analyze the importance and success of LPE transplantation in pediatric patients in both urgent and elective situations.

Ethics Committee Approval: This study was approved by the Institutional Review Board of Inonu University with approval (date: 12.08.2025, number: 2025/8266).

Informed Consent: Written informed consent was obtained from participants.

Conflict of Interest: The authors have no conflict of interest to declare.

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Author Contributions: Concept – NC, SK, ANA, SY; Design – NC, SK, ANA, SY; Supervision – NC, SK, ANA, SY; Data Collection and/or Processing – NC, ANA; Analysis and/or Interpretation – NC; Literature Search – NC; Writing – NC; Critical Reviews – NC, SY.

Peer-review: Externally peer-reviewed.

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Case Report

Pediatric biliary rhabdomyosarcoma

HEPATOLOGY FORUM

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Surgical management of pediatric biliary rhabdomyosarcoma: Importance of differential diagnosis

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Abstract

Rhabdomyosarcoma (RMS) comprises approximately 5% of all pediatric malignancies, and the biliary system is considered one of the rarest RMS locations. Awareness, knowledge, and early recognition of the disease are essential for accurate diagnosis and proper treatment of biliary RMS in a child with obstructive jaundice and suspicious radiological findings. We present two pediatric biliary RMS cases requiring different managements because of their primary evaluations at their referring facilities. A four-and-a-half-year-old boy was referred to our institution for liver transplantation following neoadjuvant chemotherapy for centrally located unresectable biliary RMS. The patient received a left lateral segment graft from a living donor with no complications during the post-transplant period. The second patient was a seven-year-old foreign boy with obstructive jaundice and a history of choledochal cyst resection. A tumoral mass was revealed during exploration, and macroscopic total resection of the lesion was performed. The final pathology result of the resected material was biliary RMS with microscopic residue on the bile duct margin and lymph node involvement. The patient was transferred to the Pediatric Oncology Division for systemic treatment following surgical recovery. Biliary RMS presents distinct challenges in terms of accurate diagnosis and successful management. A multidisciplinary approach is indispensable for effective treatment. Complete surgical resection has been proven to be the mainstay strategy in feasible cases. Contributions of pre- and postoperative chemotherapy and radiotherapy are crucial in extensive disease. Liver transplantation should be considered, with reasonable success rates, in persistent unresectable and non-metastatic cases.

Keywords: Biliary rhabdomyosarcoma; children; liver transplantation.

Introduction

The liver and biliary tract are considered very rare locations, comprising 0.5–1.5% of RMS cases. Several combinations of chemotherapy, surgery, and radiotherapy have been practiced for the treatment of biliary RMS; nevertheless, there has been no common global treatment protocol. Survival rates have been reported at approximately 50–85% at the 5-year period, depending on disease stage and histologic subtype.[1–3]

Unfortunately, 30–40% of cases present with metastatic disease at the time of biliary RMS identification due to preliminary misdiagnosis. Initial symptoms and findings—including obstructive jaundice with a suspicious liver/biliary system lesion containing cystic components—require careful workup.[3]

Here, we present two pediatric biliary RMS cases who underwent definitive surgeries at Inonu University Liver Transplantation Institute, requiring different managements because of their primary evaluations at their referring facilities.

Patients and Methods

The hospital records of two patients who were diagnosed with biliary RMS were evaluated retrospectively. This study was approved by the Institutional Review Board of Inonu University (approval no. 2025/8227).

Case Reports

Case 1 – A four-and-a-half-year-old boy with biliary RMS was referred to our institution for liver transplantation (LTx). He had initially presented to a university hospital with abdominal pain, pruritus, and diarrhea at the age of three years. Laboratory results were consistent with obstructive jaundice, and imaging studies showed a centrally located liver mass. Embryonal RMS was diagnosed following liver biopsy, and the patient received 13 cycles of chemotherapy. Control imaging showed an unresectable lesion (Fig. 1), and the patient underwent LTx as part of the first 7-way liver paired exchange (LPE) LTx in the world, 17 months after RMS diagnosis. The post-transplant period was uncomplicated, and the patient was discharged on day 18. Pathological examination of the explanted liver and surrounding lymph nodes revealed 10–15% viable periductular tumor. No recurrence has been reported according to imaging studies, with a well-functioning allograft at one year post-transplant.

Case 2 – A seven-year-old foreign boy was referred to our institution with obstructive jaundice. According to limited information obtained from his family, he had undergone surgery in his country because of jaundice and bile duct strictures. The family affirmed that he had some relief for two months, but he had been suffering from severe icterus and itching for the last month. He was transferred to Turkiye. MRI and MRCP images were evaluated as changes secondary to strictured he-

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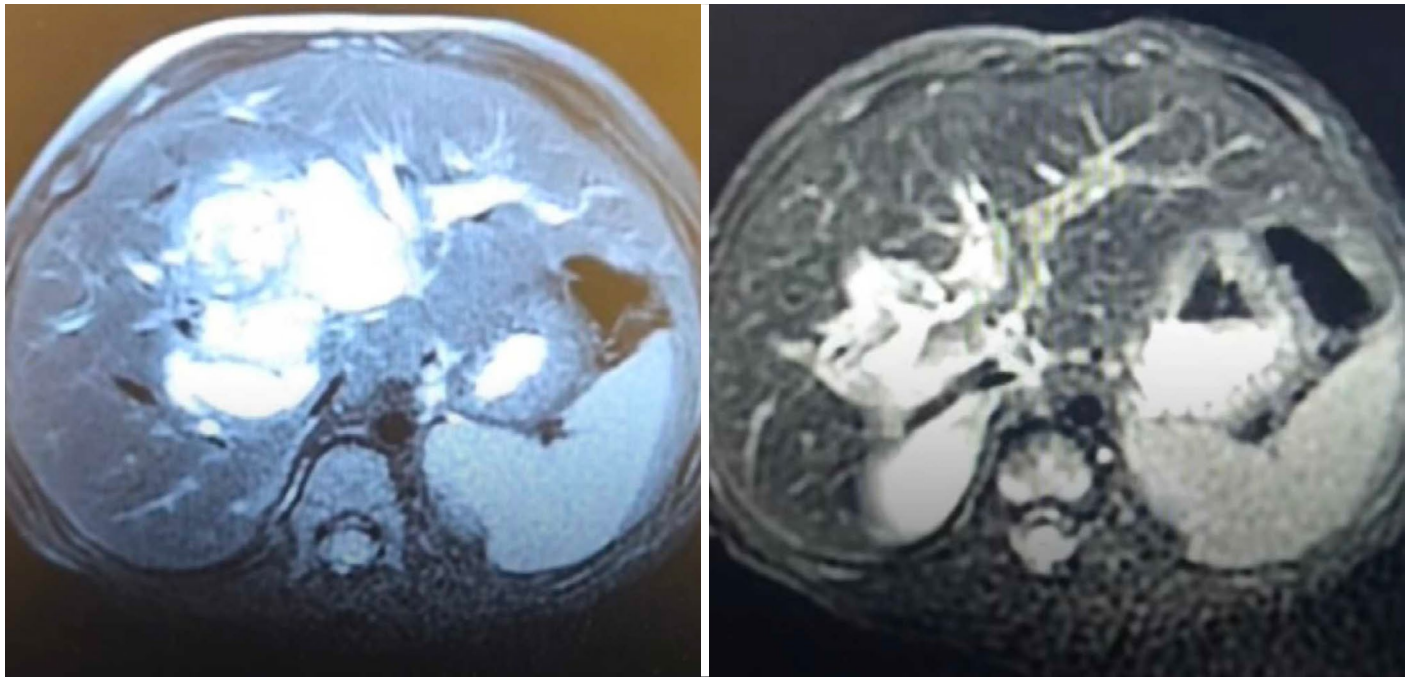

Figure 1. The MRI images of centrally located unresectable biliary RMS prior to and after the neoadjuvant chemotherapy.

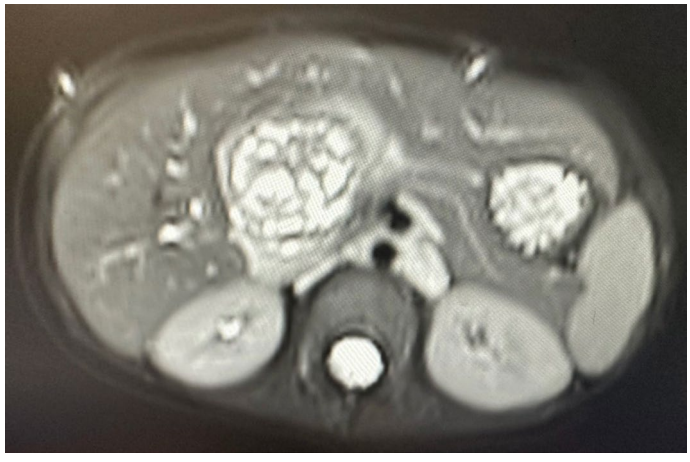

Figure 2. The MRI image of the lesion with cystic and solid components.

patico-jejunostomy anastomosis (Fig. 2). Biliary catheters were placed after dilatation of the anastomosis, and a biopsy was taken from a suspicious lesion. True-cut biopsy revealed no tumor but obstructive-type cholestasis, and hepatico-jejunostomy revision was planned.

Surgical exploration revealed suspicion of a bile duct tumor. The hepatico-jejunostomy line was totally embedded in a tumoral mass with cystic and solid components. Frozen biopsy was consistent with a sarcomal tumor. The common bile duct was totally resected, and biliary ducts were closed distally. The initial frozen pathology result was reported as RMS.

Postoperatively, bile leak was prominent from the repair area surrounding the bile ducts. A second operation was necessary to control the bile leak, and total resection of the tumor was macroscopically achieved with extended right hepatectomy during that surgery (Fig. 3). Bile duct margins were microscopically positive for botryoid-type biliary RMS, and metastatic lymph nodes were present in the final pathology report.

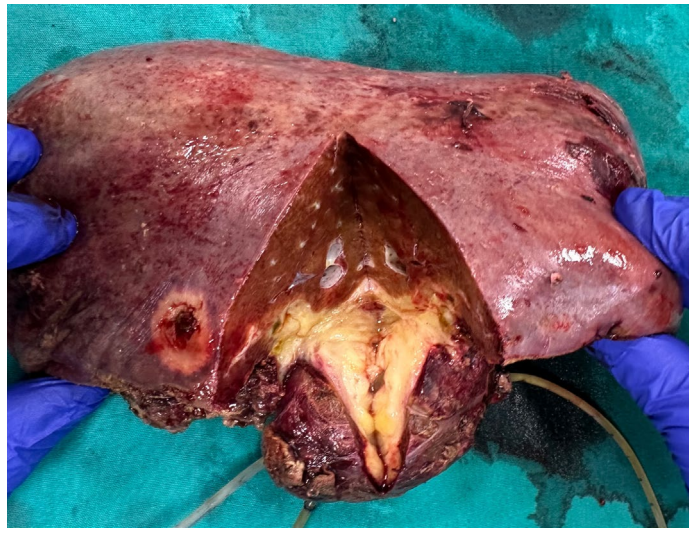

Figure 3. The picture of the extended right hepatectomy material.

The postoperative period was uncomplicated, and the patient was discharged on day 15 and referred to the Pediatric Oncology Division. There were no surgery-related complications, and the patient has been receiving systemic chemotherapy for the last six months.

Discussion

Rhabdomyosarcoma comprises approximately 5% of all pediatric malignancies, and the biliary system is one of the rarest locations. Biliary RMS presents distinct challenges in terms of accurate diagnosis and successful management. According to the literature, biliary tract RMS is typically a disease of early childhood and is most often diagnosed around 3 years of age, with boys showing a higher prevalence.[3,4] Both of our cases were boys diagnosed at approximately 4 and 7 years of age.

Patient age >10 years, alveolar histology, initial tumor size >5 cm, lymph node involvement, and distant metastasis have been reported as unfavorable features.[3–7] Reports have been controversial regarding whether the biliary system represents a favorable origin. Recent studies have drawn attention to reconsidering biliary origin as unfavorable or intermediate risk, in light of low survival and high mortality rates, especially when relapse occurs.[1,4,7–9]

We performed extended surgeries for both patients with the aim of total resection. Extended surgery in biliary RMS has been reported as ineffective in some trials.[4–6] However, if we consider the success rates of surgical resections in those studies as below standard, more acceptable outcomes in reports from Guerin et al.[1] and Fuchs et al.[7] may be more reasonable to acknowledge in the current era. We believe the importance of surgical approach is crucial in biliary RMS treatment if complete resection is feasible.

There have been reports of total remission following neoadjuvant chemotherapy. Conversely, treatment without surgery has been shown as an independent risk factor for mortality, with survival dropping to 0–17% in cases of recurrence. Some studies have presented better 5-year patient survival with extended surgical approaches compared to limited surgery. Similarly, tumor-free surgical margins have been achieved at higher rates in patients undergoing extended resection following neoadjuvant chemotherapy.[1,2,7–10]

The decision for transplantation or surgical resection in our patients was based on the possibility of total resection of the tumoral masses. Unresectable biliary RMS following neoadjuvant chemotherapy without metastasis compelled us to conclude with LTx for the first patient, mainly due to the tumor's central location. Although transplantation may be regarded as an aggressive treatment for a chemo-responsive tumor like RMS, successful reports exist of LTx for unresectable biliary RMS with no distant metastasis following neoadjuvant chemotherapy. Remarkably, most failed transplantations in the literature involved recurrent cases with extensive extrahepatic invasion, resulting in incomplete tumor resection during hepatectomy. Unfortunately, indications for resection versus LTx have not been well defined worldwide.[2,3,7]

In the second case, hepatico-jejunostomy revision was planned under conditions of uncontrolled obstructive jaundice and deteriorating liver function. Intraoperative findings were suspicious for a biliary tumor with parenchymal invasion at the previous operation site. Frozen pathology revealed a sarcoma within the resected common bile duct. Following limited resection, bile leak from fragile tissue around the resection site necessitated a second surgery to control the leak. Chemotherapy was considered too risky due to the prominent leak. Extended right hepatectomy achieved macroscopic complete resection, but final pathology revealed microscopic tumor residue at the reconstruction site with metastatic lymph nodes. Probably, the primary complete resection might have been achievable following a proper neoadjuvant chemotherapy administration in this patient. Unfortunately, absence of early imaging, operative, and pathology data, as well as distorted lesion depiction due to prior surgery and inflammation, misled the diagnosis and resulted in an additional surgical step, eliminating the chance for neoadjuvant chemotherapy before the second surgery.

Struggles in establishing the correct diagnosis have been repeatedly reported as one of the critical pitfalls in biliary RMS management.[1–5,7,8] Obstructive jaundice is a common symptom in several pathologies, including cholangitis, choledocholithiasis, choledochal malformations, pancreatic malformations, and tumors. Moreover, the radiological appearance of non-invasive biliary RMS may resemble a congenital choledochal cyst. MRCP and biopsy are especially used to differentiate obstructive benign strictures or cystic lesions from intraluminal neoplasms, but they were not beneficial in our case.[4-8]

According to the current literature, complete surgical resection remains critical whenever feasible, to minimize recurrence and mortality. However, primary total resection is not often possible due to the tumor's common location at the portal triad, necessitating chemotherapy and radiotherapy as adjunctive therapies. Neoadjuvant chemotherapy can enable tumor shrinkage to make unresectable lesions resectable, and adjuvant chemotherapy and/or radiotherapy for residual disease highlight the importance of a multimodal treatment approach to achieve tumor-free survival.[1-3,7–10]

Conclusion

Awareness, knowledge, and early recognition of the disease are essential for accurate diagnosis and proper treatment of biliary RMS in a child presenting with obstructive jaundice and suspicious radiological findings of the liver and biliary system. A multidisciplinary approach is indispensable in disease management. Complete surgical resection has been proven to be the mainstay strategy in feasible cases. Contributions of pre- and postoperative chemotherapy and/or radiotherapy are crucial in extensive disease. LTx should be considered, with reasonable success rates, in unresectable and non-metastatic cases.

Ethics Committee Approval: This study was approved by the Institutional Review Board of Inonu University with approval (date: 12.08.2025, number: 2025/8227).

Informed Consent: Written informed consent was obtained from participants.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Use of AI for Writing Assistance: Not declared.

Author Contributions: Concept – NC, SK, CK, ANA, SY; Design – NC, SK, CK, ANA, SY; Supervision – NC, SK, CK, ANA, SY; Data Collection and/or Processing – NC; Analysis and/or Interpretation – NC; Literature Search – NC; Writing – NC; Critical Reviews – NC, SY.

Peer-review: Externally peer-reviewed.

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Role of micrornas in the pathophysiology and diagnosis of metabolic dysfunction-associated steatotic liver disease: A bibliometric review

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) has become a public health problem, given its increasing incidence worldwide and strong association with metabolic syndrome components such as obesity, insulin resistance, and systemic inflammation. Recent studies have shown the relevance of microRNAs (miRNAs) as potential biomarkers and therapeutic targets in MASLD. This bibliometric review aimed to evaluate the scientific production of the last decade on miRNAs involved in the pathophysiology and diagnosis of MASLD. A total of 775 articles were initially retrieved from the PubMed database, with 51 meeting the inclusion criteria after a systematic screening process. Bibliometric analysis showed that China and the United States had the highest number of publications, with studies published mainly by the International Journal of Molecular Sciences and Hepatology. Among the most studied miRNAs were miR-122, miR-29a, miR-34a, and miR-223, which participate in lipid metabolism, inflammation, fibrosis, and insulin sensitivity. Co-authorship network analysis identified Gao Bin as the most influential author in the field. Keyword co-occurrence analysis showed growing interest in miRNAs in general, miR-29a, miR-34a, miR-122, miR-223, nonalcoholic fatty liver disease, lipogenesis, and mitochondrial stress in recent years. This review emphasizes the increasing scientific attention on miRNAs involved in MASLD and highlights their diagnostic and therapeutic potential. However, further studies are still needed for the identification and clinical validation of therapeutic targets that modulate miRNAs. Future perspectives include the integration of omics approaches and the exploration of nutritional or pharmacological strategies for miRNA modulation.

Keywords: Biomarkers; inflammation; microRNA; miR-122; miR-29a; miR-34a; nonalcoholic fatty liver disease; nonalcoholic steatohepatitis.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD) has become a relevant public health problem with increasing incidence worldwide.[1] Epidemiological studies indicate a prevalence of approximately 38% in the adult population and of 7% to 14% among children and adolescents (Elsaid et al., 2022).[2] MASLD is more prevalent in Latin America (~44%) than in Western Europe (~25%), likely due to differences in lifestyle and dietary patterns.[3,4]

Recent epidemiological studies suggest that MASLD prevalence will increase significantly by 2040, reaching approximately 55% of the world population.[3] This growth is predicted to be particularly high among individuals belonging to risk groups, including obese patients, those with type 2 diabetes (T2DM), and individuals with insulin resistance. The association of insulin resistance, T2DM, obesity, and dyslipidemia in patients with metabolic syndrome and MASLD suggests a strong link between these two conditions, pointing to shared underlying causes.[5,6]

MASLD progression can culminate in more severe conditions, such as nonalcoholic steatohepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma. A lipid accumulation of less than 5% of the hepatocyte volume is considered physiological, whereas values above this threshold are indicative of MASLD.[7] Clinical studies indicate that between 12% and 40% of individuals with MASLD progress to nonalcoholic steatohepatitis. Of these, approximately 15% to 25% progress to liver cirrhosis, and approximately 7% of patients with cirrhosis progress to hepatocellular carcinoma.[8] These data underscore the importance of early diagnosis and continuous monitoring of MASLD, especially in at-risk populations, to prevent serious complications.[9] In addition to hepatic complications, MASLD is associated with several extrahepatic manifestations, suggesting the presence of systemic pathogenic mechanisms. These complications include chronic kidney disease, extrahepatic neoplasms, and cardiovascular diseases, which contribute significantly to morbidity and mortality in this population.[10,11]

Early diagnosis of MASLD is essential for the implementation of prevention and therapeutic intervention strategies. However, diagnosis is often difficult in the early stages of the disease, as conventional hepatic serological markers, such as alanine aminotransferase (ALT) and aspartate transaminase (AST), may be within reference values. Liver biopsy remains the diagnostic gold standard but is an invasive method.[9,12] Given these limitations, recent research has focused on identifying new diagnostic and prognostic biomarkers for MASLD. Among the most promising are microRNAs (miRNAs), small non-coding RNA regulatory molecules that have been investigated for their potential value in early detection, risk stratification, and monitoring of disease progression.[13,14]

miRNAs exhibit numerous advantages as biomarkers and therapeutic targets, such as high stability, detectability in body fluids, and central regulatory roles in MASLD-associated metabolic, inflammatory, and fibrogenic processes. Recent studies indicated that some miRNAs, such as miR-122, miR-34a, and miR-29a, are highly expressed in individuals with MASLD, actively participating in the modulation of hepatic inflammation, insulin resistance, dyslipidemia, and hepatic fibrosis. miR-122 was associated with the degree of liver injury and metabolic disorders, whereas miR-34a was shown to participate in the regulation of lipid metabolism and hepatic fibrogenesis, acting on several molecular targets involved in disease progression.[14–16] Another example is miR-29a, which can regulate epigenetic mechanisms, particularly through interaction with DNA methyltransferases, directly modulating hepatic inflammatory and fibrogenic processes.[15,17] Interestingly, dietary interventions and bioactive compounds have demonstrated potential in modulating the expression of these miRNAs, offering alternative and complementary approaches for MASLD treatment and prevention.[18]

In view of these considerations, this bibliometric review aimed to evaluate the scientific production of the last 10 years on miRNAs involved in MASLD, highlighting their possible clinical and therapeutic applications. The guiding question of the research was: What are the trends and scientific contributions on the role of miRNAs in MASLD in recent years?

Materials and Methods

This study combined a bibliometric approach and a review of the literature to understand the role of miRNAs in MASLD. First, a bibliometric review was conducted to identify research trends, collaboration networks, and the academic impact of studies addressing miRNAs in the context of MASLD in the last 10 years. Next, a synthesis of the selected studies was carried out.

Bibliometric Review

This review adopted an analytical approach and focused on articles indexed by PubMed. PubMed was chosen for its broad scope, reliability, and relevance in the biomedical field. It provides open access to a vast database, including MEDLINE, which compiles publications from peer-reviewed journals with high scientific rigor. Additionally, PubMed is continuously updated, guaranteeing access to the most recent and relevant publications in the fields of health and biological sciences, enhancing the robustness of this review.

The search strategy was designed based on the research question and the Problem–Interest–Context–Outcome (PICO) framework.[19] The Problem (P) was the lack of noninvasive diagnostic and prognostic markers in MASLD. The Interest (I) was the use of serum miRNAs in the diagnosis, prognosis, and therapeutic management of MASLD in a health Context (C). No specific Outcomes (O) were included in the search. The search string comprised descriptors connected by Boolean operators: "miRNA" OR "microRNA" AND "nonalcoholic fatty liver disease" OR "NAFLD" OR "metabolic dysfunction-associated steatotic liver disease" OR "MASLD."

Initially, articles were selected based on titles and abstracts. Then, a rigorous screening was performed, excluding studies that were not original articles, such as systematic reviews, narrative reviews, commentaries, errata, and studies based exclusively on bioinformatics analyses. The remaining articles were analyzed for relevance and methodological quality, resulting in the definitive inclusion of those that met the pre-established criteria. Preference was given to applied research (experimental laboratory studies or clinical studies).

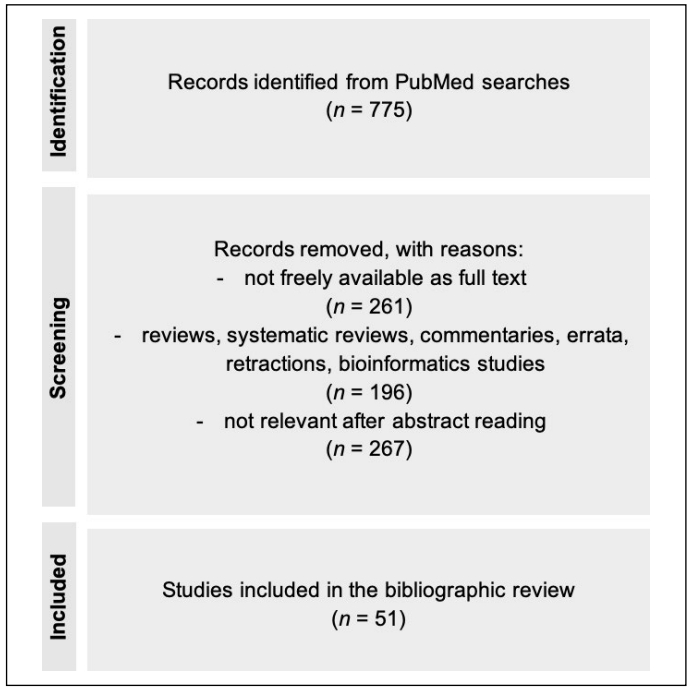

Figure 1. Flowchart detailing the steps in the bibliographic review.

The selected articles were analyzed to identify scientific collaboration networks between researchers and institutions, influential authors, emerging topics, publication patterns, frequent terms in titles and abstracts (minimum of five occurrences per term), prominent countries, and most-cited articles. These parameters were analyzed using VOSviewer® software version 1.6.19 (van Eck and Waltman, 2010).

Results and Discussion

Bibliometric Analysis

The literature search was conducted in the PubMed database, retrieving 775 articles. Of these, 261 records were removed for not being freely available in full text, and 514 articles were retained. After an initial screening, 196 articles were excluded because they were reviews, systematic reviews, commentaries, errata, retractions, or bioinformatics analyses. Of the remaining articles subjected to an in-depth screening, 51 met the eligibility criteria and were included in this review (Fig. 1). This process enabled the selection of relevant and methodologically appropriate studies to support the critical analysis of the findings, strengthening the conclusions on the topic. Appendix 1 summarizes the articles included in the review.

The synthesis of articles presented in Appendix 1 explores the role of miRNAs in the regulation of lipid metabolism, inflammation, liver fibrosis, and insulin resistance, particularly in the context of MASLD/NASH and associated conditions. Among the most studied miRNAs, miR-122, miR-29a, miR-34a, and miR-223 deserve mention for their therapeutic and diagnostic potential.

miR-122 is described as a key regulator of hepatic lipid metabolism, fibrosis, and inflammation. Several studies have shown that elevated serum levels of miR-122 positively correlate with the severity of hepatic steatosis, lobular inflammation, and fibrosis. As such, this miRNA is more sensitive than traditional liver enzymes, such as ALT and AST, in the noninvasive diagnosis of the disease. Modulation of miR-122, espe-

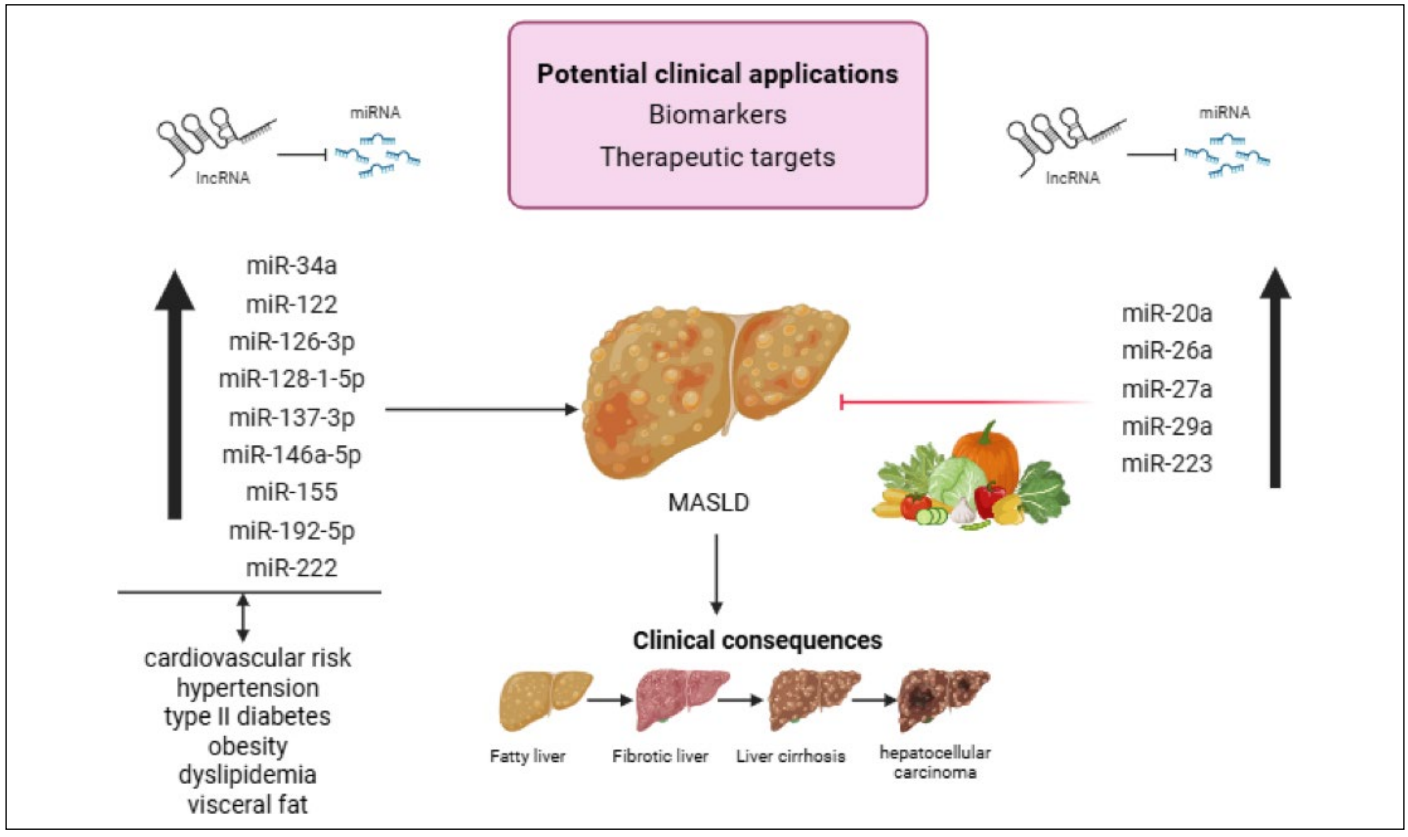

Figure 2. Role of microRNAs in the regulation of metabolic dysfunction-associated steatotic liver disease (MASLD): potential biomarkers and therapeutic targets.

cially through the LKB1/AMPK pathway and interaction with SIRT1, emerges as a promising therapeutic strategy to reduce hepatic lipid accumulation and improve metabolic homeostasis.[20,29–31,34,37,42,45,51,52,54,61,65]

The studies addressed in this review indicated that miR-29a protects against hepatic steatosis and fibrosis through the regulation of inflammatory and fibrogenic pathways. Its hepatic expression reduces lipid accumulation and inflammation, modulating pathways such as TGF-β/SMAD3, PI3K, and those of proteins associated with the inflammatory response (e.g., IL6 and MCP1). Its protective role against mitochondrial stress is also highlighted, having the potential to reduce the development of fibrosis and hepatic inflammation induced by high-fat diets.[15,17,22,23,27,54,67]

The results demonstrated that miR-34a is related to the worsening of MASLD, intensifying steatosis, inflammation, and hepatocyte apoptosis. Its elevated expression distinguishes MASLD from other liver diseases, presenting a superior diagnostic performance to conventional markers (CK-18, ALT, and indices such as FIB-4 and APRI). Therapeutic modulation of miR-34a could therefore represent a promising approach to controlling the progression of MASLD and its complications, including insulin resistance and associated cardiovascular disease.[58–60]

miR-223 demonstrates significant anti-inflammatory and antifibrotic effects, often associated with intercellular communication via extracellular vesicles. Selective transfer of miR-223 from neutrophils to hepatocytes reduces inflammation and fibrosis. It is suggested as a relevant therapeutic target to halt the progression of MASLD to NASH and hepatocellular carcinoma.[35,41,43,49]

Some studies evaluated nutritional and pharmacological interventions for miRNA modulation. The Mediterranean diet and supplementation

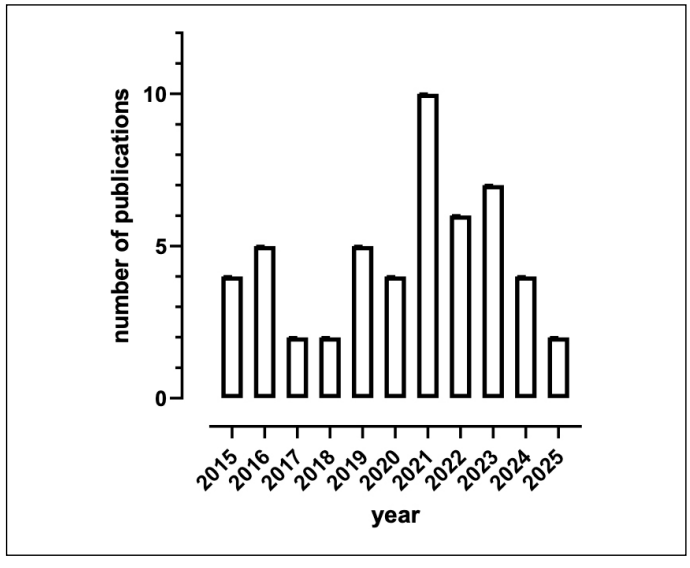

Figure 3. Temporal analysis of publications on microRNAs in the context of metabolic dysfunction-associated steatotic liver disease.

with δ-tocotrienol and resveratrol were shown to be capable of reducing the expression of inflammatory miRNAs, improving the metabolic profile in patients with metabolic syndrome. Furthermore, regular physical activity reduced pro-inflammatory miRNAs such as miR-146a-5p, highlighting the preventive potential of these approaches against metabolic and hepatic complications.[18,58]

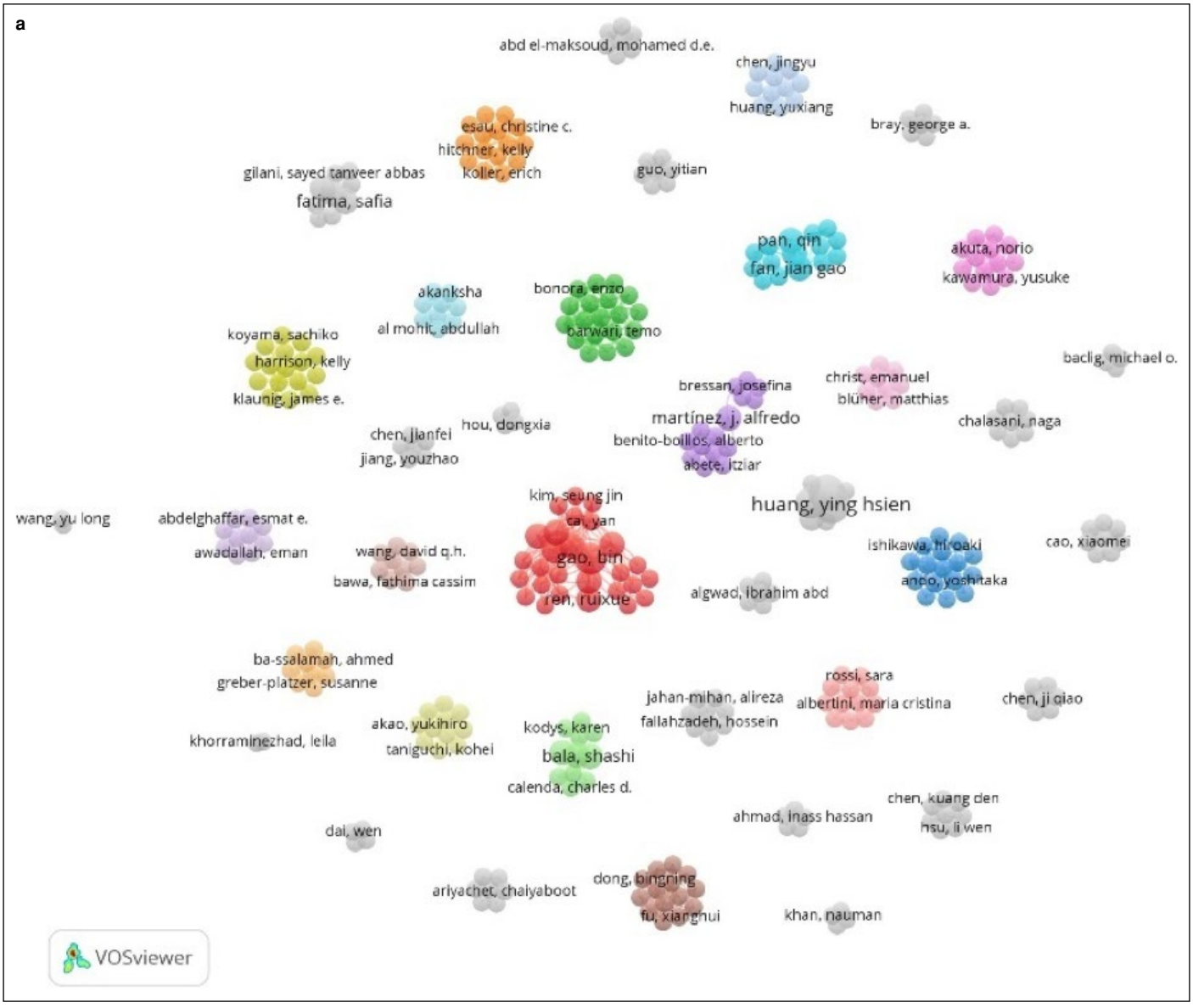

Figure 4. (a) Bibliometric network map and **(b)** overlay visualization map of the main authors of scientific publications on microRNAs in the context of metabolic dysfunction-associated steatotic liver disease.
Cont →

Taken together, these findings reinforce the importance of miRNAs as central regulators of lipid metabolism, inflammation, and progression of liver fibrosis, as well as their viability as noninvasive biomarkers for the diagnosis and therapeutic monitoring of MASLD and related metabolic diseases. Interventions targeting the modulation of these miRNAs offer promising therapeutic perspectives but require further clinical validation in studies with larger populations and different metabolic contexts. A schematic summary of the results is shown in Figure 2.

Temporal Analysis of Publications

Temporal analysis of the publications included in this review revealed an increase in the number of studies from 2020 onward (Fig. 3), reflecting the growing scientific interest in miRNAs in the context of MASLD. This increase followed the rise in the global incidence of the disease, reinforcing the relevance of the topic in recent years. According to data presented by Le et al.,[3] by 2040, more than half of the adult population will have MASLD, with the increases being more pronounced in women, smokers, and those without metabolic syndrome. Such projections are mainly associated with lifestyle changes, genetic factors, visceral fat deposition, and high consumption of sugar and saturated fat. These factors favor systemic inflammation and insulin resistance, culminating in several associated pathological conditions, such as MASLD and cardiovascular diseases, hypertension, and metabolic syndrome.[4]

Most studies were conducted in China, accounting for about 30% of all publications, followed by the United States, with approximately 20%. Studies were mainly published by the International Journal of Molecular Sciences (~14%) and Hepatology (~10%), reflecting the preference for journals with a high impact factor in the areas of molecular biology and hepatology. The impact factor of the main journals on the topic ranged from 5 (International Journal of Molecular Sciences) to 14 (Hepatology) and 20 (Journal of Hepatology).

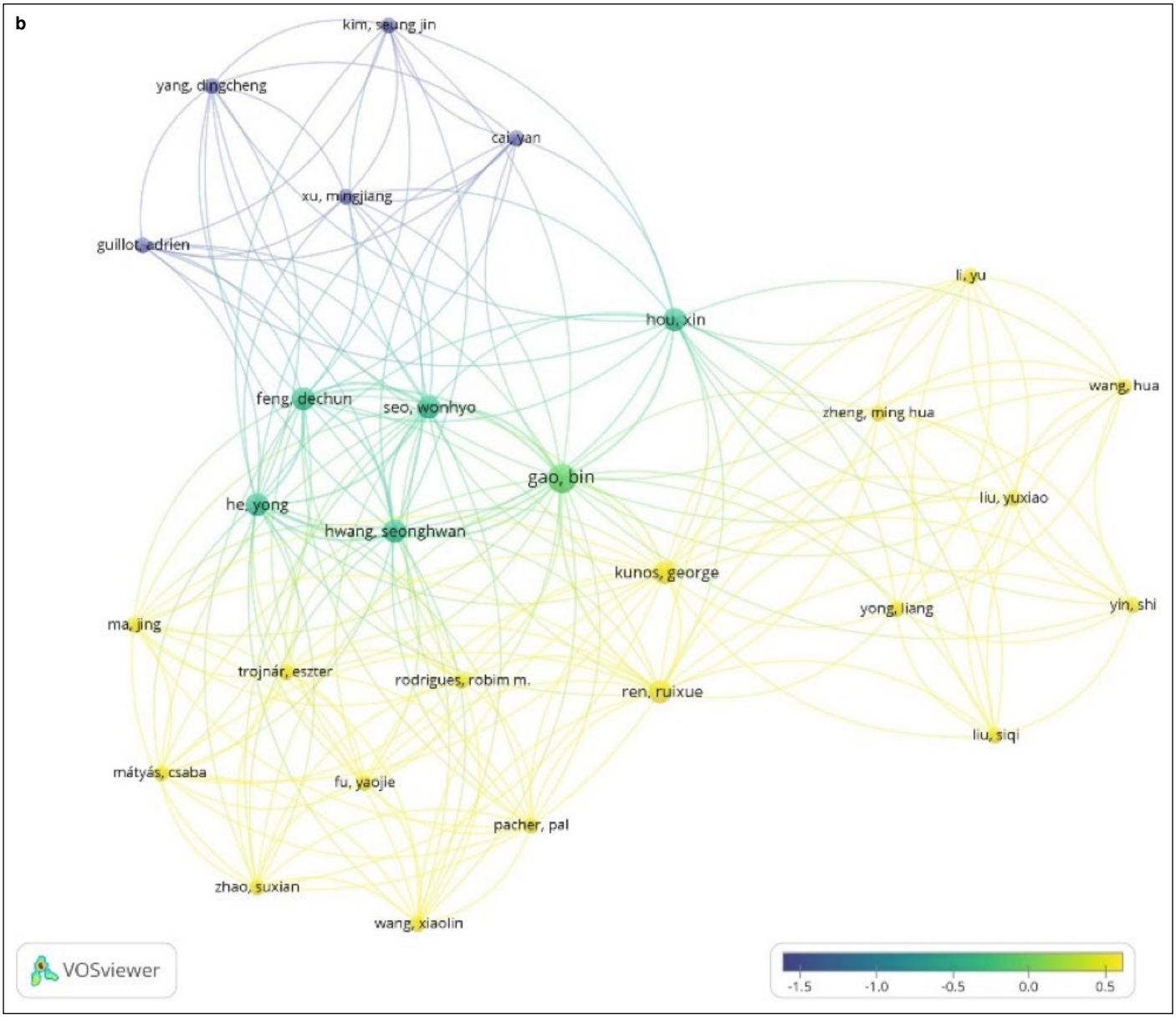

Figure 4.

The predominance of publications from China may be associated with the growing incidence of MASLD in the country, estimated at 46 new cases per 1,000 inhabitants/year, according to recent data in the literature.[68–70] In the United States, the high scientific production can be explained by the high prevalence of risk factors for MASLD, such as obesity, insulin resistance, and metabolic syndrome, which affect a significant portion of the adult population.[4]

Bibliometric analysis, performed using VOSviewer software, indicated that the authors with the highest citation strength were Gao Bin, with a link strength of 34, and Bonora Enzo, with a link strength of 18. These findings underscore the influence and protagonism of these authors in scientific production related to miRNAs in the context of MASLD.

Figure 4 shows the results of the co-authorship network analysis. Several clusters (Fig. 4a) represented by different colors can be observed, indicating communities of authors who collaborate more frequently with each other. The author Gao Bin stands out as one of the central nodes of the network, exhibiting a strong degree of connection with other authors. This finding suggests significant collaborative action and a possible leadership or reference role in the subject. Other well-defined groups include those led by Pan, Qin, Bonora, Enzo, Akuta, Norio, Koyama, and Sachiko, which reflect regional or thematic centers of scientific production. Figure 4b confirms the influence of Gao Bin, who is placed as the main central node. This high betweenness centrality is indicative of the author's strategic role in connecting different subgroups within the network. Gao's position suggests a strong influence on the production and dissemination of knowledge on the topic.

A dense cluster of highly interconnected authors was formed around Gao, including Seo, Wonhyo, Feng, Dechun, Hwang, Seonghwan, He, Yong, Hou, and Xin. This pattern suggests the existence of a well-established collaborative core, possibly linked to the same institution

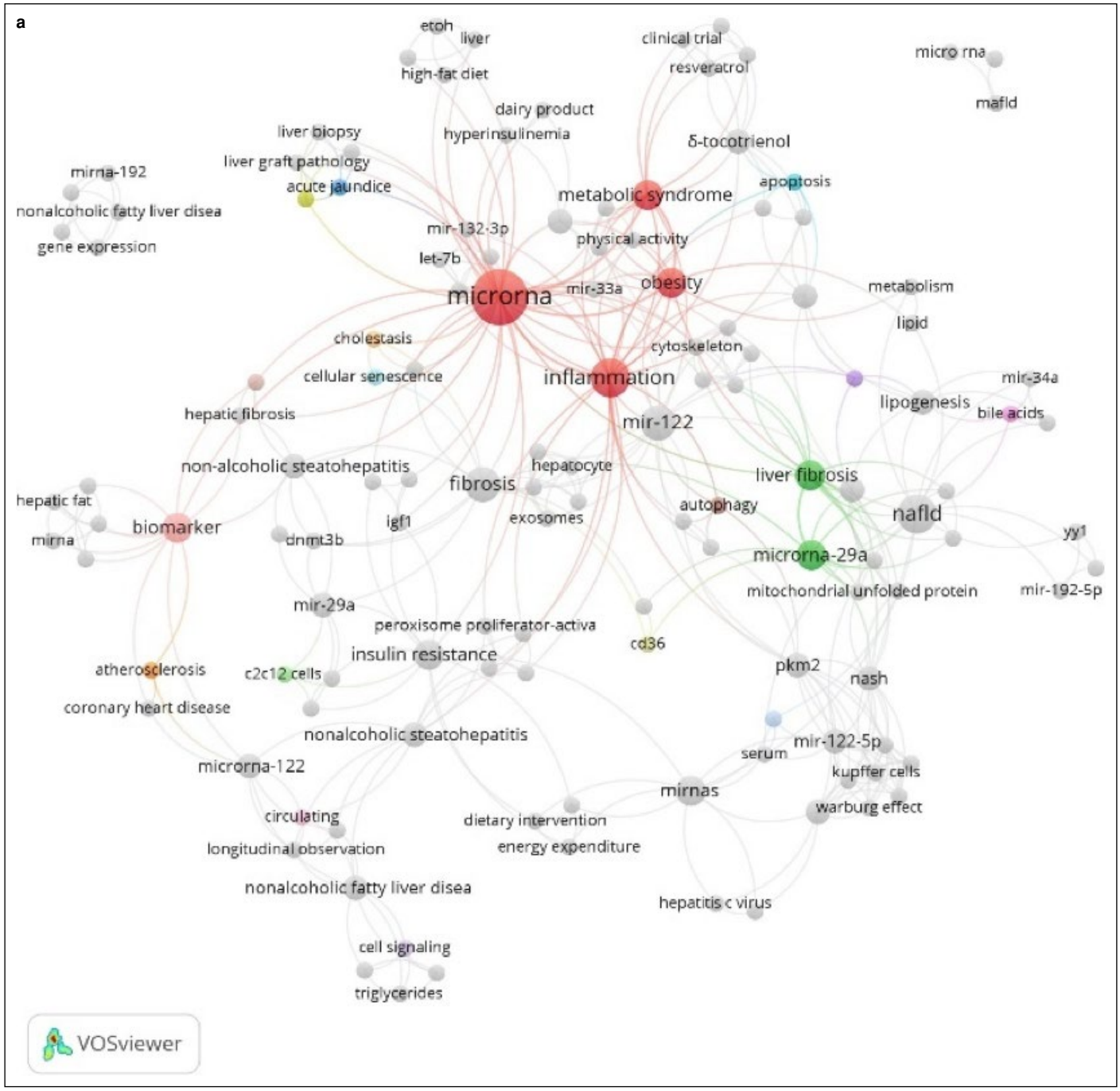

Figure 5. (a) Bibliometric network map and **(b)** overlay visualization map of the main keywords in research on microRNAs in the context of metabolic dysfunction-associated steatotic liver disease.

Cont →

or international research network, with consolidated and continuous scientific production. Furthermore, the cluster branches out into subgroups connected to authors such as Ren, Ruixue, Kunos, George, Rodrigues, Robim M., Pacher, and Pal, demonstrating interinstitutional and international collaboration. These subgroups, although less central, maintain relevant links with the core, contributing to the integration of knowledge and the methodological diversity of research.

The predominant green and yellow colors in the network (Fig. 4b) indicate positive normalization values in contribution analysis, reinforcing the active role of the authors in the generation and circulation of recent knowledge on the topic. This configuration points to a robust and integrated collaborative structure, which favors the advancement of knowledge about the role of miRNAs in the pathophysiology of MASLD and their diagnostic and therapeutic potential. Additionally, Figure 4a shows a considerable number of isolated authors, that is, those with few connections in the network (indicated in gray), suggesting independent studies with little international or interdisciplinary collaboration. This fragmentation may indicate an opportunity for strengthening collaborative networks and promoting integration between research groups.

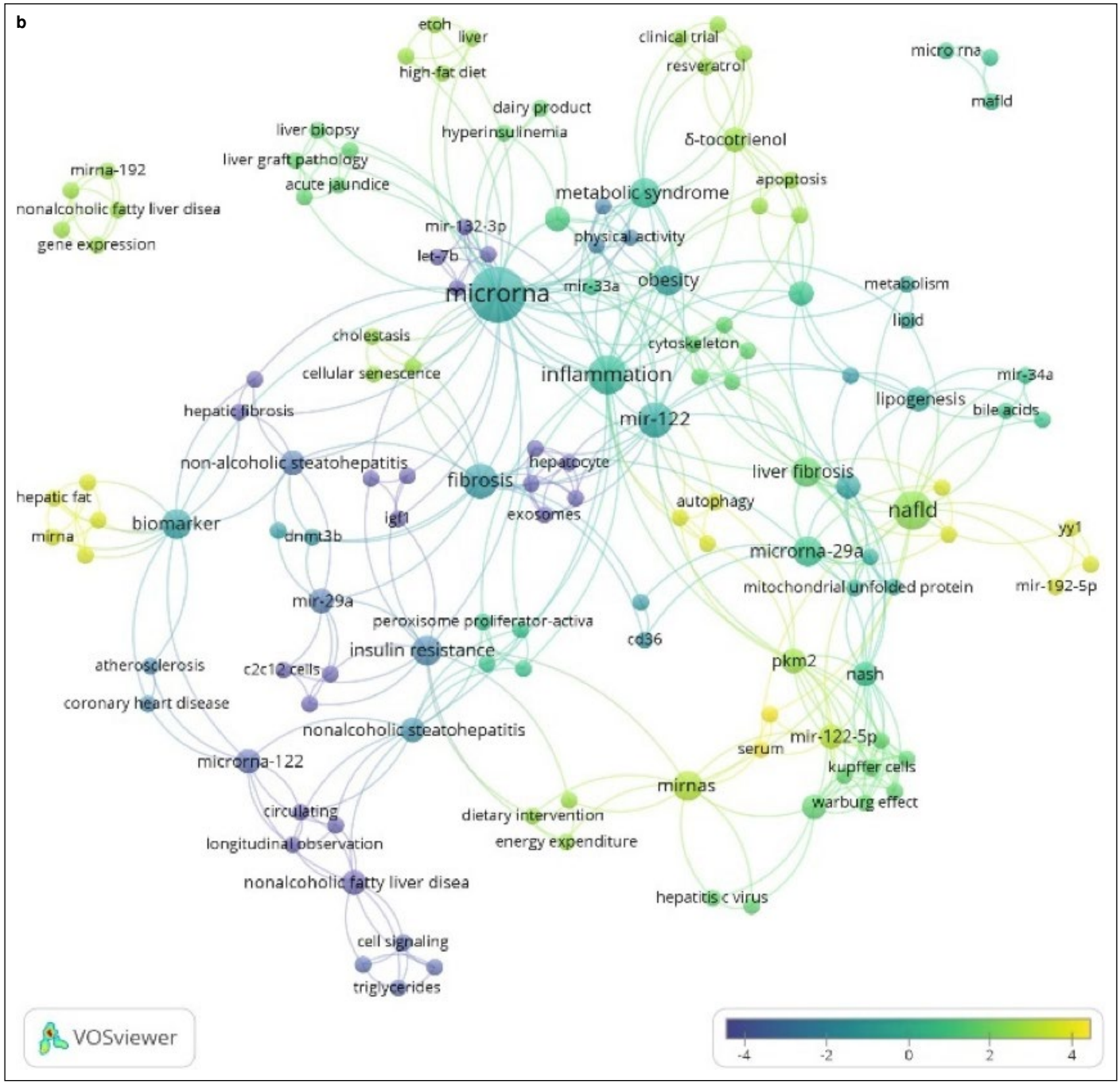

Figure 5.

The main countries of affiliation were China, the United States, and Japan. These findings reinforce the role of these nations as centers of scientific production on miRNAs and MASLD.

Keyword Analysis

Figure 5 shows the network of keyword co-occurrences extracted from the selected articles. This analysis aimed to identify the main themes, conceptual interrelations, and emerging trends in the scientific literature on miRNAs and MASLD. The cluster density map revealed multiple clusters, with the term "microRNA" appearing as a central node (Fig. 5a). This term was connected to the keywords inflammation, fibrosis, obesity, insulin resistance, NAFLD, and biomarker. Thus, studies have been exploring the role of miRNAs in inflammatory processes, hepatic fibrogenesis, and insulin resistance, which are central mechanisms in the pathophysiology of MASLD. The most recurrent miRNAs were miR-122, miR-29a, miR-34a, miR-192, miR-33a, and miR-192-5p, indicating a growing interest in their application as potential biomarkers or therapeutic targets.

The themes nonalcoholic steatohepatitis, fibrosis, autophagy, and lipogenesis have been gaining prominence, suggesting a convergence of

studies on molecular pathways that regulate both the progression and regression of liver damage. The presence of other key terms, such as clinical trial, resveratrol, and δ-tocotrienol, indicates the investigation of translational approaches and potential therapies.

Figure 5b shows the temporal overlay map of keywords. The most recent keywords appear in yellow and light green, whereas the oldest terms are displayed in blue. Terms such as miR-29a, miR-192-5p, autophagy, mitochondrial unfolded protein, Kupffer cells, serum, and NAFLD appear prominently in the most recent publications, suggesting that these themes represent current frontiers of research on miRNAs in the context of MASLD.

This analysis allowed the identification of gaps and future opportunities, particularly in the clinical validation of miRNAs as diagnostic tools and the study of their molecular mechanisms in cellular and animal models. New studies are needed to consolidate the use of miRNAs in the diagnosis, prognosis, and possibly treatment of MASLD.

Bibliometric analysis revealed a significant correlation between MASLD and metabolic syndrome, demonstrating the interrelation of MASLD with several pathological conditions, such as insulin resistance, dyslipidemia, and systemic inflammation.

It is also important to highlight the need for further investigation into the molecular mechanisms mediated by miRNAs, especially miR-122, miR-29a, and miR-223, which emerged as potential therapeutic targets. miR-122 is strongly associated with the promotion of hepatic and systemic inflammatory processes, contributing to the progression of MASLD to cirrhosis and hepatocellular carcinoma. By contrast, miR-29a and miR-223 demonstrate hepatoprotective effects, attenuating inflammation and hepatic fibrosis.

Deepening our understanding of the regulatory pathways modulated by these miRNAs is essential for elucidating the pathophysiological mechanisms of MASLD, which has increased in prevalence globally, including among children and adolescents. Furthermore, the use of these miRNAs as biomarkers may represent a promising strategy for early diagnosis. Their modulation by dietary interventions, physical activity, or specific drugs also emerges as a potentially effective therapeutic approach.

A limitation of this bibliometric review was the restricted access to the full texts of approximately one-third of the initially retrieved publications. This constraint may have resulted in the exclusion of relevant high-quality studies, potentially influencing the comprehensiveness of the analysis. Future reviews should consider strategies to improve access to full-text content, such as institutional or interlibrary collaborations, in order to ensure broader and more representative inclusion of the available literature.

Future Perspectives

This review demonstrated the relevance of miRNAs as potential biomarkers and therapeutic targets of MASLD. Despite recent advances in understanding the molecular pathways modulated mainly by miR-122 and miR-29a, important gaps remain to be explored.

Future studies should prioritize the clinical validation of these miRNAs in longitudinal and multicenter trials, aiming to consolidate their application in clinical practice for both early diagnosis and disease monitoring. Standardization of methods for collecting, extracting, and quantifying circulating miRNAs is essential for their effective use as a diagnostic tool.

Another gap in research involves investigating the therapeutic potential of modulating miRNAs through nutritional interventions, bioactive compounds, physical activity, or therapies using agomiRs or antagomiRs. The combination of pharmacological approaches with miRNA-based therapies may represent an innovative and personalized strategy for the management of MASLD and its complications. Therefore, there is a need for additional in vivo and in vitro studies, as well as in silico analyses, to elucidate the pathways regulated by miRNAs in MASLD.

Conflict of Interest: The authors have no conflict of interest to declare.

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Appendix 1. Summary of articles included in the review

Reference	Objective	Main findings
Csak et al. (2015) [20]	Evaluate miR-122 regulation of HIF-1α, vimentin, and MAP3K3 in liver fibrosis.	- ↓ liver miR-122, ↑ HIF-1α, vimentin, and MAP3K3 expression, ↑ hepatic steatosis. - ↑ serum miR-122 in hepatic steatosis associated with diet
Fu et al. (2015) [21]	Investigate miR-26a in hepatic metabolic regulation and insulin sensitivity.	- ↑ liver miR-26a, ↑ insulin sensitivity and ↓ metabolic complications of obesity, ↓ glucose production and lipid biosynthesis.
Galimov et al. (2015) [22]	Investigate miR-29a and targets in GH-induced insulin resistance.	- GH therapy, ↑ IGF-1, ↓ miR-29a, ↓ insulin signaling, COLA3A1, and ↑ myokines, fibrosis, inflammation, and insulin resistance.
Mattis et al. (2015) [23]	Evaluate how miR-29a modulates LPL and lipid handling in steatosis.	- ↓ miR-29, ↑ liver LPL, ↑ liver triglyceride (TG) and cholesterol, metabolic dysfunction-associated steatotic liver disease.
Akuta et al. (2016) [24]	Analyze circulating miR-122 in relation to NAFLD histopathology.	- ↑ serum miR-122, ↑ steatosis progression, lobular inflammation, and fibrosis progression.
Marques-Rocha et al. (2016) [25]	Evaluate how a Mediterranean diet modulates inflammatory miRNAs in metabolic syndrome.	- Mediterranean diet consumption improved miR-155 and Let-7b expression. ↑ Let-7b, ↓ reactive oxygen species (ROS) production.
Salvoza et al. (2016) [26]	Explore associations of key serum miRNAs with dyslipidemia in NAFLD.	- Patients with NAFLD had ↑ expression of miR-34a and miR-122 compared to healthy patients. ↑ miR-34a and miR-122, ↑ degree of steatosis, fibrosis, and inflammation. Serum miR-122 was higher than ALT values in patients with NAFLD.
Zhou et al. (2016) [27]	Investigate miR-29a and insulin resistance in IUGR-exposed muscle cells.	- ↑ miR-29a induced insulin resistance in C2C12 cells. ↑ miR-29a, ↓ GLUT-4 and PPARα.
Liu et al. (2016) [28]	Evaluate circulating miRNAs for NASH diagnosis and differentiation from CHB.	- miR-122, -16, -192, and -34a showed significant differential expression between NAFLD and CHB patients. miR-34a was significantly increased in NAFLD compared to CHB. - The levels of miR-122, miR-192, and especially miR-34a correlated positively with hepatic steatosis and inflammatory activity (lobular inflammation and hepatocyte ballooning). Only miR-16 showed significant correlation with hepatic fibrosis. - miR-34a showed superior diagnostic performance to the other markers (CK-18, ALT, FIB-4, and APRI) in identifying patients with NASH, reaching high specificity (0.875) and moderate sensitivity (0.704).
Wu et al. (2017) [29]	Investigate miR-122 in lipid accumulation and droplet regulation.	- ↑ miR-122, ↓ accumulation of lipids in hepatocytes, YY1-FXP-SHP axis modulation.
Willeit et al. (2017) [30]	Explore miR-122 as a biomarker for MetS and T2DM risk.	- ↑ miR-122, ↑ ALT, AST, adiposity, inflammation, insulin resistance, triglycerides and ↓ HDL-C.
Wang and Yu (2018) [31]	Explore the link between miR-122 and coronary atherosclerosis severity.	- ↑ circulating levels of miR-122, ↑ stage of coronary atherosclerotic lesion and ↑ cholesterol and TG.
Russo et al. (2018) [32]	Explore changes in miR-126 and miR-146a-5p after physical activity in obesity.	- Obesity is directly correlated with ↑ miR-146a-5p, ↑ miR-146a-5p, ↑ total cholesterol and TG, ↓ HDL-C. In U937 cells, ↑ miR-146a-5p → ↑ TLR4, NFκB, IL6, and TNFα.
Yang et al. (2019) [15]	Evaluate miR-29a in reducing hepatic inflammation and fibrosis in dietary NASH.	- ↑ miR-29a, ↓ hepatic lipid accumulation, as evidenced by ↓ AST and ROS. The mechanism associated with these effects is a decrease in the expression of SMAD3, p-PI3K, LC3B II, TGF, and IL6.
Ando et al. (2019) [33]	Evaluate the association of miR-20a, 27a, and 126 with NAFLD.	- Serum levels of miR-20a and miR-27a were significantly reduced in NAFLD patients compared with normal individuals. miR-126 showed no significant difference overall but was reduced in more severe cases in men. - There was a significant association between reduced levels of miR-20a and miR-27a and NAFLD severity, even after adjustment for multiple risk factors, such as age, sex, and metabolic indicators. - miR-126 showed a weak inverse correlation with liver fibrosis index (FIB-4) but no clear correlation with disease severity.
Long et al. (2019) [34]	Evaluate miR-122 regulation of LKB1/AMPK and Sirt1 in NAFLD lipogenesis.	- *In vivo* model of NAFLD ↑ miR-122, ↓ SIRT1 - *In vitro* model of NAFLD (HepG2 and Huh7), ↓ miR-122 promoted ↑ SIRT1 via LKB1/AMPK signaling.

Appendix 1 (cont). Summary of articles included in the review

Reference	Objective	Main findings
He et al. (2019) [35]	Evaluate miR-223 regulation of inflammation and oncogenes in NASH and HCC.	- ↑ liver and serum miR-223 after 3 months of a high-fat diet to prevent the progression from simple steatosis to NASH and liver cancer. - ↓ miR-223, ↑ proliferation and HCC markers (Ki67, CxCl10, TAZ, Gpc3, Golm1), inflammatory genes (IL6), and fibrinogenic genes (COL1A1). - miR-223-knockout mice fed a high-fat diet developed liver tumors.
Lin et al. (2019) [36]	Evaluate miR-29a in HFD-induced steatohepatitis and liver fibrosis.	- ↑ miR-29a, ↓ fat accumulation and liver mass induced by a high-fat diet and improved hepatocellular steatosis and liver fibrosis, ↓ inflammation hepatic. - ↑ miR-29a, ↓ PPAR, TFAM, MCP1, IL6, alleviation of oxidative damage and obesity reduction.
Chai et al. (2020) [37]	Evaluate how a RORA agonist modulates miR-122 and NASH severity.	- Administration of RS-2982, which binds and activates the RORA transcription factor in the liver, - ↑ miR-122 in the liver and blood, - ↓ TG in the liver and muscle tissues, ↓ inflammation and fibrosis in the liver - ↑ whole-body energy expenditure, fat oxidation, and insulin sensitivity, and ↓ weight and inflammation in adipose tissue.
Huang et al. (2020) [38]	Evaluate miR-18a-5p and miR-22-3p as stress and MetS biomarkers.	- Patients with metabolic syndrome, - ↓ miR-18a-5p and miR-22-3p, - ↑ cortisol and IL-6. - ↓ miR-18a-5p and miR-22-3p, - ↑ risk of developing metabolic syndrome.
Liu et al. (2020) [39]	Evaluate hepatic exosomal miR-192-5p in NAFLD-related macrophage activation.	- Both NAFLD and NASH patients ↑ miR-192-5p - ↑ serum ALT and AST, liver iNOS, IL6, and TNF-α. - ↑ miR-192-5p - ↓ pFox01 but not ↓ GSK3β.
Yang et al. (2020)[17]	Evaluate miR-29a in regulating mitochondrial stress in diet-induced NASH	- ↑ miR-29a - ↓ GSK3, SIRT1, mitochondrial proteostasis stress in NASH and reducing liver fat, fibrosis progression, and inflammation.
Bala et al. (2021) [40]	Evaluate miR-155 in regulating steatohepatitis and liver fibrosis in mice.	- High-fat diet increases miR-155 - ↑ TG, Cpt1α, FABA4, FAS, ACC2, TNFα, MCP1, and vimentin, resulting in progression of fibrogenesis and worsening of NASH.
He et al. (2021) [41]	Investigate neutrophil-derived EV transfer of miR-223 and effects in NASH.	- miR-223 transfer via LDLR/APOE-dependent EVs decreased hepatic inflammation and fibrosis in NASH.
Hegazy et al. (2021) [42]	Evaluate serum levels of miR-122 as a noninvasive marker to determine the severity of MAFLD.	- Serum miR-122 increased significantly with the severity of hepatic steatosis and fibrosis, correlating with ↑ lipid profile and ↑ ALT, AST, and GGT.
Hou et al. (2021) [43]	Evaluate myeloid IL-6-driven miR-223 exosomes and their role in NAFLD fibrosis.	- IL6 stimulation in myeloid cells activates macrophages to release miR-223-enriched exosomes that migrate to hepatocytes and inhibit genes such as TAZ and Cxcl10, attenuating the progression of liver fibrosis.
Lischka et al. (2021) [44]	Evaluate miRNAs associated with inflammation in obese and metabolically affected children.	- ↑ TNFα, IL-1Ra, and procalcitonin, correlated with ↑ miRNA-122 and -192 - ↑ miRNA-122, ↑ HOMA-IR.
Refeat et al. (2021) [45]	Evaluate correlation of miR-33a/miR-122 with lipid metabolism in MetS.	- Obese and diabetic patients showed increased serum levels of miR-122 and reduced levels of miR-33a - ↑ body mass index, Wc, Wt, total cholesterol, and TG.
Xu et al. (2021) [46]	Evaluate the role of hepatocyte miR-34a in the progression of NAFLD to NASH.	- Overexpression of miR-34a exacerbated NAFLD, whereas its deletion attenuated inflammation, apoptosis, and steatosis.
Yu et al. (2021) [47]	Evaluate the effect of miR-137-3p on NAFLD through activation of the AMPKα pathway.	- miR-137-3p significantly improved NAFLD through direct activation of the AMPKα pathway, reducing oxidative stress and hepatic inflammation.

Appendix 1 (cont). Summary of articles included in the review

Reference	Objective	Main findings
Zeinali et al. (2021) [48]	Evaluate miR-122, 126-3p, and 146a as inflammatory markers in prediabetes and T2DM.	- ↑ miR-122, pre-diabetic and T2DM - ↓ miR-126-3p → pre-diabetic and T2DM - ↓ miR-146a → pre-diabetic and T2DM - miR-122 potential target → interleukin 1 receptor type 1, NFκB, PRKAB1 - miR-126-3p → insulin receptor substrate 1, SPRED1, TRAF6, IL6 - miR-146a → TNFα, IL6 - ↑ miR-122 → ↑ HOMA-IR - ↑ miR-126-3p and miR-146a → ↓ HOMA-IR.
Zhang et al. (2021) [16]	Explore PPARγ-driven regulation of hepatic stress in NASH via miR-21-5p/SFRP5.	- PPARγ, ↓ miR-21-5p/SFRP5 pathway, ↓ oxidative stress and inflammation in NASH.
Ariyachet et al. (2022) [49]	Investigate miR-223's role in hepatic stellate activation and antifibrotic potential in organoids.	- ↑ miR-223, ↓ COL1A1, COL3A1, LOXL2, and ACTA2 - miR-223 suppressed hepatic stellate cell activation and reduced fibrosis.
Elghoroury et al. (2022) [50]	Explore exosomal expression of miR-18a/222 as diagnostic markers in liver disease.	- ↑ miR-18a and ↑ miR-222 ↑ ALT, AST, bilirubin, AFP, urea, and creatinine levels.
Hu et al. (2022) [51]	Assess the role of miR-122-5p in the development of NAFLD.	- ↑ miR-122-5p → correlated with the pathogenesis of NAFLD - ↓ miR-122-5p → ↑ SOD, GSH-Px, and ↓ MDA - ↓ miR-122-5p → ↓ total cholesterol, TG, liver weight, body weight, IL6, TNFα, and IL-8. - ↓ miR-122-5p → ↑ FOXO3
Inomata et al. (2022) [52]	Evaluate miR-122-5p's role in PKM2-mediated glycolysis in NASH Kupffer cells.	- ↓ miR-122-5p activated PKM2-mediated glycolysis in Kupffer cells, contributing to inflammation and worsening of NASH. miR-122-5p and PKM2 are promising therapeutic targets for controlling hepatic inflammation and NASH progression.
Khorraminezhad and Rudkowska (2022) [53]	Evaluate miRNA modulation by dairy products and its association with glycemic profile in hyperinsulinemia.	- High dairy intake modified the expression of miRNAs (miR-106-5p and miR-122-5p), affecting glycemic profile and insulin resistance.
Lin et al. (2022) [54]	Identify hepatic miRNA expression patterns in different etiologies of acute jaundice after liver transplantation.	- Acute cholangitis → ↓ miR-122, miR-301, and miR-21 - Acute rejection → ↑ miR-122 and ↓ miR-133a - Recurrent hepatitis → ↑ miR-122, miR-301, and miR-21 - Fatty change → ↑ 133a.
Fatima et al. (2023) [18]	Determine the effects of δ-tocotrienol and resveratrol on miRNAs in MetS patients.	- Supplementation increased miR-130b and miR-221 and decreased miR-122, improving components of metabolic syndrome.
Heianza et al. (2023) [55]	Evaluate miR-128-1-5p as a marker of insulin sensitivity and energy metabolism in obesity.	- ↑ miR-128-1-5p, ↑ HOMA-IR, waist circumference, and total body fat mass.
Liang et al. (2023) [56]	Analyze miR-29a modulation in liver under prolonged HFD and ethanol exposure	- Hepatic miR-29a expression initially increased with a high-fat and high-ethanol diet, subsequently decreasing with advancing liver fibrosis.
Mollet et al. (2023) [57]	Investigate which miR-193b-3p-related metabolic pathway interferes with NAFLD/MAFLD.	- ↑ miR-193b-3p, ↓ PPARGC1A, ↑ fat droplets in the liver, ↓ expression of MTTP, ↑ TRIB1, and ↑ LDLR.
Pervez et al. (2023) [58]	Explore miRNA expression changes in NAFLD with δ-tocotrienol and α-tocopherol therapy.	- δ-Tocotrienol and α-tocopherol significantly reduced the expression of miRNAs related to steatosis, inflammation, and apoptosis (miR-122, miR-21, miR-103a-2, miR-421, miR-375, and miR-34a).
Ragab et al. (2023) [59]	Evaluate the potential of miR-34a and miR-192 as early diagnostic markers of NAFLD.	- A positive correlation was observed between miR-34a and hypertension in patients with NAFLD and plasma lipid levels and a negative correlation with the hematological markers hemoglobin and leukocytes. miR-192 showed no correlation with these markers. miR-34a was elevated in early stages of liver fibrosis and reduced in advanced stages, whereas miR-192 showed a progressive increase according to fibrosis stage.

Appendix 1 (cont). Summary of articles included in the review

Reference	Objective	Main findings
Wan et al. (2023) [60]	Evaluate the role of liver-specific microRNA-34a in ductular reaction and hepatic fibrosis during experimental cholestasis.	- In murine models with liver-specific deletion of miR-34a, there was a significant reduction in ductular reaction, cellular senescence, and liver fibrosis induced by bile duct ligation. The reduction in miR-34a was accompanied by increased expression of Sirtuin-1 (Sirt1), suggesting that Sirt1 regulation may mediate the protective effects observed in the absence of miR-34a.
Hossain et al. (2024) [61]	Determine the role of miR-122 in the regulation of inflammatory and autophagic proteins mediated by PKM2 in NAFLD.	- Reduction of miR-122 increased PKM2 and liver inflammation and reduced autophagy, exacerbating NAFLD.
Ma et al. (2024) [62]	Evaluate miR-192-5p regulation of lipid metabolism through YY1 in NAFLD.	- ↑ miR-192-5p significantly reduced liver triglyceride accumulation by inhibiting factor YY1.
Tobaruela-Resola et al. (2024) [63]	Evaluate miR-122-5p, 151a-3p, 126-5p, and 21-5p for MASLD prediction.	- miR-122-5p, miR-151a-3p, miR-126-5p, and miR-21-5p significantly correlated with steatosis, liver stiffness, and liver fat content in MASLD.
Yang et al. (2024) [64]	Investigate miR-29a's role in reducing mitochondrial stress via MAVS in diet-induced NAFLD.	- miR-29a attenuated hepatic mitochondrial stress, reducing fibrosis, steatosis, and inflammation through inhibition of the MAVS pathway in a Western diet-induced NAFLD model.
Zhang et al. (2025) [65]	Analyze differential miRNA profiles in children with NAFLD and CVD risk.	- miR-122-5p showed increased expression in patients with NAFLD, significantly correlating with cardiovascular risk and metabolic alterations.
Michalak et al. (2025) [66]	Evaluate links between miRNAs and blood/serological fibrosis indicators in NAFLD.	- ↑ miR-126-3p, ↑ miR-1-3p, and ↓ miR-197-3p, correlation with hematological indices.

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Role of essential oils in preventing hepatotoxicity: A comprehensive review

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Abstract

Hepatotoxicity represents one of the significant and challenging health concerns at the worldwide level. To overcome this condition, essential oil can act as a natural therapeutic in alleviating hepatic disorders caused by toxins, drugs, alcohol, or infections. Various aromatic plants such as Citrus species, Allium species, spices, and rosemary species contain bioactive compounds—mainly terpenes, phenolics, flavonoids, and sulfur-containing compounds. These bioactive compounds possess excellent antioxidant potential to neutralize free radicals and promote the endogenous antioxidant defense system, such as SOD, catalase, Gpx, and others as well. In addition to this, they exhibit anti-inflammatory potential to regulate inflammatory cascades and cytokine levels, while their detoxification mechanism stimulates the liver's capacity to eradicate harmful substances. Although previous research has documented that essential oil exhibits the ability to protect the liver from chronic diseases—mainly fibrosis, non-alcoholic fatty liver disease (NAFLD), and drug-induced hepatotoxicity—by modulating lipid metabolism, hepatocyte integrity, the antioxidant defense system, and pathological factors, future research is still required to evaluate its efficacy, bioavailability, safe doses, and explore synergistic formulations. Keeping these perspectives in mind, the current review is planned to highlight the hepatoprotective properties of essential oils, their underlying mechanisms, and their prospective contribution to the development of natural therapeutics for liver health.

Keywords: Anti-apoptotic; anti-inflammatory; antioxidant; essential oil; hepatotoxicity; liver diseases.

Introduction

Liver disease, including cirrhosis, viral hepatitis, and liver cancer, causes more than two million fatalities a year and accounts for 4% of all deaths globally (1 out of every 25 deaths); one out of three liver-related deaths occurs in women.[1] Recently, liver disease is considered the 11th leading cause of death, but liver deaths may be underreported. The major cause of liver disease is characterized by metabolic abnormalities and histological alterations like zonal necrosis, vascular lesions, granuloma, steatosis, and cholestasis, accounting for 5% of all injuries and consequently prevalent to known injury.[2] Although the pathophysiological processes of hepatotoxicity are still poorly understood, they are primarily linked to the metabolic conversion of xenobiotics into reactive oxygen species (ROS). This contributes to the condition known as "oxidative stress" and, as a result, impairs the macromolecules within cells.[3] In the majority of liver diseases like cirrhosis, hepatitis, and hepatocellular carcinoma (HCC), oxidative stress represents one of the key factors involved in promoting these disorders.[4] Furthermore, overproduction of ROS and a decrease in the antioxidant defense system accelerate free radical formation and, as a result, attack polyunsaturated fatty acids in cellular membranes and promote lipid peroxidation.[5] Apart from that, upregulation in enzymatic markers such as serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), and aspartate aminotransferase (AST) is also interlinked with tissue necrosis and degeneration of hepatic tissues and, therefore, promotes "hepatotoxicity."[6]

As the liver plays a vital role in the metabolism, transport, and clearance of xenobiotics, it is critically susceptible to damage.[7] A wide range of toxicants such as chemicals, antibiotics, steroidal and non-steroidal drugs, viral infections, dietary and herbal supplements contribute to the onset of liver disease by triggering "oxidative stress." It plays a vital role in the pathogenesis and progression of hepatotoxicity by modulating the release of inflammatory mediators such as TNF-α, IL-1β, and apoptotic factors (Bax, Bcl-2, caspase-3, 9, etc).[8] Depending on the toxicity and mechanism of action of toxicants, it can be classified into two types: intrinsic and idiosyncratic.[9] Intrinsic toxicity is typically dose-dependent and frequently detected within hours to days after toxicant exposure. In contrast, idiosyncratic toxicity follows unpredictable behavior with variable latency of onset from weeks to months. However, a variety of synthetic and natural drugs are available on the market to reduce hepatic disease as well as to overcome the challenges associated with these disorders. Due to availability, safety, and efficacy concerns, it is necessary to explore and develop more novel drugs to counteract hepatotoxicity.[10,11]

In the recent era, the consumption of essential oils can be considered a novel approach to evaluate the potential benefits for human health. The volatile nature of essential oil allows its rapid absorption through inhalation or transdermal routes, distinguishing essential oils from non-volatile herbal extracts that rely on larger, often water-soluble molecules. These unique molecular profiles are not only responsible for the diverse pharmacological effects of essential oils but also account for the

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challenges related to their stability, standardization, and formulation. However, it can be extracted from aromatic plants by hydrodistillation, steam distillation, microwave-assisted hydrodistillation, and others as well.[12] They are typically comprised of secondary metabolites and possess oxygenated structures such as alcohols, ketones, aldehydes, and esters. They exhibit a wide range of pharmacological potential such as antioxidant, antibacterial, antiviral, and anticancer activity.[13] Due to these activities, essential oils are utilized in a variety of industries, including pharmaceutical, aroma, and food.[14] For preparing novel drugs, it is essential to consider various aspects like solubility, dose range, dosing methods, and bioactive delivery systems to ensure efficacy, safety, and stability.[15] The current research attempts to assess the composition and mechanisms of essential oils in experimental models and provides deeper insights for futuristic research in the development of folk medicine for treating liver diseases and boosting liver functions.

Essential Oil Composition

The composition of essential oils varies depending on the plant source, extraction method, and environmental factors. However, they primarily consist of volatile organic compounds that contribute to their biological activities.[16] The key components of essential oils include terpenes and their derivatives, phenolic compounds, and sulfur-containing compounds, which offer unique aroma and medicinal qualities.[17]

Pharmacokinetics, Bioavailability, and Drug Interactions

Pharmacokinetics of Essential Oils

Due to the presence of terpenoids and non-terpenoids, essential oils exhibit pharmacokinetic properties required for therapeutic potential. Their hydrophobic nature promotes absorption across biological membranes. Various routes like oral, inhalational, and transdermal allow their entry into the biological system.[18] After entry into the biological system, depending on the solubility, molecular weight, and skin barrier, absorption of essential oil and its components is rapidly distributed into lipid-rich tissues such as the liver, brain, and adipose tissues. However, the liver plays a crucial role in metabolizing the essential oil components through Phase I and Phase II reactions. Different families of cytochrome P450 (CYP450) enzymes, particularly CYP3A4 and CYP2D6, are majorly involved in the biotransformation of major EO components. The resulting metabolites are generally more water-soluble and are eliminated via renal and biliary excretion. In addition, a portion of volatile EO components can be exhaled through the lungs or secreted via sweat, contributing to their characteristic odor even after systemic administration.[19]

Bioavailability of Essential Oils

The poor bioavailability of essential oils limits their usage for therapeutic benefits. This may be due to the fact that essential oils exhibit low aqueous solubility, rapid metabolism, and instability under physiological parameters. However, orally administered EO and its components typically undergo extensive first-pass metabolism, significantly reducing their systemic concentration and therapeutic effect. Essential oil exhibits a short half-life, which limits its duration of action and may necessitate repeated or sustained delivery for therapeutic efficacy.[20] For example, curcumin extracted from turmeric essential oil exhibits significantly low oral bioavailability in response to rapid metabolism and elimination. To address these problems, a number of delivery methods have been designed to increase bioavailability, such as solid lipid nanoparticles, liposomes, and nanoemulsions. These formulations improve solubility, protect volatile constituents from degradation, and facilitate sustained release. Transdermal and sublingual routes have also gained attention as they bypass hepatic metabolism, offering improved bioavailability and prolonged systemic activity. In a previous study, lipid-based formulations of bioactive substances such as carvacrol and thymol can greatly improve their absorption and therapeutic efficacy.[21]

Drug Interactions of Essential Oils

In clinical applications, the possibility of interactions between essential oils and traditional medications is a major concern. A wide variety of EO components may alter the behavior of transport proteins and drug-metabolizing enzymes, which can either make medication combinations more hazardous or less effective. For example, one of the most studied interactions is CYP450 enzyme inhibition or induction. In a previous study, it has been demonstrated that eugenol, a phenolic molecule found in clove oil, inhibits CYP3A4, which in turn affects the activity of calcium channel blockers, statins, and some benzodiazepines. Meanwhile, limonene and other monoterpenes may induce particular CYP isoforms and lower the plasma levels of some medicinal substances.[22] Furthermore, essential oils have the ability to affect the function of efflux transporters like P-glycoprotein (P-gp), which is crucial for pharmacokinetics and drug resistance. For example, peppermint oil can improve the efficacy of NSAIDs in treating irritable bowel syndrome, but it may also prevent the absorption of iron and other minerals. The significance of assessing EO use is highlighted by these interactions, particularly for patients following polypharmacy regimens. This indicates that when EOs and medications having a limited therapeutic index, like immunosuppressants, anticoagulants, and antiepileptics, are taken together, caution is advised.[23]

Mechanism of Hepatoprotective Activity

Antioxidant

During metabolic processes or external signals such as environmental pollutants and other toxicants, the level of reactive oxygen species—mainly superoxide (O_2•−), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2)—can be increased and, as a result, promote metabolic diseases.[24] However, an increase in ROS production can be neutralized by the antioxidant defense system, or natural antioxidants and dietary supplements, to maintain redox status.[25] The use of essential oils accelerates the process of free radical scavenging to improve liver health and oxidative stress-related conditions. Due to their rich composition of bioactive compounds such as alcohols, ethers, ketones, aldehydes, and monoterpenes, essential oils exhibit significant antioxidant behaviour, preventing cellular damage and apoptosis and therefore act as hepatoprotective agents.[26] In a previous study, treatment with the essential oil of *A. campestris* has substantially reduced oxidative stress, biochemical alterations, and improved histopathological architecture in hepatic tissues of mice intoxicated with chlorpyrifos.[27] Furthermore, administration of essential oil extracted from flowers of *Tagetes patula* can be considered a natural therapeutic for relieving multiple liver disorders associated with oxidative stress. It exhibits antioxidant potential, confirmed by DPPH scavenging assay, nitric oxide, and FRAP assays at a dose of 10 mg/kg body weight in hepatic tissues intoxicated with CCl_4. Meanwhile, *Tagetes patula* essential oil restores liver activities by maintaining histological architecture and lipid profiles at two different doses.[28] This indicates that essential oil can serve as a potent therapeutic for liver illnesses due to its ability to neutralize free radicals and enhance detoxification mechanisms.

Anti-Apoptotic

Apoptosis is characterized as controlled cell death to preserve cellular homeostasis and eliminate damaged or unnecessary cells. However, in the case of certain liver illnesses, an imbalance exists between cell proliferation and death, which promotes pathophysiological conditions.[29] For example, liver diseases like acute and fulminant hepatitis and chronic illnesses like chronic hepatitis, alcoholic liver disease, cholestatic liver disease, and non-alcoholic steatohepatitis are mainly associated with excessive apoptosis.[30] It can be controlled by multiple factors like oncogenes and anti-oncogenes, death receptor–ligand binding, and excessive inflammatory cytokines. The hallmarks of apoptotic cells are membrane blebbing, cellular shrinkage, chromatin condensation and DNA fragmentation, formation of apoptotic bodies, and no release of cellular contents.[31] Collectively, it may further activate a wide range of signalling cascades for cellular apoptosis such as caspase pathways, Bcl-2 gene families, as well as immune-mediated apoptosis.[32] In order to prevent this, there is a need to explore essential oils and their bioactive compounds to evaluate their multifaceted roles in preventing hepatic damage by inhibiting apoptotic cascades. By targeting specific signaling cascades, essential oil promotes its antioxidant and anti-inflammatory potential in protecting against hepatic injuries and, as a result, acts as a novel therapeutic agent for modulating apoptotic mechanisms.[33] In a previous study, treatment with coriander essential oil upregulated the level of antioxidant enzymes and modulated Nrf2/HO-1 as well as anti-apoptotic signaling cascades in hepatic tissues of rats intoxicated with dexamethasone. Furthermore, the administration of essential oil of *Carpesium abrotanoides* L. (CAEO) significantly activated the apoptotic factors such as caspase-3 and -9 and, as a result, declined the ratio of Bcl-2/Bax protein. This suggests that CAEO acts as a potent therapeutic in preventing hepatic cancer by triggering mitochondrial-mediated apoptotic cascades involved in apoptotic processes.[34] Apart from that, the upregulation of mRNA expression of anti-apoptotic factor Bcl-2 and substantial downregulation of the nuclear and cytoplasmic apoptotic mediator p53 in hepatic tissues were observed in hepatocytes administered with thyme oil and thymol. Additionally, it ameliorates lipid peroxidation and declines the concentration of TNF-α, albumin, and IL-4 in hepatic tissues intoxicated with doxorubicin.[35] This may be due to the fact that upregulation in antioxidant enzymes (GST and Gpx) counteracts the inflammatory as well as apoptotic markers and acts as a potent hepatoprotective agent.

Anti-Inflammatory

Inflammation plays a vital role in stimulating hepatic injury by modulating cellular and molecular mechanisms followed by hepatic damage, alteration in liver function, cirrhosis, and fibrosis. Various inflammatory and immune factors such as monocytes, macrophages, neutrophil leukocytes, NK, NKT cells, Th17, and regulatory T cells are responsible for liver inflammation.[36,37] Hepatocytes represent different pattern recognition receptors (PRRs) that identify endogenous or pathogen-derived molecular sequences to enhance intracellular signalling cascades and, as a result, induce inflammatory responses. However, interaction of parenchymal and immune cells with each other produces multiple cytokines and chemokines in the local environment to promote liver inflammation.[38] To overcome this condition, essential oils offer a novel and alternative strategy to treat inflammatory diseases. Various signaling pathways upregulate the level of cytokines and other key transcription factors to activate pro-inflammatory genes and, as a result, induce pathological conditions in hepatocytes. Earlier studies have demonstrated that treatment with grape essential oil induces antioxidant enzymes, upregulates the level of trace elements, and downregulates the enzymatic activity of cytochromes, caspases, and NF-κB expression in hepatic tissues.[39] Additionally, the supplementation of lavender essential oil also minimizes hepatic destruction through downregulation of pro-inflammatory cytokines, nitric oxide (NO), and myeloperoxidase (MPO) activity in mice intoxicated with acetaminophen.[40] Furthermore, acrylamide, a major toxicant and carcinogenic agent, is involved in hepatic injury by downregulating the antioxidant defense system and modulating inflammatory factors such as the NF-κB/NLRP3 inflammasome axis and interleukins. However, treatment with *T. satureioides* essential oil highlights its therapeutic potential by targeting the NLRP3 inflammasome/NF-κB axis, which acts as a promising strategy for preventing acrylamide-induced hepatotoxicity.[41] This indicates that essential oil may serve as complementary or alternative therapy to overcome hepatic diseases by reducing inflammation through targeting a variety of signalling cascades like NF-κB, the NLRP3 inflammasome, and maintaining oxidative stress with minimal side effects (Fig. 1).

Clinical and Pre-clinical Evidences

Spice Essential Oil

Spices are classified as functional foods because they have been shown to improve specific body functions beyond just meeting basic nutritional needs in a variety of flavor, color, and aroma.[42,43] From past centuries, different spices such as bud (clove), bark (cinnamon), aromatic seed (cumin), and others have promoted flavor in foods and are considered culinary agents to preserve food and enhance health benefits.[44] From the previous literature, it has been found that alteration in biochemical parameters, oxidative markers, interleukins, caspases, and histopathological studies can be greatly influenced by the action of spice essential oils.[45,46] However, treatment with clove essential oil substantially downregulated the level of biochemical parameters (ALT, AST, and ALP) and restored histological architecture in hepatic tissues of rats. Meanwhile, it enhanced several changes such as infiltration of inflammatory cells, congestion, hyperplasia, cytoplasmic vacuolation, degeneration, and necrosis.[47] Furthermore, *S. aromaticum* L. oil ameliorated liver injury by downregulating serum biomarkers and MDA levels in rats subjected to levofloxacin. The administration of oil at a dose of 10 mg/kg reduced the level of total bilirubin and hepatic biomarkers and, as a result, acted as a hepatoprotective agent.[48] Another study revealed that chemical-induced liver injury also promotes the formation of hepatic lesions through upregulation of serum biomarkers (ALT, AST, and ALP) and lactate dehydrogenase (LDH) levels in CCl_4-intoxicated rats. Treatment with *Cinnamomum verum* essential oil (CVEO) at a dose of 100 mg/kg exhibited the potential of free radical scavenging and thus downregulated the level of serum biomarkers (ALT, AST, and ALP).[49] However, in a randomized controlled trial, treatment with *Nigella sativa* essential oil improved liver steatosis and injury and blood levels of triglycerides, LDL-C, and HDL-C in NAFLD patients.[50] Essential oils exhibit a variety of bioactive compounds such as cinnamaldehyde, cuminaldehyde, cymene, terpenes (β-pinene, γ-terpinene), eugenol, and thymoquinone, which possess multiple pharmacological activities.[51] In a recent study, it has been demonstrated that treatment with cinnamaldehyde improved liver function by significantly reducing biochemical parameters (ALT, AST, GGT), which in turn alleviates inflammation (TGF-β) and apoptosis in CCl_4-induced liver fibrosis.[52] In non-alcoholic fatty liver disease (NAFLD), the administration of cuminaldehyde, an active constituent of cumin essential oil, enhances the antioxidant defense system and

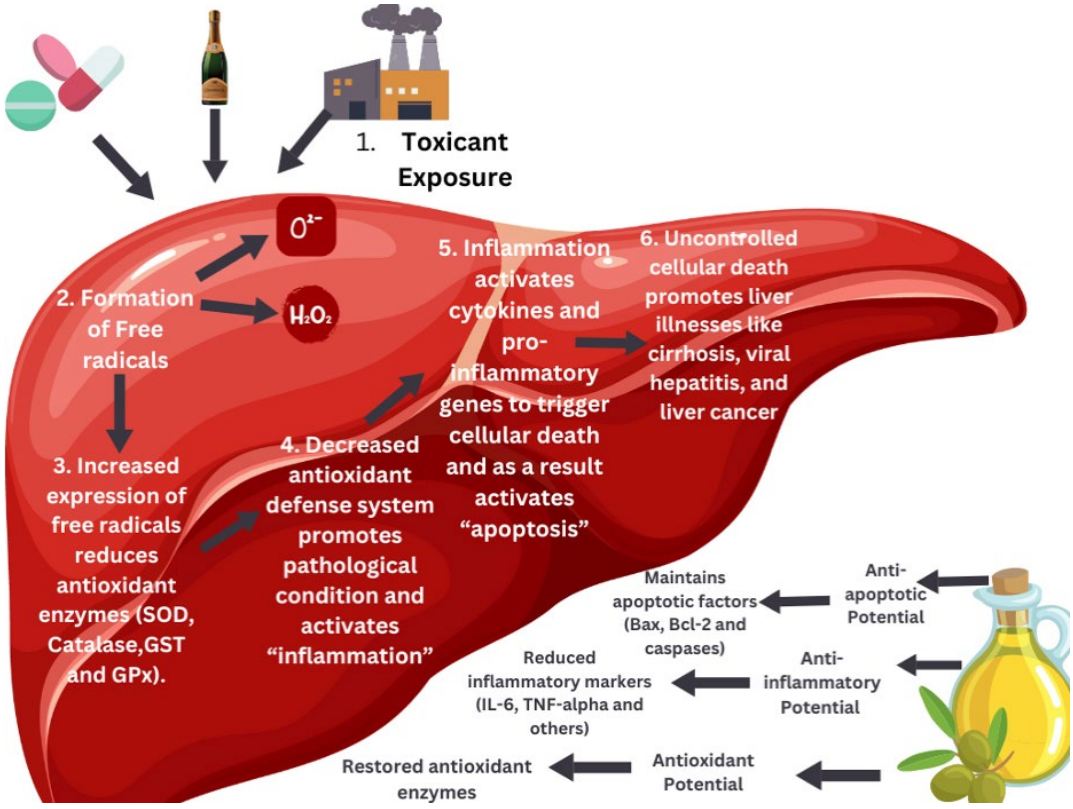

Figure 1. Mechanistic insights of essential oil in preventing hepatocytes.

significantly reduces serum levels of liver enzymes (AST and ALT) as well as hyperlipidemia in experimental rat models.[53] Besides this, other bioactive compounds like p-cymene greatly prevented the infiltration of inflammatory cells in the liver parenchyma of stressed rats. In conclusion, the study found that thymol and p-cymene have a hepatoprotective effect on immobilized rats, likely exerted by suppressing oxidative stress and inflammation, stimulating Nrf2/HO-1 signaling, and inhibiting the TNF-α/NF-κB pathway.[54] In HepG2 cell lines, eugenol protects human liver HepG2 cells from H_2O_2-induced oxidative hepatotoxicity by maintaining ROS homeostasis, increasing IL-10 levels, and upregulating cytochrome gene expression.[55] In summary, these investigations demonstrated that spice essential oils and their bioactive compounds exhibit favorable hepatoprotective benefits and require further investigations to identify safety dosage, biodistribution and potency.

Allium Species Essential Oil

Essential oils derived from *Allium* species, such as garlic (*Allium sativum*), onion (*Allium cepa*), and leek (*Allium porrum*), are renowned for their medicinal properties. These oils are highly rich in organosulfur compounds, which exhibit strong hepatoprotective properties, making them suitable for managing conditions such as drug-induced hepatotoxicity, NAFLD, and liver fibrosis.[56] In a previous study, exposure to deltamethrin upregulated biochemical parameters and downregulated the antioxidant defense system. As a result, it altered the histological architecture of liver tissues, which was confirmed by central vein congestion, necrosis, and infiltration of inflammatory leukocytes. Moreover, administration of *Allium sativum* essential oil (ASEO) significantly reduced hepatic intoxication by restoring the level of antioxidant enzymes and biochemical parameters. This highlighted that ASEO exhibits the potency to minimize hepatic damage induced by deltamethrin.[57] Furthermore, it reduces inflammation, apoptosis, and genotoxicity and modulates histological architecture in hepatic tissues of mice intoxicated with heavy metal.[58] This explains that *Allium sativum* essential oil protects against liver damage via maintaining the inflammatory pathways such as NF-κB signalling cascades and apoptotic pathways (extrinsic and intrinsic) and therefore protects against genotoxicity. Apart from that, in non-alcoholic fatty liver disease, *Allium sativum* essential oil (ASEO) and its bioactive compound DADS promote lipid-lowering and anti-obesity effects by downregulating biochemical parameters in serum and reducing body weight gain in mice exposed to a high-fat diet. However, administration with two doses of ASEO (50 and 100 mg/kg) and its bioactive compound DADS at 20 mg/kg substantially altered the mechanism of fatty acid synthesis. This indicates that ASEO and DADS dose-dependently prevented obesity in mice through minimizing lipid accumulation and oxidative stress and, as a result, protected against inflammation by alleviating metabolic abnormalities in mice fed with a high-fat diet.[59] In the case of acute liver failure, administration of DATS suppressed inflammation and apoptosis by downregulating caspase-3 and the Bax/Bcl-2 ratio in LPS/D-gal–treated mice. Further, it inhibited the increase in CD11b+ Kupffer cells and other macrophages in the liver and tumor necrosis factor-alpha in the blood.[60] Another study demonstrated that allicin exhibits a significant protective effect on CCl_4-induced liver injury via inhibiting the inflammatory response and hepatocyte apoptosis and alleviating oxidative stress associated with the progression of liver damage, highlighting the potential of allicin as a hepatoprotective agent.[61] From these findings, it has been revealed that *Allium sativum* essential oil (ASEO) and its bioactive compounds may act as a promising approach for herbal drug preparation to prevent hepatotoxicity.

Citrus Species Essential Oil

Citrus species such as grapefruit (*Citrus paradisi*), orange (*Citrus sinensis*), lemon (*Citrus limon*), and lime (*Citrus aurantiifolia*) are well-known for their therapeutic and medical qualities. The bioactive compounds such as limonene, linalool, citral, and flavonoids are mainly found in essential oils extracted from peels, leaves, or flowers.[62] One of the noteworthy uses of citrus essential oil is its hepatoprotective potential, protecting against hepatic damage induced by toxins, oxidative stress, or inflammation.[63] However, prolonged consumption of non-steroidal anti-inflammatory drugs, mainly aspirin, induces hepatotoxicity by increasing the level of biochemical parameters (AST, ALT, and LDH) and decreasing the antioxidant defense system. From the literature survey, it has been documented that citrus essential oil (CEO) exhibits antioxidant, anti-inflammatory, and anti-apoptotic potential, contributing to substantial protection against aspirin toxicity by restoring these parameters back to normal. It prevents oxidative stress and NF-κB cells from nuclear localization to restrict the activation of inflammatory genes and, as a result, inhibits inflammation and reduces hepatocyte death to shield the liver from I/R damage.[64] Furthermore, induction of HO-1 prohibits the activation of inflammatory mediators and stimulates iNOS expression and therefore promotes antioxidant defense in hepatocytes. This indicates that modulation of HO-1 can serve as a promising strategy to protect against liver damage; however, CEO treatment significantly restored the mRNA and protein expression of HO-1 to overcome cellular injuries induced by I/R. This suggests that expression of NF-κB and HO-1 signaling cascades can significantly promote liver recovery by altering metabolic abnormalities in I/R-induced liver injuries. Furthermore, in order to modify lipid metabolites and modulate genes related to lipid metabolism, CEO could potentially mediate lipid and cholesterol homeostasis and successfully prevent hypercholesterolemia and hepatic steatosis.[65,66] Bioactive compounds—mainly limonene, linalool, citral, α-terpineol, geraniol, myrcene, γ-terpinene, and β-pinene—are majorly present in citrus species essential oil.[67] In an experimental study, the administration of myrcene significantly inhibited acetaminophen-induced liver damage by normalizing several biochemical characteristics of liver function and oxidative stress and thus improved histopathological parameters due to its antioxidant activity.[68] Meanwhile, treatment with linalool ameliorated lipid accumulation in hepatocytes by promoting fatty acid oxidation, inhibiting lipid biosynthesis, and reducing oxidative stress by regulating Nrf-2/HO-1 signaling cascades.[69] Apart from that, other bioactive compounds like limonene, geraniol, citral, and others as well upregulate the antioxidant defense system and alter pathological conditions such as inflammation, apoptosis, and lipid accumulation in hepatocytes. Previous literature suggested that limonene possesses the capacity to reduce hepatic lipid peroxidation and inhibit pro-inflammatory cytokines such as TNF-α and IL-6. Moreover, geraniol exhibits anti-apoptotic properties by modulating Bcl-2 family proteins, while citral downregulates lipogenic gene expression, thereby reducing lipid accumulation.[70–73] This indicates that CEO and its bioactive compounds hold significant promise for drug development aimed at liver protection. However, further research is necessary to elucidate its precise molecular mechanisms, optimize its dosage and formulation, and evaluate its clinical safety and efficacy.

Rosemary Essential Oil

A well-known medicinal herb, rosemary (*Rosmarinus officinalis*) has been considered for its pharmacological benefits. Essential oil extracted from the plant's leaves is highly rich in bioactive components, mainly carnosol, rosmarinic acid, and carnosic acid. These bioactive compounds exhibit a variety of pharmacological potentials such as antioxidant, anti-apoptotic, anti-inflammatory, and aid in detoxification mechanisms.[74] According to a previous study, it has been indicated that rosemary essential oil (REO) acts as a potent free radical scavenger, determined by DPPH assay and, as a result, activates antioxidant defense mechanisms in hepatocytes.[75] Due to the presence of bioactive compounds such as phenolics and tocols, rosemary oil can be significantly involved in alleviating hepatotoxicity and is considered in pharmaceutical and food industries, particularly in the development of natural drug formulations and functional foods to improve overall health.[76] Moreover, the abnormal behavior of hepatocytes can be assessed by the alterations in hematological parameters, oxidative stress markers (protein carbonyl, TBARS, and H_2O_2), and a significant drop in glutathione (GSH) concentration. For example, in rats, chromium exposure upregulates biochemical parameters (ALP, AST, and ALP), total protein, and albumin levels and causes a significant decline in enzymatic antioxidants (SOD, CAT, GPx, and GST) in hepatocytes. To overcome this, treatment with REO significantly restored these parameters by improving antioxidant status and decreasing lipid peroxidation.[77] Furthermore, the assessment of histological and immunohistochemical expression of PCNA revealed the restoration of hepatic tissues. In conclusion, it has been considered that REO and its bioactive compounds can effectively modulate chromium-induced hepatotoxicity, especially in pretreated rats. Rosemary essential oil exhibits hepatoprotective activity primarily due to its bioactive compounds like 1,8-cineole, α-pinene, camphor, and borneol, which exert antioxidant, anti-inflammatory, and detoxifying effects on liver tissue.[78] In a previous study, treatment with 1,8-cineole prevented liver damage by reducing oxidative stress and inflammation in rats intoxicated with lead acetate. This hepatoprotection is probably achieved by inhibiting TLR4/MyD88/NF-κB signalling cascades.[79] Moreover, administration of alpha-pinene induces liver protective effects against N-acetyl-p-aminophenol (paracetamol, APA) damage by reducing the activity of liver enzymes, improving antioxidant/oxidative status, and reducing inflammation through the regulation of NF-κB and pro-inflammatory cytokines.[80] This indicates that rosemary essential oil and its bioactive compounds can act as promising candidates to prevent and alleviate hepatotoxicity.

Challenges, Limitations, and Contradictory Findings

Despite growing interest in the anticancer potential of essential oils and their constituents, several critical challenges limit their clinical applicability and scientific validation.

Lack of Standardization and Reproducibility

Depending on the plant species, geographical background, and extraction procedures, the chemical composition of essential oils may differ. Various studies have clearly demonstrated that in-depth analysis of compositions by techniques such as gas chromatography–mass spectrometry (GC-MS) is crucial for identifying and measuring bioactive compounds.[81–83] Due to a lack of internationally recognized quality control procedures and substantial batch-to-batch variability, it is difficult to accurately replicate experimental results. This can be a major limitation because it lacks standardization and makes it challenging to draw consistent conclusions across studies. Concerns regarding the safety and effectiveness of essential oils in clinical and therapeutic settings are also raised by this diversity, which restricts the comparability of results between investigations. Thus, standardizing extraction procedures, maintaining quality control standards, and establishing chemical characterization are important for increasing the legitimacy and relevance of essential oil research.

Limited Clinical Evidence

The effectiveness and safety of essential oils in human populations are still not well supported by high-quality clinical data. The majority of the information originates from pre-clinical or *in vitro* research, useful for comprehending mechanisms of action. Still, there is a lack of evidence representing clinical studies to examine the effect of essential oils. Study limitations include sample sizes, study durations, and methodological flaws like blinding, placebo control, or randomization. Furthermore, there is considerable variability in the dosage forms, administration routes (e.g., inhalation, topical, oral), and types of essential oils used across studies, making it difficult to establish standardized treatment protocols. Regulatory oversight is also minimal in many countries, resulting in inconsistent product quality and further complicating clinical translation. To establish essential oils as evidence-based therapeutic agents, there is an urgent need for well-designed, large-scale, and placebo-controlled clinical trials that adhere to rigorous methodological standards. These shortcomings diminish the reliability of the evidence and make it more difficult to reach strong conclusions about treatment efficacy.

Toxicity and Safety Concerns

Essential oils are not always safe and can be extremely toxic or dangerous, especially if used incorrectly or in large quantities. They contain strong bioactive chemicals which impart negative consequences like skin irritation, allergic responses, respiratory discomfort, hepatotoxicity, or neurotoxicity. Due to their lipophilic nature, they facilitate absorption through the skin and increase the risk of poisoning. People with pre-existing diseases are more vulnerable. As essential oils are widely accessible in the consumer market without any regulatory monitoring, this in turn increases the chances of misuse, such as improper dilution and consumption of oils that are not intended for internal use. Therefore, strict toxicological testing, clear labeling, uniform safety regulations, and public education are vital to ensuring the safe and responsible use of essential oils in both commercial and medical contexts. Despite the common belief that essential oils are "natural and safe," they could potentially cause unfavorable side effects.

Contradictory Findings in the Literature

Essential oils present numerous contradictory findings, mainly regarding their mechanisms of action and the establishment of efficacy and effectiveness. Literature studies suggest that essential oils exhibit promising pharmacological benefits such as antimicrobial, antioxidant, anti-inflammatory, or neuroprotective effects, while others show minimal or no activity under similar experimental conditions.[84–86] Variation in plant species, oil extraction techniques, chemical composition, and dosage are the major factors responsible for these discrepancies. Furthermore, different biological models, such as animal models, mammalian cells, or bacterial cultures, may respond differently and produce varied results. It is sometimes possible for the same essential oil to exhibit both cytotoxic and therapeutic effects, depending on the target tissue, concentration, and dosage. Such conflicting results highlight the necessity for established methodology, rigorous quality control, and reproducible techniques in essential oil research.

Conclusion

Essential oil extracted from aromatic plants offers a promising approach to protect the liver from pathological conditions such as oxidative stress, inflammation, and toxin-induced damage. Due to its pharmacological potential, it strengthens the antioxidant defense system, modulates inflammatory and apoptotic cascades to support detoxification processes, and thus serves as a valuable hepatoprotective agent. Despite these advantages, various challenges including dose optimization, oil composition consistency, and possible toxicity at higher concentrations still need to be resolved. According to recent studies, essential oils could potentially be helpful in treating liver problems, but more research is needed before they can be used in clinical settings. By tackling these avenues, essential oils provide enormous promise as sustainable, safe, and efficient alternatives for managing and preventing liver illnesses, facilitating a holistic approach to liver health.

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