ISSN 1948-5182 (online)

# World Journal of *Hepatology*

*World J Hepatol* 2019 August 27; 11(8): 613-662

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

Contents

Monthly Volume 11 Number 8 August 27, 2019

## OPINION REVIEW



## ORIGINAL ARTICLE

### Basic Study



### Retrospective Study



### Observational Study



## CASE REPORT

**Contents**

*World Journal of Hepatology*

**Volume 11 Number 8 August 27, 2019**

## ABOUT COVER

Editorial Board Member of *World Journal of Hepatology*, Kazuto Tajiri, MD, PhD, Lecturer, Department of Gastroenterology, Toyama University Hospital, Toyama 930-0194, Japan

## AIMS AND SCOPE

*World Journal of Hepatology* (*World J Hepatol*, *WJH*, online ISSN 1948-5182, DOI: 10.4254), is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

The *WJH* covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, *etc*. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, *etc*.

We encourage authors to submit their manuscripts to *WJH*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great basic and clinical significance.

## INDEXING/ABSTRACTING

The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, China National Knowledge Infrastructure (CNKI), China Science and Technology Journal Database (CSTJ), and Superstar Journals Database.

## RESPONSIBLE EDITORS FOR THIS ISSUE

**Responsible Electronic Editor:** *Yu-Jie Ma*

**Proofing Production Department Director:** *Yun-Xiaojian Wu*

**NAME OF JOURNAL**
*World Journal of Hepatology*

**ISSN**
ISSN 1948-5182 (online)

**LAUNCH DATE**
October 31, 2009

**FREQUENCY**
Monthly

**EDITORS-IN-CHIEF**
Ke-Qin Hu, Koo Jeong Kang, Nikolaos T Pyrsopoulos

**EDITORIAL BOARD MEMBERS**
https://www.wjgnet.com/1948-5182/editorialboard.htm

**EDITORIAL OFFICE**
Ya-Juan Ma, Director

**PUBLICATION DATE**
August 27, 2019



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

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

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

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

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

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

**ONLINE SUBMISSION**
https://www.f6publishing.com

World Journal of Hepatology

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

*World J Hepatol* 2019 August 27; 11(8): 613-618

DOI: 10.4254/wjh.v11.i8.613

ISSN 1948-5182 (online)

OPINION REVIEW

# Is porto sinusoidal vascular disease to be actively searched in patients with portal vein thrombosis?

Stefania Gioia, Silvia Nardelli, Lorenzo Ridola, Giulia d'Amati, Oliviero Riggio

**ORCID number:** Stefania Gioia (0000-0002-3940-4390); Silvia Nardelli (0000-0002-7038-9539); Lorenzo Ridola (0000-0002-8596-2609); Giulia d'Amati (0000-0002-6959-691X); Oliviero Riggio (0000-0000-2241-3223).

**Author contributions:** Gioia S contributed to study concept and manuscript preparation; Nardelli S contributed to manuscript preparation; Ridola L contributed to manuscript preparation; d'Amati G contributed to manuscript preparation and final drafting of the manuscript; Riggio O contributed to study concept, manuscript preparation, final drafting of the manuscript, and study supervision.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.



**Manuscript source**: Invited manuscript

**Stefania Gioia, Silvia Nardelli, Lorenzo Ridola, Oliviero Riggio,** Dipartimento di Medicina Traslazionale e di Precisione, "Sapienza" Università di Roma, Roma 00185, Italy

**Giulia d'Amati,** Dipartimento di Scienze Radiologiche, Oncologiche e Anatomo Patologiche, "Sapienza" Università di Roma, Roma 00185, Italy

**Corresponding author:** Stefania Gioia, MD, Dipartimento di Medicina Traslazionale e di Precisione, "Sapienza" Università di Roma, Viale dell'Università 37, Roma 00185, Italy.
stefania.gioia@uniroma1.it
**Telephone:** +39-6-49970463
**Fax:** +39-6-49972006

## Abstract

Porto sinusoidal vascular liver disease (PSVD) and portal vein thrombosis (PVT) are distinct vascular liver diseases characterized, respectively, by an intrahepatic and a prehepatic obstacle to the flow in the liver portal system. PVT may also occur as a complication of the natural history of PSVD, especially if a prothrombotic condition coexists. In other cases, it is associated to local and systemic pro-thrombotic conditions, even if its cause remains unknown in up to 25% despite an active search. In our opinion, the presence of PSVD should be suspected in patients with PVT especially in those with PVT "sine causa" and the active search of this condition should be included in their diagnostic work-out. However, sometimes the diagnosis of pre-existing PSVD is very hard. Biopsy cannot be fully discriminant as similar histological data have been described in both conditions. Liver stiffness may help as it has been shown to be higher in PSVD than in "pure" PVT, due to the presence of sclerosis in the portal venous radicles observable in PSVD patients. Nevertheless, comparing liver stiffness between PVT and PSVD has until now been restricted to very limited series of patients. In conclusion, even if it is still totally hypothetical, our point of view may have clinical consequences, especially when deciding to perform a liver biopsy in patients with a higher liver stiffness and suspending the anticoagulation in patients with PVT and no detectable prothrombotic factors.

**Key words:** Porto sinusoidal vascular liver disease; Obliterative portal venopathy; Portal vein thrombosis; Anticoagulant therapy



**Core tip:** Porto sinusoidal vascular liver disease (PSVD) and chronic portal vein

**Received:** March 26, 2019
**Peer-review started:** March 26, 2019
**First decision:** June 16, 2019
**Revised:** July 3, 2019
**Accepted:** July 16, 2019
**Article in press:** July 17, 2019
**Published online:** August 27, 2019

**P-Reviewer:** Garbuzenko DV
**S-Editor:** Ma YJ
**L-Editor:** Filipodia
**E-Editor:** Ma YJ

thrombosis (PVT) are vascular liver diseases. This review aims to discuss the possibility that (PSVD) should be suspected in patients with PVT and to analyse the possible diagnostic tools able to differentiate between these two conditions. Moreover, the review focuses on the possible and relevant clinical consequences of missing a diagnosis of PSVD in patients with PVT.

**Citation:** Gioia S, Nardelli S, Ridola L, d'Amati G, Riggio O. Is porto sinusoidal vascular disease to be actively searched in patients with portal vein thrombosis? *World J Hepatol* 2019; 11(8): 613-618
**URL**: https://www.wjgnet.com/1948-5182/full/v11/i8/613.htm
**DOI**: https://dx.doi.org/10.4254/wjh.v11.i8.613

## INTRODUCTION

Porto sinusoidal vascular liver disease (PSVD) is a vascular liver disease characterized by portal hypertension in the absence of cirrhosis and other causes of liver disease[1]. Contrary to the Eastern World and Developing Countries, in the Western World PSVD is considered infrequent. However, a low grade of suspicion can contribute, at least partially, to the low frequency of PSVD in Western Countries. Indeed, in the last years, the deepening of the knowledge on PSVD and its clinical presentation led to the recognition of an increasing number of cases and to the awareness that, in the past, some patients affected by PSVD were misdiagnosed and classified as affected by cryptogenic cirrhosis[2]. Moreover, it has been recently shown that a number of disorders and drugs (Table 1) are associated with PSVD[1,2], and this observation suggests that an active search of signs of portal hypertension in patients affected by those diseases could lead to the identification of PSVD patients.

### *Patients with portal vein thrombosis (PVT)*

This review aims to discuss the possibility that PSVD should be suspected in patients with PVT. In fact, a particular relationship exists between PSVD and PVT. In a considerable number of patients[3], about 40%, PSVD is associated to prothrombotic conditions, including myeloproliferative disorders. The prothrombotic state may represent a pathogenic factor leading the occlusion of the small branches of the portal vein, contributing to the so called obliterative portal venopathy (Figure 1A), which is considered the primary histological lesion of PSVD[4]. In turn, in patients with PSVD, the prothrombotic state may favour, together with the low portal flow, the development of an extrahepatic portal vein thrombosis. PVT actually occurs in up to 40% of PSVD patients[5,6], especially if a prothrombotic condition coexists. Whether the anticoagulant therapy is useful for the prevention of portal vein thrombosis or to improve the natural history of porto sinusoidal vascular liver disease idiopathic non-cirrhotic portal hypertension is still unknown[7].

The strong relationship between PSVD, hypercoagulability and incidence of PVT led to the hypothesis that a subset of patients with acute or chronic PVT could be affected by a pre-existing, undiagnosed PSVD. Actually, despite an active search[8] of local or systemic predisposing conditions (Table 2), the cause of PVT is not recognizable in up to 25% of the patients. Moreover, in patients in whom a PVT is diagnosed, the evidence of a prothrombotic state usually leads to the diagnosis of a PVT secondary to that condition without taking into consideration the possibility of a pre-existing and undiagnosed PSVD. The possibility of missing a diagnosis of PSVD in a patient with PVT, especially if acute, may have relevant clinical consequences.

Unfortunately, the distinction between these two conditions is not so easy and well defined, and the usefulness of liver biopsy to establish a differential diagnosis is questionable. In fact, while liver biopsy is adequate to exclude the presence of cirrhosis in patients with portal hypertension, the specific diagnostic features of PSVD are much more difficult to recognise[2]. More to the point, the histological distinction between the modification of the liver structure occurring in patients with PSVD (an intrahepatic cause of low portal perfusion) or PVT (in which the low portal perfusion is due to extrahepatic obstruction) may be difficult. Studies reporting a direct comparison of the histology features of PSVD and PVT are rare, mainly because the patients with PVT are not usually submitted to liver biopsy. Moreover, some modifications of liver histology, such as nodular regeneration and sinusoidal dilatation, could be due to compensatory adaptation to the reduction of portal flow

**Table 1 Diseases associated to porto sinusoidal vascular liver disease**

| |
|---|
| **Thrombophilia** |
| Myeloproliferative neoplasm |
| Protein S or C deficiency |
| Antiphospholipid antibodies |
| Lupus anticoagulant |
| Factor V Leiden |
| Prothrombin mutation |
| **Hematologic disease** |
| Myeloproliferative neoplasm (polycythemia vera, chronic myelogenous leukaemia, essential thrombocythemia) |
| Myeloid metaplasia |
| Lymphoproliferative conditions (Hodgkin's disease, non-Hodgkin's lymphoma, chronic lymphocytic leukaemia and multiple myeloma) |
| Spherocytosis |
| **Genetic disorders** |
| Cystic fibrosis |
| Adams Oliver syndrome |
| Turner's disease |
| **Autoimmune disease** |
| Rheumatoid arthritis |
| Systemic lupus erythematosus |
| Systemic sclerosis |
| Scleroderma |
| **Gut diseases** |
| Celiac disease |
| Inflammatory bowel disease |
| **Drug and toxics** |
| Oxaliplatin |
| Azathioprine |
| 6-thioguanine |
| Arsenic |
| Busulfan |
| Cytosine arabinoside |
| Cyclophosphamide |
| Bleomycin |
| Chlorambucil |
| Doxyrubicin |
| Carmustine |
| **Acquired and congenital immunodeficiency** |
| Human immunodeficiency virus |
| Primary antibody-deficiency syndrome |

that is common to both conditions. Verheij *et al*[4] compared liver biopsies from 70 patients with PSVD and 23 patients with PVT and observed that phlebosclerosis (*i.e*. obliterative portal venopathy), nodular regeneration and portal tract remnants (Figure 1A-C) were significantly more frequent in patients with PSVD than in those with PVT, while portal vein dilatation was more frequently observed in patients with PVT. These results are in line with the hypothesis proposed by Wanless, which considers PSVD a microvascular disorder resulting from injury to the small portal vein branches, whereas PVT is, by definition, a macrovascular disorder. However, the applicability of the above described observations to the distinction of PVT secondary or not to PSVD in the single patient is arguable. In fact, histological features, such as sinusoidal dilatation, para-portal shunts and increased portal vessels, although with different prevalence, are actually observed in both conditions. Moreover, the inclusion of patients affected by a pre-existing PSVD in the PVT group in the study by Verheji *et al*[4] cannot be completely ruled out. Consequently, due to this existing bias, it is hard to really differentiate the first and the second group on the basis of histology.

Indeed, luminal narrowing and disappearance or sclerosis of the portal venous

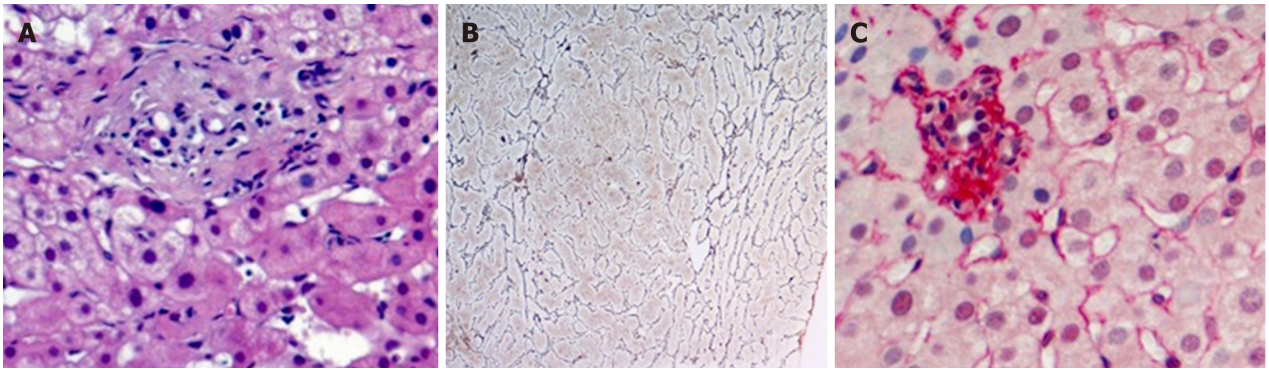


**Figure 1 The three histologic features more frequent in porto sinusoidal vascular liver disease.** A: Obliterative venopathy with small, rounded and fibrotic portal tracts, without evidence of the portal vein branch; B: Portal tract remnant; C: Nodular regeneration (parenchymal micronodular transformation is indicated with an asterisk).

radicles are typical changes observed in the fibrotic portal tract of PSVD patients[9]. Moreover, according to the endothelial-mesenchymal transition theory proposed by Sato *et al*[10], which would explain in part the pathogenesis of the obliterative portal venopathy, an endothelial dysfunction is present in patients with PSVD. The vascular endothelial cells of portal venules acquire myofibroblastic features and start to synthesize type I collagen. Thus, as a consequence of the presence of a certain degree of liver fibrosis in patients with PSVD, it can be hypothesised that the use of liver stiffness measurement would be helpful to distinguish patients with PSVD and patients with PVT. The latter patients, at least theoretically, would be characterized by a lower liver stiffness. A higher liver stiffness was actually observed in a group of 22 patients affected by regenerative nodular hyperplasia in comparison with 13 patients with PVT[11,12].

In patients with PVT, especially if acute, the correct identification of a pre-existing PSVD as the condition predisposing to PVT has relevant practical therapeutic implications. In fact, even in the absence of consistent clinical studies, in patients with PSVD, a lifelong anticoagulation is strongly suggested in presence of a prothrombotic condition and/or a portal vein thrombosis[13]. On the contrary, current guidelines on non-cirrhotic PVT do not suggest prolonging anticoagulation treatment lifelong in the absence of a prothrombotic state and/or extension of the thrombosis to mesenteric and splenic veins[13]. Thus, in a patient with PVT and an underlying undiagnosed PSVD without a prothrombotic state and without extension of the thrombosis to mesenteric and splenic veins, the anticoagulant therapy could be erroneously stopped once the PVT is resolved. However, as in PSVD, the slowing down of the portal flow is probably the principal condition leading to PVT, and as this condition is irreversible, the stop of anticoagulation may induce the rapid relapse of PVT.

Figure 2 reports the computed tomography scan obtained in a 17-year-old male patient referred to our ward for acute portal vein thrombosis. Because of the negativity of any predisposing cause of portal vein thrombosis, including acquired and congenital prothrombotic states, he was treated with oral anticoagulation for only 1 year, and complete portal vein recanalization was documented at the computed tomography scan. However, after the withdrawal of anticoagulation, the patient had a rapid relapse of PVT with the extension of the thrombosis to splenic vein. The patient was firstly treated with low-molecular-weight-heparin and after with systemic thrombolysis with alteplase reaching the complete resolution of the thrombosis. With suspicion of porto sinusoidal vascular liver disease, the patient was submitted to liver biopsy, and finally the diagnosis of PSVD was made by observing perivenular and perisinusoidal fibrosis, sinusoidal dilatation, para-portal shunts and nodular regenerative hyperplasia.

In conclusion, PSVD is probably a not so rare condition that should be actively searched not only by the hepatologists in the patients with signs of portal hypertension but also by the specialists who manage the patients affected by diseases associated with PSVD. Among the conditions associated with PSVD, PVT could be also included, and the suspicion of a pre-existing and missed PSVD should arise in any patient with PVT included (and maybe especially) in those affected by a prothrombotic state. However, more studies are needed to clarify the distinctive features of PSVD and PVT both histologically and elastographically, and new studies are needed to clarify the indications and the benefits of anticoagulant therapy in patients affected by PSVD.

**Table 2 Predisposing causes of portal vein thrombosis**

| Systemic prothrombotic conditions | Recommended checks |
|---|---|
| **Inherited** | Resistance to activated C protein/molecular biology for |
| Factor V Leiden | G1691A polymorphism |
| Prothrombin gene mutation | Molecular biology for G20210A polymorphism |
| Protein C, protein S and antithrombin deficiency | Ratio with F II, V, VII or X after correction for vitamin K deficiency; family survey (recommended) |
| **Acquired** | |
| Antiphospholipid syndrome | Anticardiolipin ELISA; LLAC |
| Myeloproliferative neoplasm (MPN) | JAK2 and CALR mutations; osteomidullary biopsy |
| Paroxysomal nocturnal hemoglobinuria | Flow cytometry (deficient cells of CD55 and CD59) |
| Oral contraceptive use | Anamnesis |
| Obesity | BMI > 30 kg/m$^2$ |
| CMV infection | CMV IgG, CMV IgM |
| Pregnancy | Beta-HCG, anamnesis |
| Celiac disease | Anti-transglutaminase IgA/IgG |
| **Local prothrombotic conditions** | Anamnesis and radiological examination |
| **Surgery and trauma** | |
| Umbilical cannulation | |
| Splenectomy, cholecystectomy | |
| Hepatic resection | |
| Abdominal trauma | |
| Bariatric surgery | |
| Portosystemic shunts including TIPS | Anamnesis and radiological examination |
| **Inflammatory and infectious diseases** | |
| Neonatal omphalitis | |
| Appendicitis, diverticulitis, pancreatitis | |
| Inflammatory bowel disease | |
| Cholecystitis, cholangitis | |
| **Other** | |
| Cirrhosis | |
| Porto sinusoidal vascular liver disease | |

LLAC: Lupus-like anticoagulant; ELISA: Enzyme-linked immunosorbent assay; BMI: Body mass index; CMV, Cytomegalovirus; HCG: Human chorionic gonadotropin; Ig: Immunoglobulin; TIPS: Transjugular portosystemic intrahepatic shunt.

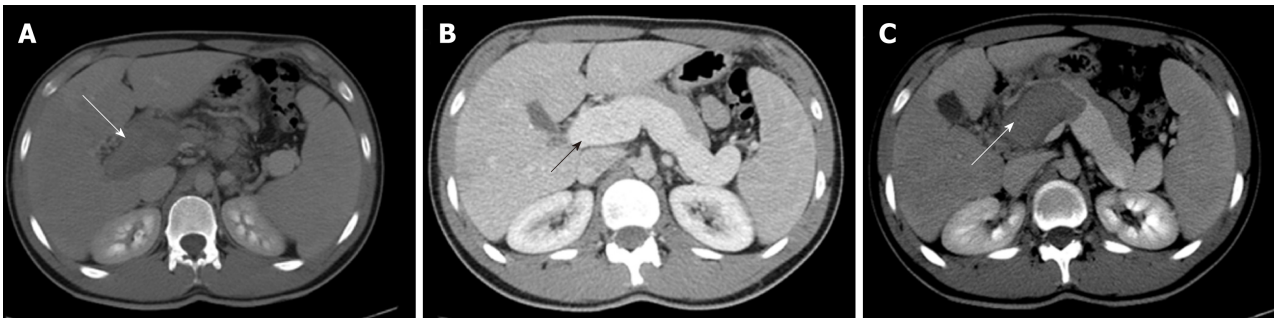


**Figure 2 Contrast-enhanced computed tomography scan of a 17 year-old patient.** A: Occlusive thrombosis of the trunk of portal vein; B: Complete resolution of the portal vein thrombosis after 1 year of anticoagulant therapy; C: Relapse of the portal vein thrombosis with extension to splenic vein 1 months after the stop of the anticoagulant therapy.

## REFERENCES

1 **Schouten JN**, Garcia-Pagan JC, Valla DC, Janssen HL. Idiopathic noncirrhotic portal hypertension. *Hepatology* 2011; **54**: 1071-1081 [PMID: 21574171 DOI: 10.1002/hep.24422]
2 **Riggio O**, Gioia S, Pentassuglio I, Nicoletti V, Valente M, d'Amati G. Idiopathic noncirrhotic portal hypertension: current perspectives. *Hepat Med* 2016; **8**: 81-88 [PMID: 27555800 DOI:

10.2147/HMER.S85544]
3 **Hillaire S**, Bonte E, Denninger MH, Casadevall N, Cadranel JF, Lebrec D, Valla D, Degott C. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: a re-evaluation in 28 patients. *Gut* 2002; **51**: 275-280 [PMID: 12117894 DOI: 10.1136/gut.51.2.275]
4 **Verheij J**, Schouten JN, Komuta M, Nevens F, Hansen BE, Janssen HL, Roskams T. Histological features in western patients with idiopathic non-cirrhotic portal hypertension. *Histopathology* 2013; **62**: 1083-1091 [PMID: 23600724 DOI: 10.1111/his.12114]
5 **Gioia S**, Nardelli S, Pasquale C, Pentassuglio I, Nicoletti V, Aprile F, Merli M, Riggio O. Natural history of patients with non cirrhotic portal hypertension: Comparison with patients with compensated cirrhosis. *Dig Liver Dis* 2018; **50**: 839-844 [PMID: 29429910 DOI: 10.1016/j.dld.2018.01.132]
6 **Siramolpiwat S**, Seijo S, Miquel R, Berzigotti A, Garcia-Criado A, Darnell A, Turon F, Hernandez-Gea V, Bosch J, Garcia-Pagán JC. Idiopathic portal hypertension: natural history and long-term outcome. *Hepatology* 2014; **59**: 2276-2285 [PMID: 24155091 DOI: 10.1002/hep.26904]
7 **Valla DC**, Cazals-Hatem D. Vascular liver diseases on the clinical side: definitions and diagnosis, new concepts. *Virchows Arch* 2018; **473**: 3-13 [PMID: 29572606 DOI: 10.1007/s00428-018-2331-3]
8 **DeLeve LD**, Valla DC, Garcia-Tsao G; American Association for the Study Liver Diseases. Vascular disorders of the liver. *Hepatology* 2009; **49**: 1729-1764 [PMID: 19399912 DOI: 10.1002/hep.22772]
9 **Guido M**, Alves VAF, Balabaud C, Bathal PS, Bioulac-Sage P, Colombari R, Crawford JM, Dhillon AP, Ferrell LD, Gill RM, Hytiroglou P, Nakanuma Y, Paradis V, Quaglia A, Rautou PE, Theise ND, Thung S, Tsui WMS, Sempoux C, Snover D, van Leeuwen DJ; International Liver Pathology Study Group. Histology of portal vascular changes associated with idiopathic non-cirrhotic portal hypertension: nomenclature and definition. *Histopathology* 2019; **74**: 219-226 [PMID: 30129657 DOI: 10.1111/his.13738]
10 **Sato Y**, Nakanuma Y. Role of endothelial-mesenchymal transition in idiopathic portal hypertension. *Histol Histopathol* 2013; **28**: 145-154 [PMID: 23275299 DOI: 10.14670/HH-28.145]
11 **Vuppalanchi R**, Mathur K, Pyko M, Samala N, Chalasani N. Liver Stiffness Measurements in Patients with Noncirrhotic Portal Hypertension-The Devil Is in the Details. *Hepatology* 2018; **68**: 2438-2440 [PMID: 30014586 DOI: 10.1002/hep.30167]
12 **Gioia S**, Nardelli S, Riggio O. Letter to the Editor: Liver Stiffness in Noncirrhotic Portal Hypertension: The Devil Is in the Diagnosis. *Hepatology* 2019; **70**: 444-445 [PMID: 30506573 DOI: 10.1002/hep.30367]
13 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: Vascular diseases of the liver. *J Hepatol* 2016; **64**: 179-202 [PMID: 26516032 DOI: 10.1016/j.jhep.2015.07.040]

World Journal of Hepatology

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

*World J Hepatol* 2019 August 27; 11(8): 619-637

DOI: 10.4254/wjh.v11.i8.619

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

# Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice

Kandy T Velázquez, Reilly T Enos, Jackie E Bader, Alexander T Sougiannis, Meredith S Carson, Ioulia Chatzistamou, James A Carson, Prakash S Nagarkatti, Mitzi Nagarkatti, E Angela Murphy

**ORCID number:** Kandy T Velázquez (0000-0003-2427-165X); Reilly T Enos (0000-0001-5571-4586); Jackie E Bader (0000-0002-8383-7898); Alexander T Sougiannis (0000-0002-8512-7291); Meredith S Carson (0000-0002-2186-0195); Ioulia Chatzistamou (0000-0002-6632-8469); James A Carson (0000-0003-3733-8796); Prakash S Nagarkatti (0000-0003-2663-0759); Mitzi Nagarkatti (0000-0002-5977-5615); E Angela Murphy (0000-0002-4803-5822).

**Author contributions:** Velázquez KT performed the majority of experiments and analyzed the data; Chatzistamou I performed histopathological analysis; Velázquez KT performed staining; Velázquez KT, Enos RT, Carson MS, and Sougiannis AT performed molecular investigations; Velázquez KT, Bader JE, Nagarkatti PS, Nagarkatti M and Murphy EA designed and coordinated the microbiome research; Velázquez KT, Enos RT, Carson JA and Murphy EA designed and coordinated the obesity and non-alcoholic fatty liver disease research; Velázquez KT, Enos RT, and Murphy EA wrote the paper; all authors were involved in editing the manuscript.

**Supported by** National Institute of Health, No. NCI-3R01CA121249-08S1, NCCIH-K99AT009206 and No. NCI-1R21CA191966.

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the

**Kandy T Velázquez, Reilly T Enos, Jackie E Bader, Alexander T Sougiannis, Meredith S Carson, Ioulia Chatzistamou, James A Carson, Prakash S Nagarkatti, Mitzi Nagarkatti, E Angela Murphy,** Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, Columbia, SC 29209, United States

**James A Carson,** College of Health Professions, University of Tennessee Health Sciences Center, Memphis, TN 38163, United States

**Corresponding author:** E Angela Murphy, PhD, Associate Professor, Department of Pathology, Microbiology and Immunology, School of Medicine, University of South Carolina, 6439 Garners Ferry Rd, Columbia, SC 29209, United States. angela.murphy@uscmed.sc.edu
**Telephone:** +1-803-2163414
**Fax:** +1-803-2163414

## Abstract

### *BACKGROUND*

Non-alcoholic fatty liver disease (NAFLD) has become an epidemic largely due to the worldwide increase in obesity. While lifestyle modifications and pharmacotherapies have been used to alleviate NAFLD, successful treatment options are limited. One of the main barriers to finding safe and effective drugs for long-term use in NAFLD is the fast initiation and progression of disease in the available preclinical models. Therefore, we are in need of preclinical models that (1) mimic the human manifestation of NAFLD and (2) have a longer progression time to allow for the design of superior treatments.

### *AIM*

To characterize a model of prolonged high-fat diet (HFD) feeding for investigation of the long-term progression of NAFLD.

### *METHODS*

In this study, we utilized prolonged HFD feeding to examine NAFLD features in C57BL/6 male mice. We fed mice with a HFD (60% fat, 20% protein, and 20% carbohydrate) for 80 wk to promote obesity (Old-HFD group, $n$ = 18). A low-fat diet (LFD) (14% fat, 32% protein, and 54% carbohydrate) was administered for the same duration to age-matched mice (Old-LFD group, $n$ = 15). An additional group of mice was maintained on the LFD (Young-LFD, $n$ = 20) for a shorter duration (6 wk) to distinguish between age-dependent and age-independent effects. Liver, colon, adipose tissue, and feces were collected for histological and molecular assessments.

Institutional Animal Care and Use Committee at the University of South Carolina.

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

**Data sharing statement:** Data from this manuscript will be available upon request.

**ARRIVE guidelines statement:** The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.



**Manuscript source**: Unsolicited Manuscript

**Received:** March 7, 2019
**Peer-review started:** March 11, 2019
**First decision:** April 11, 2019
**Revised:** July 5, 2019
**Accepted:** July 16, 2019
**Article in press:** July 17, 2019
**Published online:** August 27, 2019

**P-Reviewer:** Bridle KR, Luo G
**S-Editor:** Cui LJ
**L-Editor:** A
**E-Editor:** Ma YJ

***RESULTS***

Prolonged HFD feeding led to obesity and insulin resistance. Histological analysis in the liver of HFD mice demonstrated steatosis, cell injury, portal and lobular inflammation and fibrosis. In addition, molecular analysis for markers of endoplasmic reticulum stress established that the liver tissue of HFD mice have increased phosphorylated Jnk and CHOP. Lastly, we evaluated the gut microbial composition of Old-LFD and Old-HFD. We observed that prolonged HFD feeding in mice increased the relative abundance of the *Firmicutes* phylum. At the genus level, we observed a significant increase in the abundance of *Adercreutzia, Coprococcus*, *Dorea*, and *Ruminococcus* and decreased relative abundance of *Turicibacter* and *Anaeroplasma* in HFD mice.

***CONCLUSION***

Overall, these data suggest that chronic HFD consumption in mice can mimic pathophysiological and some microbial events observed in NAFLD patients.

**Key words:** High-fat diet; Obesity; Non-alcoholic fatty liver disease; Gut microbiome; Endoplasmic reticulum stress; Inflammation; Fibrosis



**Core tip:** This work describes how mice consuming a chronic high-fat diet can mimic the clinical characteristics of non-alcoholic fatty liver disease. We used histopathological, metabolic, and molecular approaches to establish that prolonged high-fat-diet feedings in mice may be used as a pre-clinical model to study long-term interventions involving steatosis, steatohepatitis, fibrosis, glucose disturbances, endoplasmic reticulum stress, and gut microbial dysbiosis.

**Citation:** Velázquez KT, Enos RT, Bader JE, Sougiannis AT, Carson MS, Chatzistamou I, Carson JA, Nagarkatti PS, Nagarkatti M, Murphy EA. Prolonged high-fat-diet feeding promotes non-alcoholic fatty liver disease and alters gut microbiota in mice. *World J Hepatol* 2019; 11(8): 619-637
**URL**: https://www.wjgnet.com/1948-5182/full/v11/i8/619.htm
**DOI**: https://dx.doi.org/10.4254/wjh.v11.i8.619

## INTRODUCTION

Advanced stages of non-alcoholic fatty liver disease (NAFLD) can be classified as a form of chronic hepatitis. An estimated 25% of individuals are affected globally[1]. In the United States alone, approximately 95 million adults have NAFLD and the prevalence has continued to rise[2]. In fact, it is now considered the most common cause of liver disorder in the United States and other Western industrialized countries. NAFLD exists as a spectrum and is best categorized histologically. Characteristic features include steatosis, inflammation, hepatocellular ballooning, and fibrosis[3]. NAFLD can be further classified as non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)[3]. NAFL is a less severe form and is defined as the presence of steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes[3]. NASH, on the other hand, is characterized by the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis[3]. These characteristics of NAFLD can facilitate the risk of further disease progression[1]. For example, NAFLD has been linked to the progression and development of hepatocellular carcinoma (HCC), the major histological subtype of liver cancer[1]. Similarly, it has been associated with metabolic disorders as up to 75% of individuals with NAFLD have been reported to have type II diabetes[1]. Thus, understanding the risk factors for NAFLD is of critical public health importance.

Obesity is a well-characterized risk factor for the development of NAFLD. Although it is well known that obesity can be prevented through healthy dietary habits and physical activity[4,5], interventions in a clinical setting have largely been unsuccessful, especially in the long-term[6,7]. Thus, recent research has focused on understanding the pathways driving the pathologic processes associated with obesity-induced NAFLD so that therapeutic targets can be identified. Animal models are

critical to this mission and have greatly enhanced our understanding of NAFLD development. Although multiple animal models of NAFLD exist, high-fat-diet (HFD) administration has been a widely used model[8]. However, a limitation of this approach is that HFD administration to mice does not appear to induce significant NAFLD progression (*i.e.*, liver cell death, inflammation, or fibrosis) despite reproducibly inciting obesity, the metabolic syndrome, and hepatic steatosis[8]. A potential explanation for this phenomenon is that the duration of HFD feedings is not long enough to produce significant NASH even when diet treatment is administered for six months. Therefore, a longer duration of HFD administration may be necessary to recapitulate the pathology seen in the human condition.

The gut microbiome has recently emerged as a culprit in the development of chronic diseases, such as, obesity[9,10], diabetes[11,12], liver disease[13,14], and cancer[15]. In the case of NAFLD, the location of the portal vein allows easy access for bacteria and microbial-derived products to translocate from the gut to the liver[16]. In support of this hypothesis, studies conducted in obese humans with NAFLD revealed gut dysbiosis when compared to healthy humans[17,18]. Raman *et al*[17] reported an enrichment of *Lactobacillus* species and various microbes from the *Firmicutes* phylum in obese humans with NAFLD. Meanwhile, Wong *et al*[18], observed that NASH patients had lower fecal abundance of *Firmicutes*. Although studies examining the link between human gut microbiota and liver diseases have advanced our understanding of this relationship, preclinical models mimicking gut dysbiosis in NAFLD are still lacking[19].

We sought to examine the effects of chronic HFD feeding on NAFLD in mice. *C57BL/6* mice were used given their susceptibility to HFD-induced obesity. We utilized a diet consisting of 60% fat, 20% protein, and 20% carbohydrate, which was fed to the mice for a period of 80 wk-a protocol designed to mimic lifetime consumption of a diet high in fat. Our analysis focused largely on liver pathology, fibrosis, inflammation, and endoplasmic reticulum (ER) stress. We also measured metabolic outcomes and characterized fecal microbiota using 16S rRNA sequencing. Our data indicate that chronic HFD consumption does result in significant NAFLD and gut-bacterial dysbiosis. Specifically, we report a significant increase in steatosis, inflammation, cell injury, fibrosis, and ER stress, which was associated with increases in the *Actinobacteria* and *Firmicutes* phylum and decreases in the *Bacteriodetes* and *Tenericutes* phylum.

## MATERIALS AND METHODS

### Animals and diet

All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee at the University of South Carolina (animal protocol number, 2044-100482-093011). A total of 53 *C57BL/6* male mice were obtained from Jackson Laboratories. Mice (3-5 per cage, wood bedding with nesting material) were kept in a 12:12 h dark/light cycle, in a humidity and temperature control room, and *ad libitum* access to food and water. Animal handling and experiments were performed to minimize pain and discomfort. At 10 wk of age, male mice were randomly assigned to one of two diets for the duration of 80 wk: Low-fat diet (old-LFD, $n$ = 15) from Harlan Teklad Rodent Diet, no 8604 (14% Fat, 54% carbohydrate, 32% protein) or High-Fat Diet (Old-HFD, $n$ = 18) from Research Diets, D12492 (60% Fat, 20% carbohydrate, 20% protein). An additional group of male mice (10 wk of age) was maintained on the LFD (Young-LFD, $n$ = 20) but for a shorter duration (6 wk) to distinguish between age-dependent and age-independent effects of obesity on metabolic, molecular, and histological measures. All three groups of mice were euthanized (overdose of isoflurane) the same day, and fat depots (epididymal, retro-peritoneal, and mesentery), liver, and spleen were removed and weighed. The livers were collected and stored at -80 °C or fixed in 4% paraformaldehyde for further analysis.

### Metabolic measurements and assays

A day prior to euthanasia, ten mice per each group were fasted for five hours (light cycle). Blood collection was carried out in conscious animals, the tip of the tail was cut with scissors and heparinized capillary tubes (0.12 cm diameter, 7.5 cm length) were used to collect 70 μL of blood from the tail vein for the measurement of glucose and insulin. Blood glucose was assessed using a glucometer (Bayer Counter, New Jersey, United States) and plasma insulin was determined using a mouse ELISA assay from Mercodia (Uppsala, Sweden). Insulin resistance was calculated by HOMA index using the following equation IR = (insulin uU/mL) (glucose mmol/L) /22.5.

### Staining

Liver, colon, and adipose tissue were fixed in 4% paraformaldehyde, paraffin-embedded, sectioned, and then stained with hematoxylin eosin (HE). Picro-sirius red stain kit (Cat ab150681, abcam, Cambridge, MA, United States) was used according to the manufacturer's instructions to stain the liver for histological evaluation of fibrosis. For Oil red O staining, frozen liver tissues were cut (10 μm) using a cryostat (Leica Biosystems, Nussloch, Germany) and staining was performed as previously described[20].

### Histopathology

Histological scoring system for NAFLD was achieved based on HE and picro-sirius red staining in the liver of Young-LFD ($n$ = 6), Old-LFD ($n$ = 10), and Old-HFD ($n$ = 12) mice as previously described[21]. A certified pathologist (I.C.) blindly evaluated the histological findings of steatosis (0-3), portal and lobular inflammation (0-3), cell injury (0-2), and fibrosis (0-4) in the liver sections. NAFLD Activity Score (NAS) was calculated by adding the unweighted scores for steatosis, lobular inflammation, cell injury (0-8).

### Western blotting

Briefly, liver was homogenized in Mueller Buffer containing a protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO, United States). Total protein concentrations were determined by the Bradford method. Equal amounts of crude protein homogenates (20 μg) were fractioned on hand-casted SDS-polyacrylamide gels and electrophoretically transferred to a PVDF membrane using a Royal Genie Blotter (IDEA Scientific, Minneapolis, MN, United States). Membranes were stained with a Ponceau S solution in order to verify equal protein loading and transfer efficiency. Western blots were performed using primary antibodies from Cell Signaling (Danvers, MA, United States) (phosphorylated-IRE1α, IRE1α, XBP1, phosphorylated-EIF2α, EIF2α, phosphorylated-Jnk, Jnk, CHOP, β-actin, phosphorylated-NFκB, NFκB), AbD Serotec Raleigh, NC (F4/80), and Novus Biologicals Littleton, CO (phosphorylated-IRE1α).

### Quantitative real-time PCR

Gene expression in liver and colon tissue was performed in duplicate after RNA isolation with trizol reagent. The following Taqman gene expression assays from Applied Biosystems were used: Monocyte chemoattractant protein-1 (MCP-1), interleukin 10 (IL-10), interleukin 17-alpha (IL-17α), interleukin 6 (IL-6), forkhead box P3 (Foxp3), or tumor necrosis factor alpha (TNF-α). All primers were normalized to 18s rRNA.

### Microbiome analysis

Before sacrifice, mice were individually placed in autoclaved cages with no bedding for the collection of three or more fecal pellets per mouse. Fecal pellets were frozen immediately after collection and stored at -80 °C. Isolation and concentration of microbial DNA was achieved using the QIAamp Fast DNA Stool Mini Kit (cat number: 51604, QIAGEN) and a nanophotometer Pearl, respectively. Characterization of the fecal microbiota via 16S rRNA sequencing was performed via the amplification of the 16S rRNA V3 and V4 hypervariable regions. We used 16S V3 314F forward and V4 805R reverse primers, respectively (5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG3′&5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG GACTACHVGG GTATCTAATCC3′) with added Ilumina adapter overhang nucleotide sequences[22]. The PCR conditions used were 3 min at 95 °C followed by 25 cycles of 30 s at 95 °C, 30 sat 55 °C, 30 s at 72 °C, and a final extension at 72°C for 5 min. Each reaction mixture (25 μL) contained 50 ng of genomic DNA, 0.5 μL of amplicon PCR forward primer (0.2 μmol/L), 0.5 μl of amplicon PCR reverse primer (0.2 μmol/L) and 12.5 μL of 2× KAPA Hifi Hot Start Ready Mix. Each reaction was cleaned up using Agencourt AMPure XP beads. Attachment of dual indices and Illumina sequencing adapters was performed using 5 μL of amplicon PCR product DNA, 5 μL of Illumina Nextera XT Index Primer 1 (N7xx), 5 μL of Nextera XT Index Primer 2 (S5xx), 25 μL of 2× KAPA HiFi Hot Start Ready Mix, and 10 μL of PCR-grade water. Amplification was carried out under the following conditions: 3 min at 95 °C, followed by 8 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C, and a final extension at 72 °C for 5 min. Constructed 16S metagenomic libraries were purified with Agencourt AM Pure XP beads and quantified with Quant-iTPicoGreen. Library quality control and average size distribution were determined using an Agilent Technologies 2100 Bioanalyzer. Libraries were normalized and pooled to 40 nmol/L based on quantified values. Pooled samples were denatured and diluted to a final concentration of 6 pmol/L with a 30% PhiX (Illumina) control. Amplicons were

subjected to pyrosequencing using the MiSeq Reagent Kit V3 in the Illumina MiSeq System. The online 16S analysis software from NIH was used to analyze sequencing data collected on the Illumina Miseq. FASTQ sequences were uploaded to Nephele and the 16S metagenomics application was executed. The groups of related DNA sequences were assigned to operational taxonomic units (OTUs), and output files were analyzed to determine gut microbial composition.

### Statistical analysis

Data from this experiment were analyzed using commercially available statistical software, Prism 5 (GraphPad Software). The statistician, Dr. Bo Cai, from the Department Epidemiology and Biostatistics at the University of South Carolina reviewed the statistical methods of this study. Morphometric measurements, metabolic assays, western blot analysis, and gene expression results were analyzed using a one-way ANOVA followed by Newman-Keuls post hoc test. Kruskal-Wallis test followed by a Dunn's Post-Hoc was used to assess differences in histopathology. A two-tailed Student's t-test and a Mann-Whitney *U* test were used to determine differences in microbiota phylum, family, and genus between Old-LFD and Old-HFD mice. Data that did not pass Barlett's test for equal variances was log-transformed and then re-analyzed. Data are presented as the mean ± SE or median with interquartile range. The level of significance was set at $P < 0.05$.

## RESULTS

### Morphometric and metabolic analysis of diet-induced NAFLD

To evaluate whether prolonged HFD feeding leads to morphometric and metabolic changes, we determined the mass of three visceral fat depots (epididymal, kidney, and mesentery) and evaluated basal glucose metabolism. As expected, 80 wk of HFD feedings augmented body weight (Figure 1A) in Old-HFD mice when compared to Young-LFD and Old-LFD mice ($P < 0.05$). This increase in body weight was in part due to the significant expansion of epididymal (Figure 1B), kidney (Figure 1C), and mesentery (Figure 1D) fat pads ($P < 0.05$) as well as liver (Figure 1E) and spleen (Figure 1F) tissues. In addition, Old-LFD mice showed a higher body weight and epididymal fat accumulation than Young-LFD mice. Figure 1G, shows a representative histological image of epididymal fat tissue stained with HE; we can observe larger adipocytes surrounded by infiltrated immune cell in Old-HFD mice compared to Old-LFD and Young-LFD mice.

We next addressed whether Old-HFD mice were insulin resistant. As anticipated, Old-HFD mice showed significantly elevated fasting blood glucose (Figure 2A) and insulin (Figure 2B) when compared to Young-LFD and Old-LFD mice ($P < 0.05$). These results were consistent with the HOMA index in which Old-HFD mice exhibited insulin resistance relative to Young-LFD and Old-LFD mice ($P < 0.05$) (Figure 2C). No differences in glucose, insulin, and HOMA index were observed between Old-LFD and Young-LFD mice even though Old-LFD mice displayed an increase in body weight and epididymal fat mass.

### Histopathological assessment of diet-induced NAFLD

In order to evaluate whether prolonged HFD promotes NAFLD, we performed HE, picro-sirius red, and Oil Red O stains in the liver tissue of Young-LFD, Old-LFD, and Old-HFD mice (Figure 3A-C). The analysis of the specimens showed that livers of the Young-LFD mice had minimal focal inflammation, minimal perisinusoidal fibrosis, and no signs of steatosis (NAS score 0-1) (Figure 3D-J). Old-LFD mice displayed mild focal inflammation with focal steatosis, and perisinusoidal fibrosis (NAS score 1-2). In the case of Old-HFD mice, we observed extensive steatosis, portal and lobular inflammation with cell injury (ballooning degeneration of the hepatocytes), and evident fibrosis (NAS score 5-6). We next confirmed fat accumulation in the liver with Oil Red O staining (Figure 3C). Overall, Oil Red O staining provided evidence of macrovesicular accumulation of triglycerides in the hepatocytes of Old-HFD mice when compared to Young-LFD and Old-LFD groups.

### Examination of inflammatory markers in the liver tissue of mice following prolonged HFD feedings

Since inflammation is one of the hallmarks of obesity and NAFLD, we next addressed the question of whether prolonged HFD consumption increases liver inflammation. To assess this, we examined gene expression of MCP-1, IL-6, and TNF-α and the protein concentration of F4/80 and p-NFκB (Figure 4A-E). MCP-1 was significantly elevated in Old-HFD and Old-LFD mice when compared to Young-LFD mice ($P <$

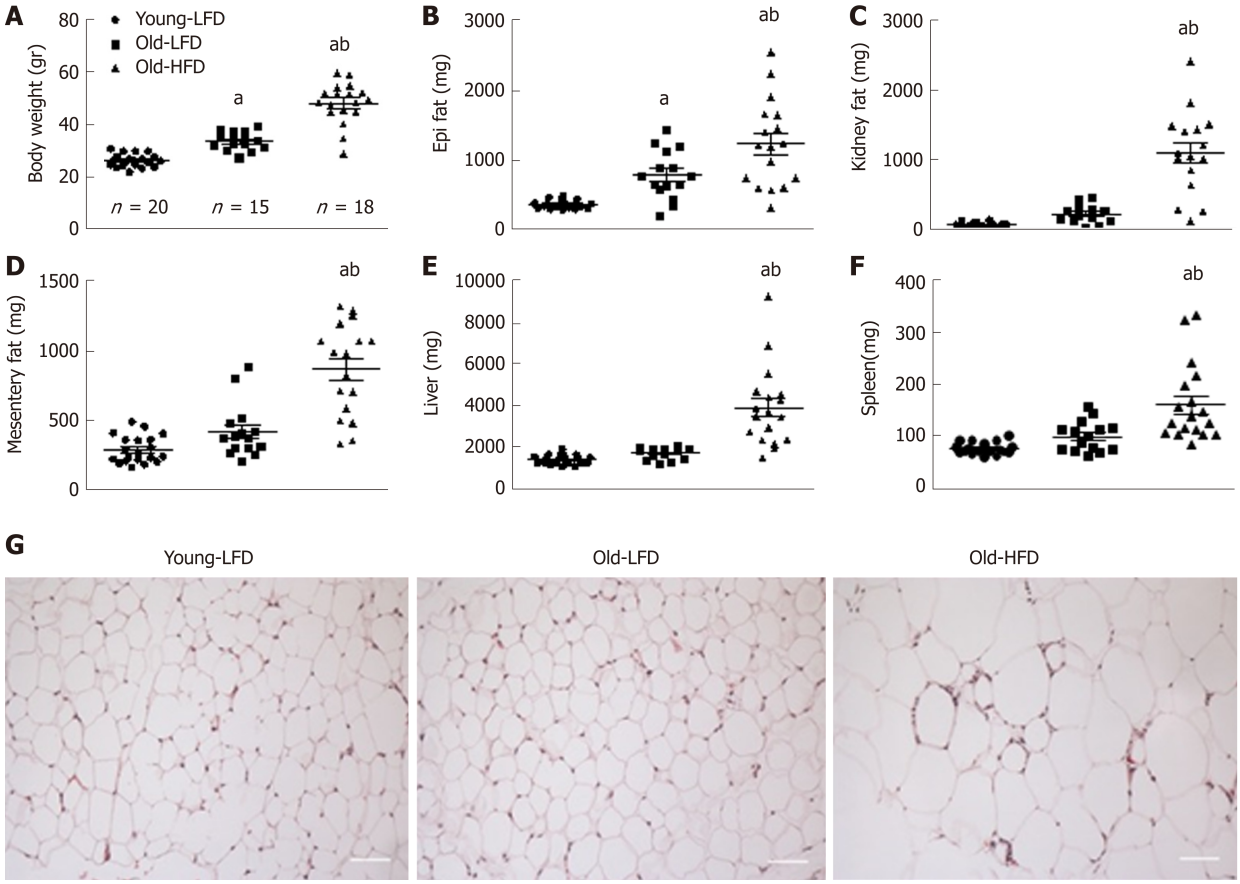


**Figure 1 Morphometric characterization of mice subjected to prolonged high-fat diet-feeding.** A: Average body weight; B: Average mass of epididymal fat pad; C: Kidney fat pad; D: Mesenteric fat pad; E: Liver tissue; F: Spleen tissue. G: Representative hematoxylin and eosin images of epididymal adipose tissue (200 X). Data are expressed as mean ± SE. *n* = 15-20 mice per group. [a]Significantly different from Young-LFD ($P < 0.05$). [b] Significantly different from Old-LFD ($P < 0.05$). HFD: High-fat diet-feeding; LFD: Low-fat diet.

0.05). Interestingly, F4/80 was significantly decreased with age with a further decrease observed with HFD feeding ($P < 0.05$). No significant changes were observed in the gene expression of IL-6 and TNF-α or the protein expression of p-NFκB.

### *Effects of prolonged HFD feeding on* ER *stress in the liver*

Previous studies have reported that ER stress is involved in the development and progression of steatosis[23-25]. Therefore, we next investigated the signaling pathways activated by ER stress, such as, binding immunoglobulin (Bip), inositol-requiring enzyme-1 (IRE1α), X-box-binding protein-1 (XBP1s), eukaryotic translation initiation factor 2α (EIF2α), c-Jun-N-terminal kinase (Jnk), and C/EBP-homologous protein (CHOP). There was no change in Bip across the groups (Figure 5A). However, both of the aged groups showed a significant reduction in phosphorylated IRE1α (Figure 5B) and XBP1s (Figure 5C) when compared to the Young-LFD mice ($P < 0.05$) and there was a further reduction in pIRE1α with HFD feeding (Old-LFD versus Old-HFD) ($P < 0.05$). For phosphorylated EIF2α protein expression, there was an elevation in the Old-LFD group compared to Young-LFD and Old-HFD (Figure 5D) ($P < 0.05$). Phosphorylated Jnk was increased in both of the aged groups and there was a further increase with HFD feeding (Figure 5E) ($P < 0.05$). Only the Old-HFD group exhibited an increase in CHOP when compared to Old-LFD and Young-LFD (Figure 5F) ($P < 0.05$).

### *Effects of prolonged HFD feeding on colon inflammation and the gut microbiome profile*

To examine whether prolonged HFD feeding produced an inflammatory environment in the colon and/or disrupted the gut microbiome, we analyzed gene expression of inflammatory markers in the colonic tissue and fecal microbial DNA using pyrosequencing. In this study, we did not assess the bacterial composition of the Young-LFD mice, because short-term manipulation of diet is transient[26]. A significant increase in IL-6 was observed with age and there was a further increase with HFD feedings (Figure 6C) ($P < 0.05$). Foxp3 was increased in Old-HFD mice when

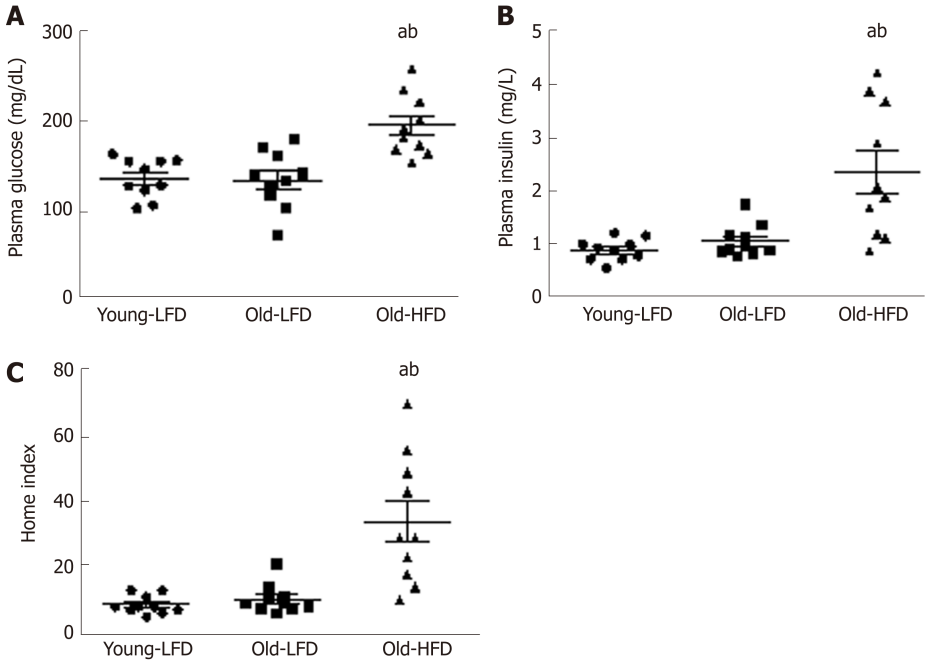


**Figure 2 Metabolic characterization of mice subjected to prolonged high-fat diet-feeding.** A: Five hours fasting blood glucose; B: Five hours fasting plasma insulin; C: Homeostasis model of assessment-insulin resistance index. Data are expressed as mean ± SE. *n* = 10 mice per group. [a]Significantly different from Young-LFD ($P < 0.05$). [b]Significantly different from Old-LFD ($P < 0.05$). HOMA-IR: Homeostasis model of assessment-insulin resistance; LFD: Low-fat diet.

compared to Young-LFD and Old-LFD mice ($P < 0.05$) (Figure 6C and E). However, we found no changes in MCP-1, IL-10, or IL-17α (Figure 6AB, and D) between any of the groups. Representative histological images of the colon tissue in Figure 6F shows similar colonic morphology between groups.

In Figure 7, we observed similar species richness at the taxonomic level (Figure 7A). However, principal coordinate (PC) analysis based on OTU showed distinct gut microbiota composition between Old-LFD and Old-HFD mice (Figure 7B). *Firmicutes* and *Bacteriodetes* were the most predominant phyla in both groups, comprising of 61% and 32% of gut microbiota in Old-LFD mice and 73% and 21% in Old-HFD mice, respectively (Figure 8A and B). Consistently, the ratio of *Firmicutes* to *Bacteriodetes* in Old-HFD mice was altered to favor *Firmicutes* when compared with Old-LFD (Figure 8C, $P < 0.05$). The next two prevalent phylum included *Tenericutes* and *Verrucumicrobia* with 5% and 0.2% of microbiomes in Old-LFD mice and 0.8% and 3.4% in Old-HFD mice, respectively. Prolonged HFD feeding significantly increased the abundance of *Actinobacteria* and *Firmicutes* and decreased the abundance of *Bacteriodetes* and *Tenericutes* when compared to Old-LFD mice ($P < 0.05$). In figure 8D, a positive correlation was observed between body weight and *Firmicutes* abundance ($P < 0.05$), while a negative correlation was observed between body weight and *Bacteriodetes* abundance ($P < 0.05$). At the genus level (Figure 9), we observed a significant increase in the abundance of *Adercreutzia* (Phylum-*Actinobacteria*), *Coprococcus* (Phylum-*Firmicutes*), *Dorea* (Phylum-*Firmicutes*), and *Ruminococcus* (Phylum-*Firmicutes*) in Old-HFD mice when compared to Old-LFD mice ($P < 0.05$). Further, Old-HFD mice showed a decrease in the abundance of *Turicibacter* (Phylum-*Firmicutes*) and *Anaeroplasma* (Phylum-*Tenericutes*) ($P < 0.05$).

## DISCUSSION

In humans, chronic HFD consumption is associated with systemic inflammation, obesity, metabolic dysfunction, NAFLD, and an altered gut microbial profile[27-29]. However, animal studies using standard HFD paradigms (16 wk feeding) which mimic obesity, metabolic disorders, and gut microbiota disruption have failed to produce consistent NAFLD pathology[8,30] limiting our advancement of mechanistic insights and the development of therapeutic strategies. Therefore, the present study utilized a prolonged HFD feeding (80 wk) to examine NAFLD evolvement. Given that the progression of steatosis to NASH has been linked with changes in inflammation, ER stress[23], and fecal bacterial composition[17], we sought to examine these outcomes in our pre-clinical diet-induced obesity model. In general, we found that prolonged

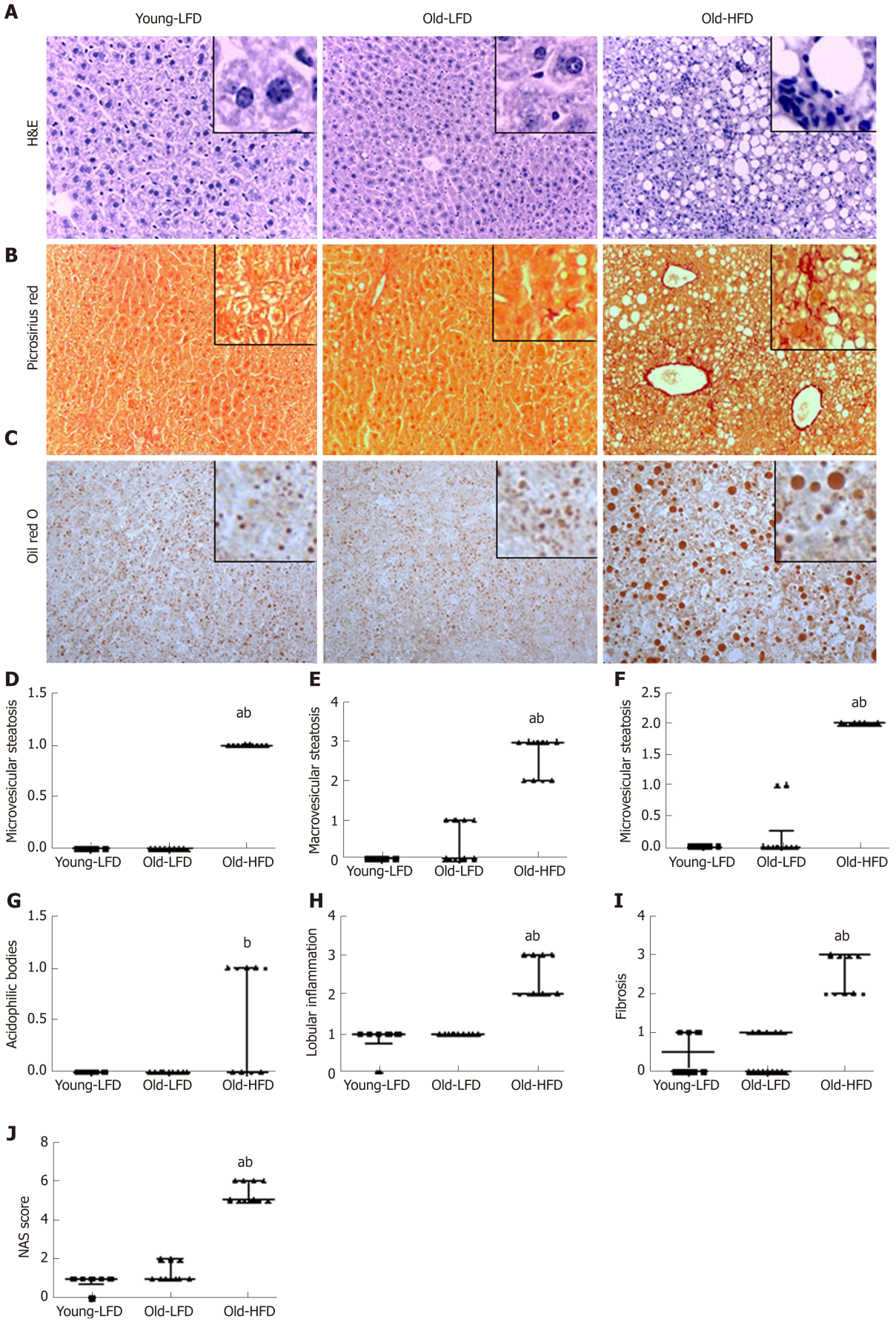


**Figure 3 Histological assessment of the liver tissue in mice subjected to prolonged high-fat diet-feeding.** A: Hematoxylin and eosin; B: Picrossirious red; C: Oil red O images of liver tissue (200 X); D: Histological score of microvesicular steatosis; E: Microvesicular steatosis; F: Ballooing degeneration of hepatocytes; G: Acidophilic bodies; H: Lobular inflammation; I: Fibrosis; J: Non-alcoholic steatohepatitis score. Data are expressed as median with interquartile range. $n$ = 6-11 mice per group. [a]Significantly different from Young-LFD ($P < 0.05$), [b]Significantly different from Old-LFD ($P < 0.05$). LFD: Low-fat diet.

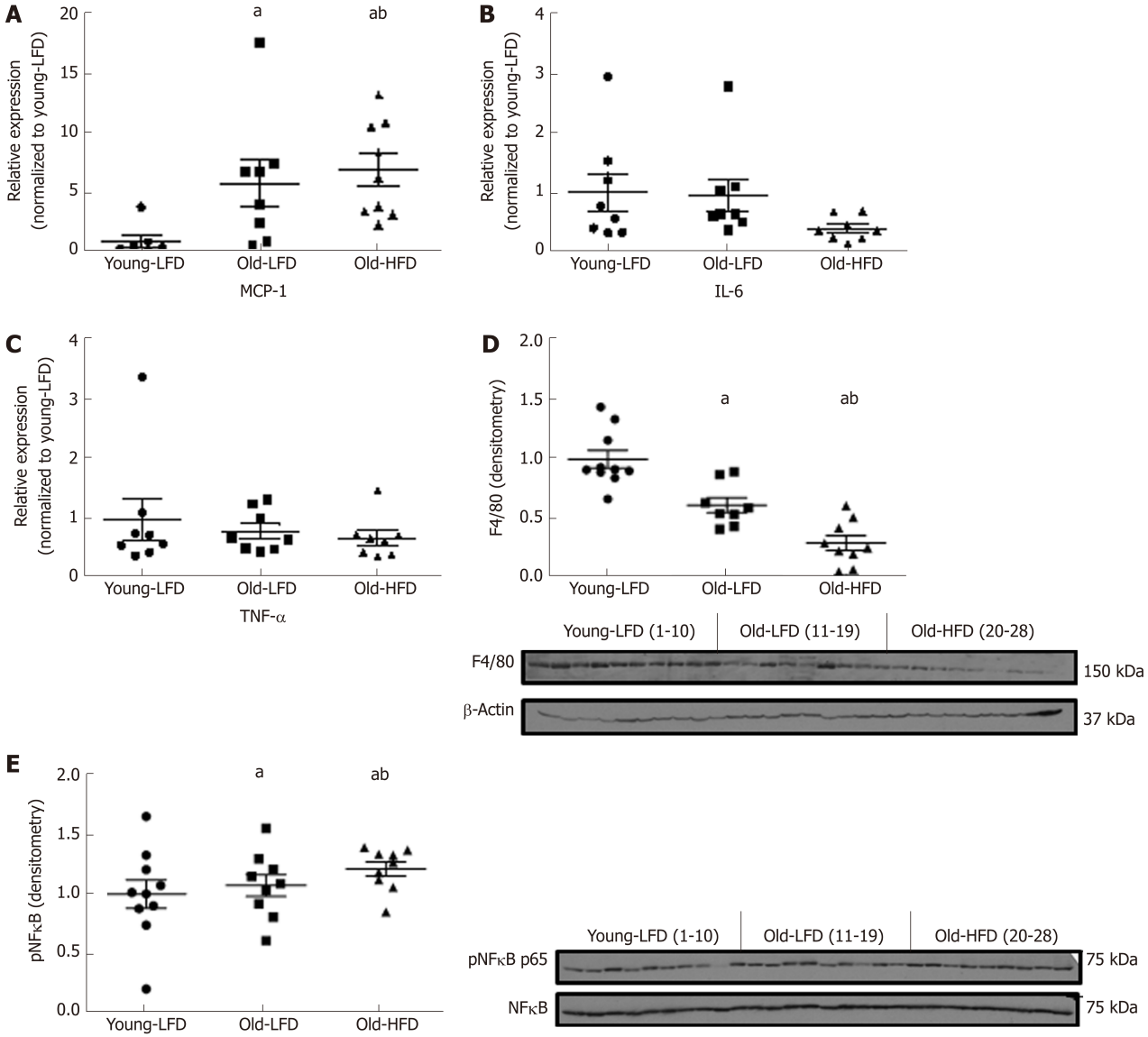


**Figure 4 Inflammatory signaling in the liver tissue of mice subjected to prolonged high-fat diet-feeding.** A: Gene expression of monocyte chemoattractant protein-1; B: Gene expression of interleukin; C: Gene expression of tumor necrosis factor alpha; D: Protein concentration of epidermal-gowth factor like-like module-containing mucin-like hormone receptor-like 1 also known as F4/80; E: Phosphorylated and total nuclear factor kappa-light-chain-enhancer of activated B cells. Data are expressed as mean ± SEM. $n$ = 8-10 mice per group. [a] Significantly different from Young-LFD ($P < 0.05$). [b] Significantly different from Old-LFD ($P < 0.05$). MCP-1: Monocyte chemoattractant protein-1; IL-6: Interleukin 6; TNFα: Tumor necrosis factor alpha; NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells; LFD: Low-fat diet.

HFD feeding promotes obesity, insulin resistance, ER stress, alterations in gut bacterial composition, and NAFLD in male C57BL/6 mice.

In order to determine if lifetime consumption of a HFD can reliably reproduce NAFLD, we fed mice for a period of 80 wk with a 60% (total kcals) fat diet. NAFLD with 14% kcal from fat and an equal amount of choline as the HFD was used to compare disease outcomes independent of choline deficiency. We controlled for choline given that choline deficient diets have been widely used to induce NAFLD/NASH in rodents[31,32], despite the fact that these diets do not show physiological characteristics involved in the progression of NAFLD/NASH[33]. Consistent with our previous studies[21,34-36], we report that HFD consumption increased morphometric parameters (body weight, liver mass, fat pads, and spleen mass) and fasting blood glucose and insulin. Histopathological analysis of the liver indicated that prolonged HFD feeding produced extensive steatosis with portal and lobular inflammation and parenchymal fibrosis in all mice in the Old-HFD group (NAS ≥ 5). Similar to our findings, others have reported metabolic disturbances and NAFLD pathology using an extended period of HFD feedings[37,38]. For example, VanSaun *et al*[37] reported pericellular fibrosis, advanced stage of fibrosis, and NASH after 36, 56, and 80 wk of HFD, respectively. In addition, they observed non-invasive dysplastic tumors in seven of eight mice fed HFD for 36-80 wk. Interestingly, we also

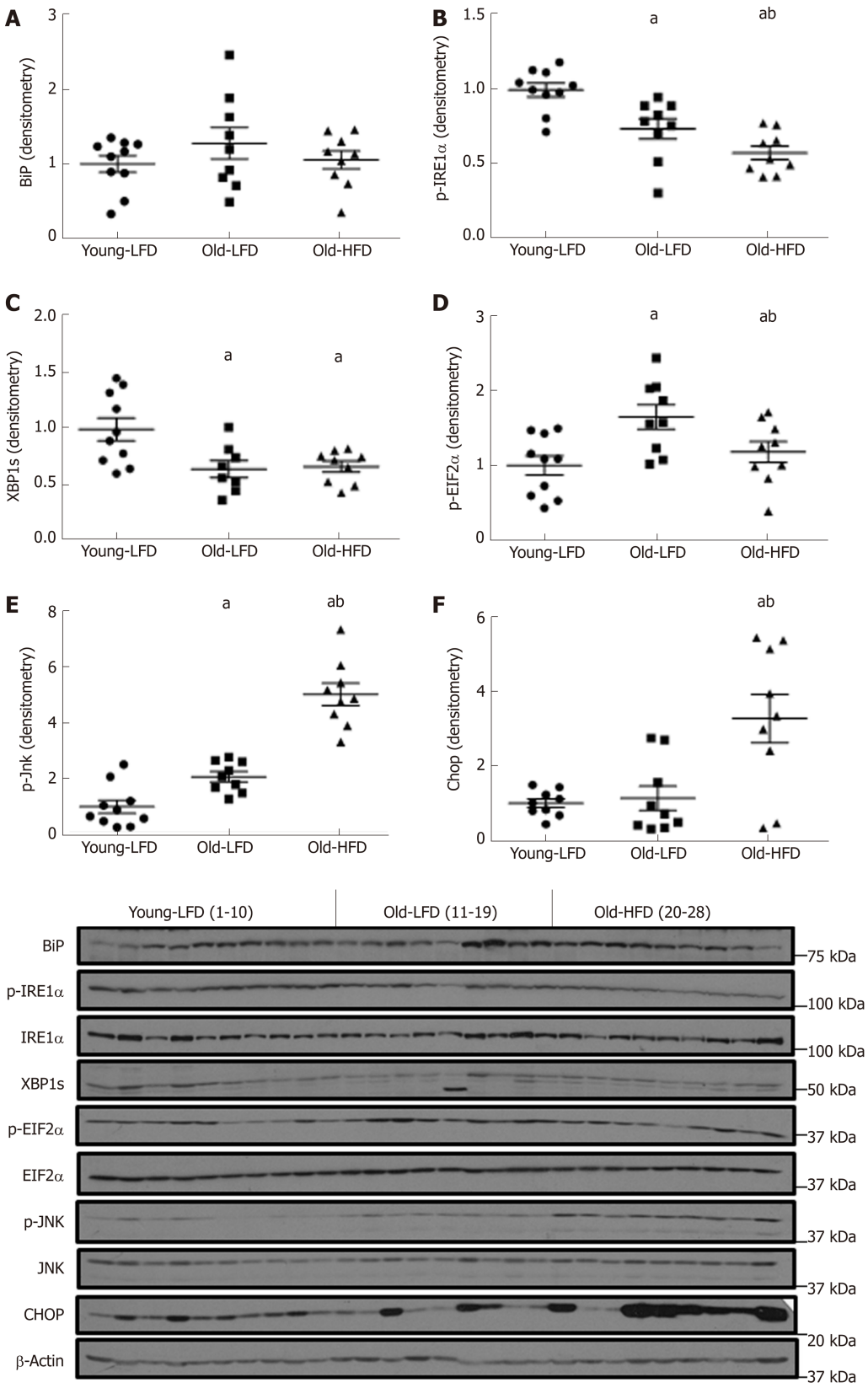


**Figure 5 Endoplasmic reticulum stress signaling in the liver tissue of mice subjected to prolonged high-fat diet-feeding.** A: Western blot densitometry analysis of binding immunoglobulin; B: Western blot densitometry analysis of phosphorylated inositol-requiring enzyme-1; C: Western blot densitometry analysis of X-box-binding protein-1; D: Western blot densitometry analysis of phosphorylated eukaryotic translation initiation factor 2α; E: Western blot densitometry analysis of phosphorylated c-Jun-N-terminal kinase; F: Western blot densitometry analysis of C/EBP-homologous protein. Data are expressed as mean ± SE. *n* = 9-10 mice per group. [a]Significantly different from Young-LFD ($P < 0.05$). [b]Significantly different from Old-LFD ($P < 0.05$). Bip: Binding immunoglobulin; IRE1α: Inositol-requiring enzyme-1; XBP1s: X-box-binding protein-1; EIF2α: Eukaryotic translation initiation factor 2α; Jnk: Jun-N-terminal kinas; CHOP: C/EBP-homologous protein; LFD: Low-fat diet.

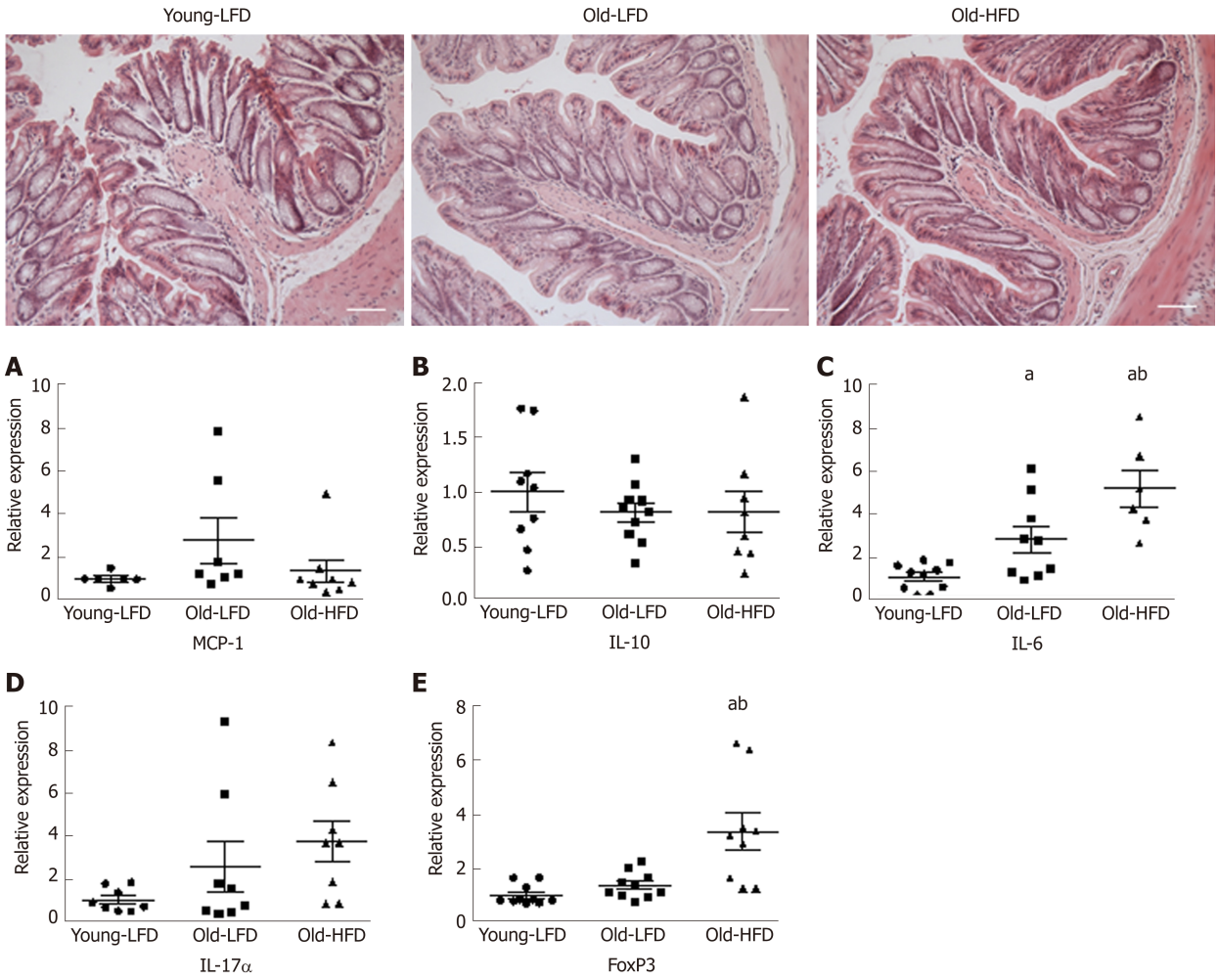


**Figure 6 Inflammatory signaling in the colon tissue of mice subjected to prolonged high-fat diet-feeding.** Representative histology images of colonic tissue (200 X) from Young-low-fat diet (LFD), Old-LFD, and Old-HFD mice. A: Gene expression of Monocyte chemoattractant protein-1 (MCP-1); B: Interleukin 10 (IL-10); C: Interleukin 6 (IL-6); D: Interleukin 17 (IL-17); E: Forkhead box P3 (FoxP3). Data are expressed as mean ± SE. $n$ = 10 mice per group. [a] Significantly different from Young-LFD ($P < 0.05$). [b] Significantly different from Old-LFD ($P < 0.05$). HFD: High-fat diet-feeding; LFD: Low-fat diet.

observed non-invasive focal masses in the liver of Old-HFD mice. Young-LFD and Old-LFD mice had an average NAS score of 1, which is not considered diagnostic of steatohepatitis.

Other HFD feeding regimes have been used to mimic the NAFLD condition. However, variability in the degree of NAFLD pathology has been a limitation of these models. For example, the use of the Amylin liver NASH model (AMLN) diet, which is based on a high content of fructose, cholesterol, and trans-fat (partially hydrogenated vegetable oil), has been shown to induce NAFLD stage heterogeneity in mice when the duration of feeding is between 12-30 wk[39-41]. However, one study reported that only twenty percent of mice develop liver fibrosis when fed an AMLN diet for a duration of 12 wk[40]. Another study reported that key hallmarks of NASH (macrovesicular steatosis and high levels of aspartate aminotransferase) are detected as early as 26 wk after AMLN diet consumption in $Lep^{ob/ob}$ and C57BL6 mice[41]. Nonetheless, not all C57BL/6 mice developed NASH when mice were maintained on an AMLN diet for 30 wk[39]. Thus, prolonging the duration of HFD consumption, as in the current study, may be enough to promote a more homogeneous pathology of NAFLD.

The role of pro-inflammatory cytokines in obesity and NAFLD remains unclear. Human studies investigating the action of plasma TNF-α has shown positive or no correlation with insulin resistance in NAFLD and obese patients[42,43]. In the case of IL-6, a more clear association has been stipulated between elevated plasma IL-6, insulin resistance, and NAFLD progression[44,45]. However, there is no clinical evidence that blocking IL-6 may serve as a treatment for NAFLD and/or metabolic diseases. Additionally, elevated levels of the chemokine MCP-1 also has been observed in NASH and NAFLD patients, which has been correlated with liver fat content[46-48]. Our current findings show no differences in the gene expression of TNFα and IL-6 in

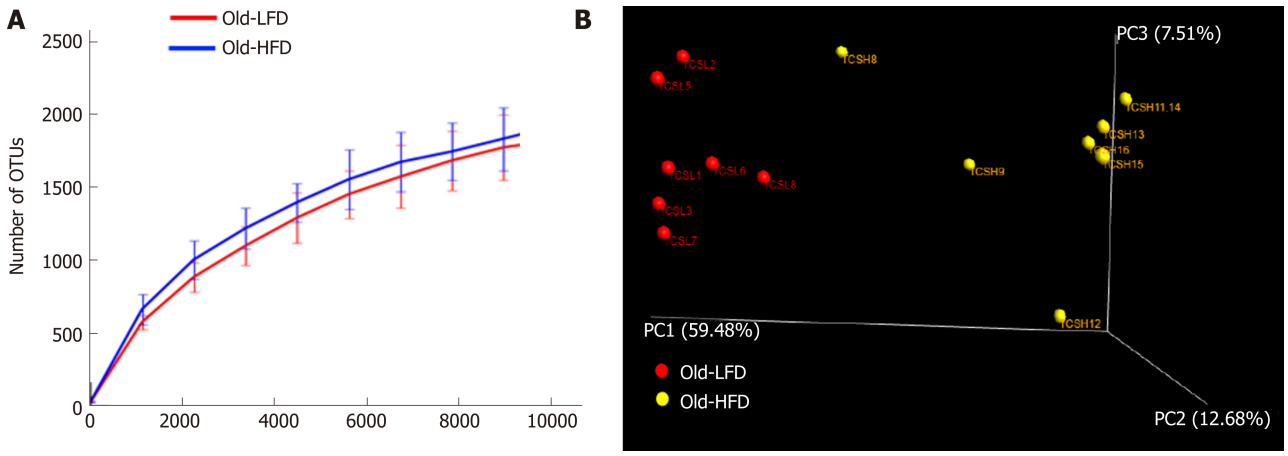


**Figure 7 Gut microbial composition of mice subjected to prolonged high-fat diet-feeding.** A: Chao1 and sequence per sample; B: Principal coordinates analysis (PCoA). *n* = 7 mice per group. Only the feces of Old-high-fat diet-feeding and Old-high-fat diet-feeding mice were analyzed. Data are expressed as mean ± SE. *n* = 7 per group. HFD: High-fat diet-feeding; LFD: Low-fat diet.

mouse liver with NASH pathology. These data coincide with no changes in protein expression of NFκB, a transcription factor involved in inflammatory signaling pathways. Contrary to our hypothesis, a reduction in F4/80 protein expression, a pan macrophage and Kupffer cells marker, was observed in aged mice. This decrease in F4/80 may be a result of aging, as macrophage immune function declines in senescence[49,50]. Old-HFD mice showed an even more significant drop in F4/80 than Old-LFD mice, which may be an indication of a depressed immune system related to NAFLD. Interestingly, the gene expression of MCP-1, a major recruiter of macrophages was increased in the liver of both Old-LFD and Old-HFD mice. Given that there was no difference between Old-HFD and Old-LFD groups we believe that this effect is due to aging and not obesity nor NAFLD and likely a compensatory response to the decrease in F4/80 seen in aged mice. It is important to mention that even though we did not find significant changes in the gene expression of TNF-α and IL-6, we did observe significant lobular inflammation in the liver of Old-HFD mice, histologically.

ER stress is associated with obesity, insulin resistance, and NAFLD[23-25,51]. It is believed that ER stress can lead to hepatic steatosis by altering lipid metabolism[52]. The ER stress response is regulated by three transmembrane proteins: ATF6, IRE1α, and PKR-like ER kinase (PERK), the molecular chaperone, BiP, and their downstream signaling cascades, including EIF2α (PERK pathway) and XBP1 activation (IRE1α pathway), which attempt to bring homeostasis back to the cell under stressful conditions. However, if this is not achieved, chronic ER stress ensues eventually leading to cell death via Jnk and CHOP signaling[53] and downregulation of the IRE1α -XBP1 axis[54]. A rapid response to acute ER stress is a significant increase in the production of molecular chaperones, such as BiP[54]. We found no differences in the protein expression across groups with respect to BiP; however, we did find a significant decrease in p-IRE1α and XBP1s in both of the Old groups suggesting that the livers of these mice were experiencing a certain degree of chronic ER stress. Furthermore, the fact that the Old-HFD mice displayed increases in both p-JNK and CHOP protein expression compared to all other groups provides evidence that the chronic consumption of the HFD led to a more advanced stage of chronic ER stress than the Old LFD-treated mice. This is corroborated by the significant increase in p-EIF2α displayed by the Old-LFD mice and not the Old-HFD mice, as activation of EIF2α, which inhibits protein translation, typically occurs during the earlier stages of chronic ER stress[55]. In fact, Choi *et al*[56] demonstrated that inhibition of EIF2α phosphorylation exacerbates macro-vesicular steatosis, leukocyte infiltration, and fibrosis in mice. Thus, the increased EIF2α phosphorylation displayed by the Old-LFD mice may be a protective mechanism against the more advance stages of NAFLD as displayed by the Old-HFD mice.

We also sought to investigate colonic inflammatory cytokines involved in the pathology of colitis due to the co-existence of NAFLD with inflammatory bowel disease (IBD)[57-61]. We observed an increase in IL-6 and FoxP3 in the colon tissue of Old-HFD mice, but no changes were observed in MCP-1, IL-10, and IL-17α between groups. Our findings of an increase in IL-6 is consistent with Jiang *et al*[62], that reported an increase in IL-6 mRNA expression in the intestinal mucosa of NAFLD patients when compared to healthy subjects. Since systemic inflammation including

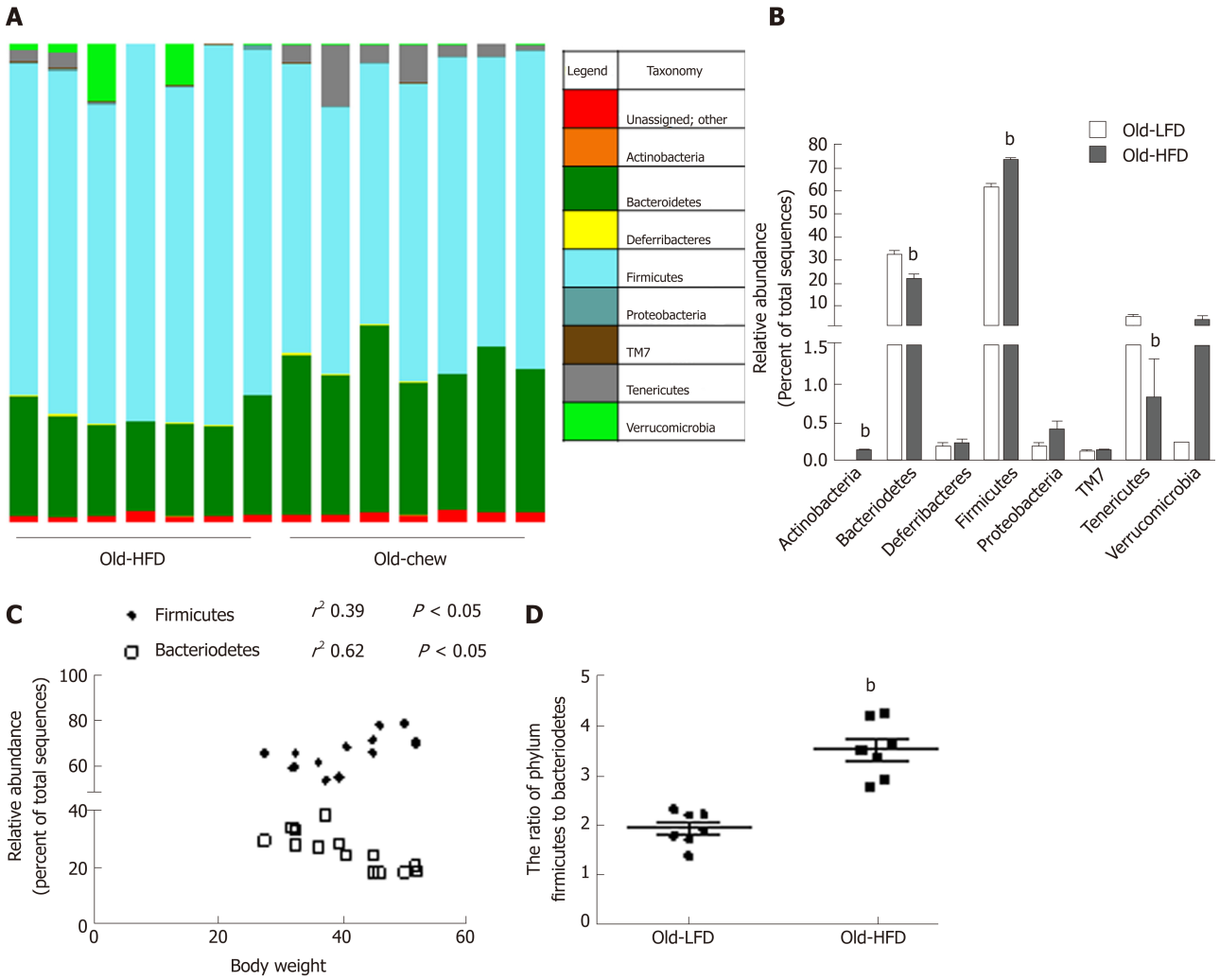


**Figure 8 Relative abundance of gut microbial at the phylum level of mice subjected to prolonged high-fat diet-feeding.** A: Bar chart presenting the relative abundance of operational taxonomic units in bacterial phyla; B: Mean relative abundance of phyla; C: Mean relative ratio between *firmicutes* and *bacteroidetes* in Old-low-fat diet (LFD) and Old-high-fat diet-feeding (HFD) mice; D: Correlation between mean relative abundance of *firmicutes* and *bacteroidetes* and mouse body weight. Data are expressed as mean ± SE. $n = 7$ mice per group. [b]Significantly different from Old-LFD ($P < 0.05$). HFD: High-fat diet-feeding; LFD: Low-fat diet.

IL-6 and TNF-α has been involved in both NAFLD and IBS in humans[57-61], we cannot discard that systemic inflammation and/or gut permeability may play an important role in the development of these disorders. However, we did not measure systemic cytokines nor intestinal permeability in our study. Thus, whether prolonged HFD consumption for 80 wk, as in the current study, exhibits these characteristics remains to be determined. The increase in FoxP3 expression is likely a consequence of the chronic low-grade inflammation in the colon and may explain, in part, the absence of a difference in colon histology.

Studies have reported that the taxonomic composition of gut microbiota in obese individuals indicates a higher proportion of *Firmicutes* and a lower amount of *Bacteroidetes*[14,19]. Interestingly, these differences in the ratio of *Firmicutes* and *Bacteroidetes* at the phylum level has been not observed in obese NAFLD patients when compared with age matched healthy control subjects[17,18,62-68]. In fact, most of the gut microbiota associated changes in NAFLD and NASH occur at the family and the genus level (Table 1). In general, there is not consistent evidence of a specific family or genus involved in NAFLD/NASH. This could be due, in part, to discrepancies in body mass index, stratification of the disease, sex, diet, and the exclusion or inclusion criteria used across studies. Significant findings in our study include an increase in the abundance of *Firmicutes* and *Actinobacteria* and a decrease in *Bacteriodetes* and *Tenericutes* phylotypes in Old-HFD mice. Consistent with the obesity literature, changes in the *Firmicutes* phylum were positively correlated with body weight while differences in the *Bacteriodetes* phylum were negatively associated with body weight. Since changes in *Firmicutes*, *Actinobacteria* and *Bacteriodetes* have been found when comparing obese NAFLD/NASH and lean healthy children and adolescent patients, we believe that the changes observed in these phyla may be related to the body

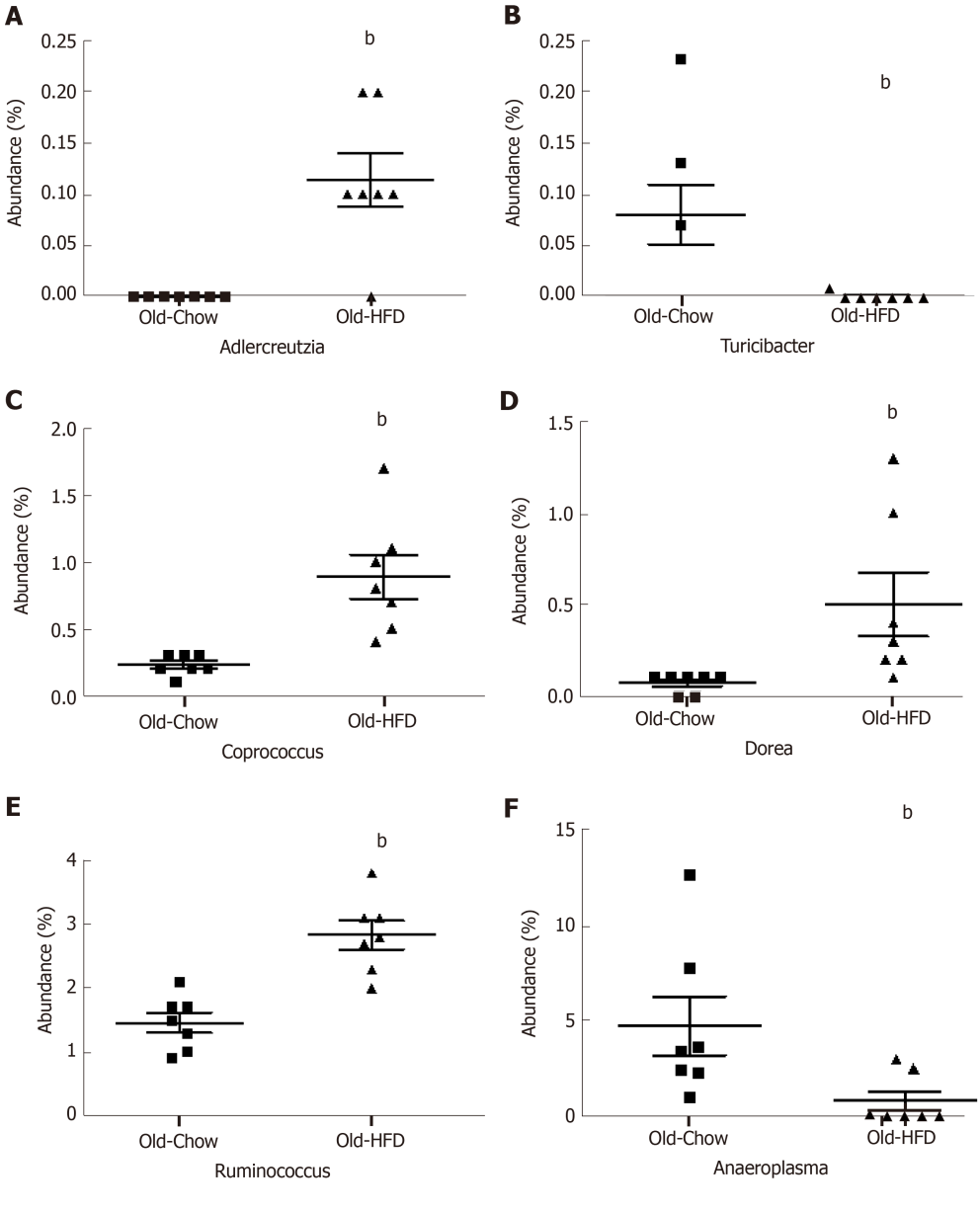


**Figure 9 Relative abundance of gut microbial at the genus level of mice subjected to prolonged high-fat diet-feeding.** A: Mean relative abundance of *Adlercreutzia*; B: Mean relative abundance of *Turicibacter*; C: Mean relative abundance of *Coprococcus*; D: Mean relative abundance of *Dorea*; E: Mean relative abundance of *Ruminococcus*; F: Mean relative abundance of *Anaerosplasma*. Data are expressed as mean ± SE. $n = 7$ mice per group. $^{b}P < 0.05$, *vs* Old-LFD. HFD: High-fat diet-feeding; LFD: Low-fat diet.

composition and not to the NAFLD pathology. At the genus level, we report an over-representation of *Adlercreutzia, Coprococcus, Dorea*, and *Ruminococcus* and under-representation of *Turicibacter* and *Anaeroplasma* abundance in Old-HFD mice. These findings are consistent with some, but not all, of the reported human NAFLD/NASH associated organisms. For example, Raman *et al*[17] and Del Chierico *et al*[65] reported elevated representation of *Dorea* in NAFLD/NASH children, adolescents, and adult subjects, but Da Silva *et al*[63] observed lower abundance of *Dorea* in NAFLD patients. In obese post-menopausal women, *Dorea* genera has been negatively associated with insulin resistance and positively correlated with markers of inflammation[66]. Taken together, these studies suggest an association between increased obesity, inflammation, and elevated *Dorea* genera – which may or may not be related to NAFLD. Consistent with our findings, Boursier *et al*[19] and Del Chierico *et al*[65] observed an increase in the abundance of *Ruminococcus*. Meanwhile, Da Silva *et al*[63] observed that NAFLD patients had lower fecal abundance of *Ruminococcus*. Other genera that were altered in the Old-HFD group including *Adlercreutzia*, *Turicibacter*, and *Anaerosplasma* have not been associated with NAFLD or NASH in human patients. Thus, whether changes in abundance of these microbes is due to diet, obesity, or other factors independent of NAFLD warrants further investigation.

**Table 1 Gut microbiota associated changes in non-alcoholic fatty liver disease and Non-alcoholic steatohepatitis subjects**

| Study | Subjects | Family | Genus |
|---|---|---|---|
| Zhu *et al*[14], 2012 | [3]HC, BMI = 20 kg/m$^2$ | ↑ Prevotellaceae | ↑ Prevotella |
| | | ↑ Enterobacteriaceae[1] | ↑ Escherichia[1] |
| | [3]Obese, BMI > 30 kg/m$^2$ | ↓ Rikenellaceae | ↓ Alistipes |
| | | ↓ Actinobacteria | ↓ Bifidobacterium |
| | [3]NASH, BMI > 30 kg/m$^2$ | ↓ Lachnospiraceae | ↓ Blautia |
| | | ↓ Ruminococcaceae | ↓ Faecalibacterium |
| Mouzaki *et al*[64], 2013 | HC, BMI = 26 kg/m$^2$ | ↑ Enterobacteriaceae | ↑ Clostridium coccoides[2] |
| | SS, BMI = 29 kg/m$^2$ | ↓ Lachnospiraceae[2] | |
| | NASH, BMI > 30 kg/m$^2$ | | |
| Raman *et al*[17], 2013 | HC, BMI < 25 kg/m$^2$ | ↑ Kiloniellaceae | ↑ Lactobacillus |
| | | ↑ Pasteurellaceae | ↑ Robinsoniella |
| | NAFLD, BMI > 30 kg/m$^2$ | ↑ Lactobacillaceae | ↑ Roseburia |
| | | ↑ Lachnospiraceae | ↑ Dorea |
| | | ↑ Veillonellaceae | ↓ Oscillibacter |
| | | ↓ Ruminococcaceae | |
| | | ↓ Porphyromonadaceae | |
| | | Clostridiales | ↓ Anaerosporobacter |
| Wong *et al*[18], 2013 | HC, BMI = 22 kg/m$^2$ | ↑ Succinivibronaceae | ↑ Parabacteriodes |
| | | ↑ Porphyromonadaceae | ↑ Allisonella |
| | NASH, BMI > 30 kg/m$^2$ | ↓ Unclassified | ↓ Faecalibacterium |
| Jiang *et al*[62], 2015 | HC, BMI = 23 kg/m$^2$ | ↑ Peptostreptococcaceae | ↑ Anaerobacter |
| | | ↓ Ruminococcaceae | ↑ Streptococcus |
| | NAFLD, BMI = 26 kg/m$^2$ | | ↑ Lactobacillus |
| | | | ↑Escherichia |
| | | | ↑ Clostridium XI |
| | | | ↓ Oscillibacter |
| | | | ↓ Odoribater |
| | | | ↓ Alistipes |
| | | | ↓ Flavonifractor |
| Boursier *et al*[19], 2016 | HC, BMI > 30 kg/m$^2$ | ↑ Bacteroidaceae | ↑ Bacteroides |
| | NASH, BMI > 30 kg/m$^2$ | ↓ Prevotellaceae | ↑ Ruminoccocus |
| | | ↓Erysipelotrichaceae | ↓ Prevotella |
| Mouzaki *et al*[67], 2016 | HC, BMI = 27 kg/m$^2$ | | ↓ Clostridium leptum |
| | NASH, BMI > 30 kg/m$^2$ | | |
| Del Chierico *et al*[65], 2017 | [3]HC, BMI = 18 kg/m$^2$ | ↓ Rikenellaceae[4] | ↑ Bradyrhizobium[4] |
| | | | ↑ Anaerococcus[4] |
| | [3]Obese, BMI = 26 kg/m$^2$ | | ↑ Peptoniphilus[4] |
| | [3]NAFLD, BMI = 26 kg/m$^2$ | | ↑ Propionibacterium acnes[4] |
| | [3]NASH, BMI = 27 kg/m$^2$ | | ↑ Dorea[4] |
| | | | ↑ Ruminococcus[4] |
| | | | ↓ Oscillospira[4] |
| Nobili *et al*[68], 2018 | [3]HC, BMI = 18 kg/m$^2$ | | ↑ Lactobacillus mucosae *[12] |
| | [3]Obese, BMI = 26 kg/m$^2$ | | |
| | [3]NAFLD, BMI = 26 kg/m$^2$ | | |
| | [3]NASH, BMI = 27 kg/m$^2$ | | |
| Da Silva *et al*[63], 2018 | HC, BMI = 27 kg/m$^2$ | ↓Bacteroidaceae | ↓ Alistipes |
| | | ↓ Lachnospiraceae | ↓ Anaerostipes |
| | NAFLD, BMI > 30 kg/m$^2$ | ↓ Lactobacillaceae | ↓ Bacteroides |
| | | ↓ Porphyromonadaceae | ↓Blautia |
| | | ↓ Rikenellaceae | ↓ Coprococcus |
| | | | ↓ Dorea |
| | | | ↓ Faecalibacterium |

| | | |
|---|---|---|
| | | ↓ Lactobacillus<br>↓Parabacteroides<br>↓ Roseburia<br>↓ Ruminococcus |

[1]Different from Obese;
[2]Different from Steatosis or non-alcoholic fatty liver disease;
[3]Children and adolescent;
[4]Differences between grouping patients and healthy control. BMI: Body mass index; HC: Healthy control; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis; SS: Steatosis.

A limitation in our study is that we were not able to pinpoint a causal relationship between NAFLD and specific microbes. This is in part due to an inability to dissect changes independent of long-term dietary macronutrient manipulation or obesity – both of which directly impact the composition of gut microbiota[26]. In addition, it is important to note that our findings did not recapitulate all of the microbial findings that have been reported in the clinical NAFLD/NASH literature. Therefore, additional mechanistic studies are necessary to determine if any of the intestinal bacteria changes that we observed can promote NAFLD pre-clinically. Further, our study was limited to one-time point; all mice were exposed to chronic HFD feeding for the duration of 80 wk. However, it is certainly possible that a shorter duration of HFD feedings (less than 80 wk) may incite NAFLD pathophysiology; although most likely not to the same degree. It also would have been informative to examine changes across time to fully evaluate factors involved in progression of the disease. A third limitation in our study is that we are not able to conclude if the macronutrient composition of the diet is responsible for NAFLD development or rather the excess calories that intrinsically promotes NAFLD. Thus, it may be advantageous to study energy-dense isocaloric diets with high carbohydrate or fat macronutrient content.

In summary, our study examined the effects of prolonged HFD feeding on NAFLD. In particular, we focused on determining the common features of this animal model with NAFLD and human manifestation of the disease. Our prolonged HFD feeding led to the development of obesity, steatosis, non-alcoholic stetohepatitis, insulin resistance, steatosis, liver ER stress, and gut dysbiosis making it a suitable model for the study of NAFLD. Our results suggest that chronic HFD can mimic most, but not all, of the pathophysiological events observed in NAFLD.

## ARTICLE HIGHLIGHTS

### *Research background*

Animal models that can exhibit characteristics seen in non-alcoholic fatty liver disease (NAFLD) have the potential to drive the discovery of new drugs to treat this disease.

### *Research motivation*

Most animal models used to investigate NAFLD misrepresent typical characteristics seen in human patients with NAFLD. Therefore, any successful treatments documented in these animal models may not be clinically translatable.

### *Research objectives*

To evaluate if mice consuming a high calorie diet for a prolonged time can mimic clinical characteristics of NAFLD.

### *Research methods*

Male mice (10 wk old) were assigned to the following groups: Young- low-fat diet (LFD) ($n$ = 20; low calorie diet for), Old-LFD ($n$ = 15; low calorie diet), and Old-HFD ($n$ = 18; high calorie diet). Mice in the LFD consumed a diet rich in carbohydrates, meanwhile the HFD was abundant in fat content. Liver, colon, adipose tissue, and feces were collected at 16 wk of age in Young-LFD mice and at 90 wk of age in Old-LFD and Old-HFD to evaluate microscopic features, glucose metabolism, inflammation, endoplasmic reticulum (ER) stress, and microbiome profile seen in NAFLD.

### *Research results*

Old-HFD mice showed increased body weight, blood glucose, plasma insulin, HOMA index, steatosis, hepatocellular ballooning, inflammation, fibrosis, NAFLD activity score, ER stress markers (CHOP and p-Jnk), and abundance of *Firmicutes, Adlercreutzia, Turicibacter, Coprococcus, Dorea, and Ruminococcus*.

### *Research conclusions*

Mice fed a high calorie diet for 80-wk (Old-HFD) mimicked microscopic characteristic and

microbial events that have been previously observed in NAFLD patients.

### *Research perspectives*

It is important to critically select animal models to study any disease including NAFLD. Future research dedicated to investigation of new treatments for NAFLD should consider prolonged-HFD feedings as their animal model.

## REFERENCES


1 **Younossi ZM**, Otgonsuren M, Henry L, Venkatesan C, Mishra A, Erario M, Hunt S. Association of nonalcoholic fatty liver disease (NAFLD) with hepatocellular carcinoma (HCC) in the United States from 2004 to 2009. *Hepatology* 2015; **62**: 1723-1730 [PMID: 26274335 DOI: 10.1002/hep.28123]

2 **Federico A**, Zulli C, de Sio I, Del Prete A, Dallio M, Masarone M, Loguercio C. Focus on emerging drugs for the treatment of patients with non-alcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 16841-16857 [PMID: 25492998 DOI: 10.3748/wjg.v20.i45.16841]

3 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ; American Gastroenterological Association; American Association for the Study of Liver Diseases; American College of Gastroenterologyh. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

4 **Fox KR**, Hillsdon M. Physical activity and obesity. *Obes Rev* 2007; **8** Suppl 1: 115-121 [PMID: 17316313 DOI: 10.1111/j.1467-789X.2007.00329.x]

5 **Wareham NJ**, van Sluijs EM, Ekelund U. Physical activity and obesity prevention: a review of the current evidence. *Proc Nutr Soc* 2005; **64**: 229-247 [PMID: 15960868]

6 **Roberts DL**, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med* 2010; **61**: 301-316 [PMID: 19824817 DOI: 10.1146/annurev.med.080708.082713]

7 **Lemmens VE**, Oenema A, Klepp KI, Henriksen HB, Brug J. A systematic review of the evidence regarding efficacy of obesity prevention interventions among adults. *Obes Rev* 2008; **9**: 446-455 [PMID: 18298429 DOI: 10.1111/j.1467-789X.2008.00468.x]

8 **Asai A**, Chou PM, Bu HF, Wang X, Rao MS, Jiang A, DiDonato CJ, Tan XD. Dissociation of hepatic insulin resistance from susceptibility of nonalcoholic fatty liver disease induced by a high-fat and high-carbohydrate diet in mice. *Am J Physiol Gastrointest Liver Physiol* 2014; **306**: G496-G504 [PMID: 24436353 DOI: 10.1152/ajpgi.00291.2013]

9 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404 DOI: 10.1038/nature07540]

10 **Murphy EF**, Cotter PD, Hogan A, O'Sullivan O, Joyce A, Fouhy F, Clarke SF, Marques TM, O'Toole PW, Stanton C, Quigley EM, Daly C, Ross PR, O'Doherty RM, Shanahan F. Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. *Gut* 2013; **62**: 220-226 [PMID: 22345653 DOI: 10.1136/gutjnl-2011-300705]

11 **Larsen N**, Vogensen FK, van den Berg FW, Nielsen DS, Andreasen AS, Pedersen BK, Al-Soud WA, Sørensen SJ, Hansen LH, Jakobsen M. Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLoS One* 2010; **5**: e9085 [PMID: 20140211 DOI: 10.1371/journal.pone.0009085]

12 **Qin J**, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, Peng Y, Zhang D, Jie Z, Wu W, Qin Y, Xue W, Li J, Han L, Lu D, Wu P, Dai Y, Sun X, Li Z, Tang A, Zhong S, Li X, Chen W, Xu R, Wang M, Feng Q, Gong M, Yu J, Zhang Y, Zhang M, Hansen T, Sanchez G, Raes J, Falony G, Okuda S, Almeida M, LeChatelier E, Renault P, Pons N, Batto JM, Zhang Z, Chen H, Yang R, Zheng W, Li S, Yang H, Wang J, Ehrlich SD, Nielsen R, Pedersen O, Kristiansen K, Wang J. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012; **490**: 55-60 [PMID: 23023125 DOI: 10.1038/nature11450]

13 **Boursier J**, Diehl AM. Implication of gut microbiota in nonalcoholic fatty liver disease. *PLoS Pathog* 2015; **11**: e1004559 [PMID: 25625278 DOI: 10.1371/journal.ppat.1004559]

14 **Zhu L**, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* 2013; **57**: 601-609 [PMID: 23055155 DOI: 10.1002/hep.26093]

15 **Yu LX**, Schwabe RF. The gut microbiome and liver cancer: mechanisms and clinical translation. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 527-539 [PMID: 28676707 DOI: 10.1038/nrgastro.2017.72]

16 **Tilg H**, Cani PD, Mayer EA. Gut microbiome and liver diseases. *Gut* 2016; **65**: 2035-2044 [PMID: 27802157 DOI: 10.1136/gutjnl-2016-312729]

17 **Raman M**, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, Bailey J, Myers RP, Rioux KP. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013; **11**: 868-875.e1-3 [PMID: 23454028 DOI: 10.1016/j.cgh.2013.02.015]

18 **Wong VW**, Tse CH, Lam TT, Wong GL, Chim AM, Chu WC, Yeung DK, Law PT, Kwan HS, Yu J, Sung JJ, Chan HL. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis--a longitudinal study. *PLoS One* 2013; **8**: e62885 [PMID: 23638162 DOI: 10.1371/journal.pone.0062885]

19 **Boursier J**, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, Guy CD, Seed PC, Rawls JF, David LA, Hunault G, Oberti F, Calès P, Diehl AM. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016; **63**: 764-775 [PMID: 26600078 DOI: 10.1002/hep.28356]

20 **Koopman R**, Schaart G, Hesselink MK. Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. *Histochem Cell Biol* 2001; **116**: 63-68 [PMID: 11479724]

21 **Velázquez KT**, Enos RT, Carson MS, Cranford TL, Bader JE, Chatzistamou I, Singh UP, Nagarkatti PS, Nagarkatti M, Davis JM, Carson JA, Murphy EA. Weight loss following diet-induced obesity does not

alter colon tumorigenesis in the AOM mouse model. *Am J Physiol Gastrointest Liver Physiol* 2016; **311**: G699-G712 [PMID: 27609769 DOI: 10.1152/ajpgi.00207.2016]
22 **Klindworth A**, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, Glöckner FO. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013; **41**: e1 [PMID: 22933715 DOI: 10.1093/nar/gks808]
23 **Zhang XQ**, Xu CF, Yu CH, Chen WX, Li YM. Role of endoplasmic reticulum stress in the pathogenesis of nonalcoholic fatty liver disease. *World J Gastroenterol* 2014; **20**: 1768-1776 [PMID: 24587654 DOI: 10.3748/wjg.v20.i7.1768]
24 **Chambers JE**, Marciniak SJ. Cellular mechanisms of endoplasmic reticulum stress signaling in health and disease. 2. Protein misfolding and ER stress. *Am J Physiol Cell Physiol* 2014; **307**: C657-C670 [PMID: 24944205 DOI: 10.1152/ajpcell.00183.2014]
25 **Pagliassotti MJ**. Endoplasmic reticulum stress in nonalcoholic fatty liver disease. *Annu Rev Nutr* 2012; **32**: 17-33 [PMID: 22809102 DOI: 10.1146/annurev-nutr-071811-150644]
26 **Wu GD**, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**: 105-108 [PMID: 21885731 DOI: 10.1126/science.1208344]
27 **Martinez KB**, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: Are they linked? *Gut Microbes* 2017; **8**: 130-142 [PMID: 28059614 DOI: 10.1080/19490976.2016.1270811]
28 **Manzel A**, Muller DN, Hafler DA, Erdman SE, Linker RA, Kleinewietfeld M. Role of "Western diet" in inflammatory autoimmune diseases. *Curr Allergy Asthma Rep* 2014; **14**: 404 [PMID: 24338487 DOI: 10.1007/s11882-013-0404-6]
29 **Minihane AM**, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, Teeling JL, Blaak EE, Fenech M, Vauzour D, McArdle HJ, Kremer BH, Sterkman L, Vafeiadou K, Benedetti MM, Williams CM, Calder PC. Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br J Nutr* 2015; **114**: 999-1012 [PMID: 26228057 DOI: 10.1017/S0007114515002093]
30 **Le Roy T**, Llopis M, Lepage P, Bruneau A, Rabot S, Bevilacqua C, Martin P, Philippe C, Walker F, Bado A, Perlemuter G, Cassard-Doulcier AM, Gérard P. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* 2013; **62**: 1787-1794 [PMID: 23197411 DOI: 10.1136/gutjnl-2012-303816]
31 **Nakae D**, Mizumoto Y, Andoh N, Tamura K, Horiguchi K, Endoh T, Kobayashi E, Tsujiuchi T, Denda A, Lombardi B. Comparative changes in the liver of female Fischer-344 rats after short-term feeding of a semipurified or a semisynthetic L-amino acid-defined choline-deficient diet. *Toxicol Pathol* 1995; **23**: 583-590 [PMID: 8578101 DOI: 10.1177/019262339502300504]
32 **Itagaki H**, Shimizu K, Morikawa S, Ogawa K, Ezaki T. Morphological and functional characterization of non-alcoholic fatty liver disease induced by a methionine-choline-deficient diet in C57BL/6 mice. *Int J Clin Exp Pathol* 2013; **6**: 2683-2696 [PMID: 24294355]
33 **Machado MV**, Michelotti GA, Xie G, Almeida Pereira T, Boursier J, Bohnic B, Guy CD, Diehl AM. Mouse models of diet-induced nonalcoholic steatohepatitis reproduce the heterogeneity of the human disease. *PLoS One* 2015; **10**: e0127991 [PMID: 26017539 DOI: 10.1371/journal.pone.0127991]
34 **Cranford TL**, Velázquez KT, Enos RT, Sougiannis AT, Bader JE, Carson MS, Bellone RR, Chatzistamou I, Nagarkatti M, Murphy EA. Effects of high fat diet-induced obesity on mammary tumorigenesis in the PyMT/MMTV murine model. *Cancer Biol Ther* 2019; **20**: 487-496 [PMID: 30388923 DOI: 10.1080/15384047.2018.1537574]
35 **Velázquez KT**, Enos RT, Carson MS, Cranford TL, Bader JE, Sougiannis AT, Pritchett C, Fan D, Carson JA, Murphy EA. miR155 deficiency aggravates high-fat diet-induced adipose tissue fibrosis in male mice. *Physiol Rep* 2017; 5 [PMID: 28947593 DOI: 10.14814/phy2.13412]
36 **Enos RT**, Davis JM, Velázquez KT, McClellan JL, Day SD, Carnevale KA, Murphy EA. Influence of dietary saturated fat content on adiposity, macrophage behavior, inflammation, and metabolism: composition matters. *J Lipid Res* 2013; **54**: 152-163 [PMID: 23103474 DOI: 10.1194/jlr.M030700]
37 **VanSaun MN**, Lee IK, Washington MK, Matrisian L, Gorden DL. High fat diet induced hepatic steatosis establishes a permissive microenvironment for colorectal metastases and promotes primary dysplasia in a murine model. *Am J Pathol* 2009; **175**: 355-364 [PMID: 19541928 DOI: 10.2353/ajpath.2009.080703]
38 **Hill-Baskin AE**, Markiewski MM, Buchner DA, Shao H, DeSantis D, Hsiao G, Subramaniam S, Berger NA, Croniger C, Lambris JD, Nadeau JH. Diet-induced hepatocellular carcinoma in genetically predisposed mice. *Hum Mol Genet* 2009; **18**: 2975-2988 [PMID: 19454484 DOI: 10.1093/hmg/ddp236]
39 **Clapper JR**, Hendricks MD, Gu G, Wittmer C, Dolman CS, Herich J, Athanacio J, Villescaz C, Ghosh SS, Heilig JS, Lowe C, Roth JD. Diet-induced mouse model of fatty liver disease and nonalcoholic steatohepatitis reflecting clinical disease progression and methods of assessment. *Am J Physiol Gastrointest Liver Physiol* 2013; **305**: G483-G495 [PMID: 23886860 DOI: 10.1152/ajpgi.00079.2013]
40 **Trevaskis JL**, Griffin PS, Wittmer C, Neuschwander-Tetri BA, Brunt EM, Dolman CS, Erickson MR, Napora J, Parkes DG, Roth JD. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G762-G772 [PMID: 22268099 DOI: 10.1152/ajpgi.00476.2011]
41 **Kristiansen MN**, Veidal SS, Rigbolt KT, Tølbøl KS, Roth JD, Jelsing J, Vrang N, Feigh M. Obese diet-induced mouse models of nonalcoholic steatohepatitis-tracking disease by liver biopsy. *World J Hepatol* 2016; **8**: 673-684 [PMID: 27326314 DOI: 10.4254/wjh.v8.i16.673]
42 **Lesmana CR**, Hasan I, Budihusodo U, Gani RA, Krisnuhoni E, Akbar N, Lesmana LA. Diagnostic value of a group of biochemical markers of liver fibrosis in patients with non-alcoholic steatohepatitis. *J Dig Dis* 2009; **10**: 201-206 [PMID: 19659788 DOI: 10.1111/j.1751-2980.2009.00386.x]
43 **Li W**, Zheng L, Sheng C, Cheng X, Qing L, Qu S. Systematic review on the treatment of pentoxifylline in patients with non-alcoholic fatty liver disease. *Lipids Health Dis* 2011; **10**: 49 [PMID: 21477300 DOI: 10.1186/1476-511X-10-49]
44 **Kugelmas M**, Hill DB, Vivian B, Marsano L, McClain CJ. Cytokines and NASH: a pilot study of the effects of lifestyle modification and vitamin E. *Hepatology* 2003; **38**: 413-419 [PMID: 12883485 DOI: 10.1053/jhep.2003.50316]
45 **Wieckowska A**, Papouchado BG, Li Z, Lopez R, Zein NN, Feldstein AE. Increased hepatic and circulating interleukin-6 levels in human nonalcoholic steatohepatitis. *Am J Gastroenterol* 2008; **103**: 1372-1379 [PMID: 18510618 DOI: 10.1111/j.1572-0241.2007.01774.x]
46 **Haukeland JW**, Damås JK, Konopski Z, Løberg EM, Haaland T, Goverud I, Torjesen PA, Birkeland K, Bjøro K, Aukrust P. Systemic inflammation in nonalcoholic fatty liver disease is characterized by elevated

levels of CCL2. *J Hepatol* 2006; **44**: 1167-1174 [PMID: 16618517 DOI: 10.1016/j.jhep.2006.02.011]
47 **Westerbacka J**, Kolak M, Kiviluoto T, Arkkila P, Sirén J, Hamsten A, Fisher RM, Yki-Järvinen H. Genes involved in fatty acid partitioning and binding, lipolysis, monocyte/macrophage recruitment, and inflammation are overexpressed in the human fatty liver of insulin-resistant subjects. *Diabetes* 2007; **56**: 2759-2765 [PMID: 17704301 DOI: 10.2337/db07-0156]
48 **Greco D**, Kotronen A, Westerbacka J, Puig O, Arkkila P, Kiviluoto T, Laitinen S, Kolak M, Fisher RM, Hamsten A, Auvinen P, Yki-Järvinen H. Gene expression in human NAFLD. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1281-G1287 [PMID: 18388185 DOI: 10.1152/ajpgi.00074.2008]
49 **Linehan E**, Fitzgerald DC. Ageing and the immune system: focus on macrophages. *Eur J Microbiol Immunol (Bp)* 2015; **5**: 14-24 [PMID: 25883791 DOI: 10.1556/EUJMI-D-14-00035]
50 **Stahl EC**, Haschak MJ, Popovic B, Brown BN. Macrophages in the Aging Liver and Age-Related Liver Disease. *Front Immunol* 2018; **9**: 2795 [PMID: 30555477 DOI: 10.3389/fimmu.2018.02795]
51 **Kim OK**, Jun W, Lee J. Mechanism of ER Stress and Inflammation for Hepatic Insulin Resistance in Obesity. *Ann Nutr Metab* 2015; **67**: 218-227 [PMID: 26452040 DOI: 10.1159/000440905]
52 **Oyadomari S**, Harding HP, Zhang Y, Oyadomari M, Ron D. Dephosphorylation of translation initiation factor 2alpha enhances glucose tolerance and attenuates hepatosteatosis in mice. *Cell Metab* 2008; **7**: 520-532 [PMID: 18522833 DOI: 10.1016/j.cmet.2008.04.011]
53 **Schwabe RF**, Uchinami H, Qian T, Bennett BL, Lemasters JJ, Brenner DA. Differential requirement for c-Jun NH2-terminal kinase in TNFalpha- and Fas-mediated apoptosis in hepatocytes. *FASEB J* 2004; **18**: 720-722 [PMID: 14766793 DOI: 10.1096/fj.03-0771fje]
54 **Chen Y**, Brandizzi F. IRE1: ER stress sensor and cell fate executor. *Trends Cell Biol* 2013; **23**: 547-555 [PMID: 23880584 DOI: 10.1016/j.tcb.2013.06.005]
55 **Guan BJ**, Krokowski D, Majumder M, Schmotzer CL, Kimball SR, Merrick WC, Koromilas AE, Hatzoglou M. Translational control during endoplasmic reticulum stress beyond phosphorylation of the translation initiation factor eIF2α. *J Biol Chem* 2014; **289**: 12593-12611 [PMID: 24648524 DOI: 10.1074/jbc.M113.543215]
56 **Choi WG**, Han J, Kim JH, Kim MJ, Park JW, Song B, Cha HJ, Choi HS, Chung HT, Lee IK, Park TS, Hatzoglou M, Choi HS, Yoo HJ, Kaufman RJ, Back SH. eIF2α phosphorylation is required to prevent hepatocyte death and liver fibrosis in mice challenged with a high fructose diet. *Nutr Metab (Lond)* 2017; **14**: 48 [PMID: 28781602 DOI: 10.1186/s12986-017-0202-6]
57 **Chao CY**, Battat R, Al Khoury A, Restellini S, Sebastiani G, Bessissow T. Co-existence of non-alcoholic fatty liver disease and inflammatory bowel disease: A review article. *World J Gastroenterol* 2016; **22**: 7727-7734 [PMID: 27678354 DOI: 10.3748/wjg.v22.i34.7727]
58 **Gisbert JP**, Luna M, González-Lama Y, Pousa ID, Velasco M, Moreno-Otero R, Maté J. Liver injury in inflammatory bowel disease: long-term follow-up study of 786 patients. *Inflamm Bowel Dis* 2007; **13**: 1106-1114 [PMID: 17455203 DOI: 10.1002/ibd.20160]
59 **Bargiggia S**, Maconi G, Elli M, Molteni P, Ardizzone S, Parente F, Todaro I, Greco S, Manzionna G, Bianchi Porro G. Sonographic prevalence of liver steatosis and biliary tract stones in patients with inflammatory bowel disease: study of 511 subjects at a single center. *J Clin Gastroenterol* 2003; **36**: 417-420 [PMID: 12702985]
60 **Riegler G**, D'Incà R, Sturniolo GC, Corrao G, Del Vecchio Blanco C, Di Leo V, Carratù R, Ingrosso M, Pelli MA, Morini S, Valpiani D, Cantarini D, Usai P, Papi C, Caprilli R. Hepatobiliary alterations in patients with inflammatory bowel disease: a multicenter study. Caprilli amp; Gruppo Italiano Studio Colon-Retto. *Scand J Gastroenterol* 1998; **33**: 93-98 [PMID: 9489915]
61 **Bessissow T**, Le NH, Rollet K, Afif W, Bitton A, Sebastiani G. Incidence and Predictors of Nonalcoholic Fatty Liver Disease by Serum Biomarkers in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis* 2016; **22**: 1937-1944 [PMID: 27379445 DOI: 10.1097/MIB.0000000000000832]
62 **Jiang W**, Wu N, Wang X, Chi Y, Zhang Y, Qiu X, Hu Y, Li J, Liu Y. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep* 2015; **5**: 8096 [PMID: 25644696 DOI: 10.1038/srep08096]
63 **Da Silva HE**, Teterina A, Comelli EM, Taibi A, Arendt BM, Fischer SE, Lou W, Allard JP. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* 2018; **8**: 1466 [PMID: 29362454 DOI: 10.1038/s41598-018-19753-9]
64 **Mouzaki M**, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013; **58**: 120-127 [PMID: 23401313 DOI: 10.1002/hep.26319]
65 **Del Chierico F**, Nobili V, Vernocchi P, Russo A, Stefanis C, Gnani D, Furlanello C, Zandonà A, Paci P, Capuani G, Dallapiccola B, Miccheli A, Alisi A, Putignani L. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017; **65**: 451-464 [PMID: 27028797 DOI: 10.1002/hep.28572]
66 **Brahe LK**, Le Chatelier E, Prifti E, Pons N, Kennedy S, Hansen T, Pedersen O, Astrup A, Ehrlich SD, Larsen LH. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutr Diabetes* 2015; **5**: e159 [PMID: 26075636 DOI: 10.1038/nutd.2015.9]
67 **Mouzaki M**, Wang AY, Bandsma R, Comelli EM, Arendt BM, Zhang L, Fung S, Fischer SE, McGilvray IG, Allard JP. Bile Acids and Dysbiosis in Non-Alcoholic Fatty Liver Disease. *PLoS One* 2016; **11**: e0151829 [PMID: 27203081 DOI: 10.1371/journal.pone.0151829]
68 **Nobili V**, Putignani L, Mosca A, Chierico FD, Vernocchi P, Alisi A, Stronati L, Cucchiara S, Toscano M, Drago L. Bifidobacteria and lactobacilli in the gut microbiome of children with non-alcoholic fatty liver disease: which strains act as health players? *Arch Med Sci* 2018; **14**: 81-87 [PMID: 29379536 DOI: 10.5114/aoms.2016.62150]

World Journal of Hepatology

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

*World J Hepatol* 2019 August 27; 11(8): 638-645

DOI: 10.4254/wjh.v11.i8.638

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

# Impact of psychosocial comorbidities on clinical outcomes after liver transplantation: Stratification of a high-risk population

Neil Bhogal, Amaninder Dhaliwal, Elizabeth Lyden, Fedja Rochling, Marco Olivera-Martinez

**ORCID number:** Neil Bhogal (0000-0002-9223-1943); Amaninder Dhaliwal (0000-0002-9761-437X); Elizabeth Lyden (0000-0002-1635-6365); Fedja Rochling (0000-0002-6668-8982); Marco Olivera-Martinez (0000-0003-1376-3739).

**Author contributions:** Bhogal N contributed to study formulation, study design, data compilation, data analysis, and manuscript preparation; Dhaliwal A contributed to study formulation, study design, data analysis, and manuscript preparation; Lyden E contributed to study design and statistical analysis; Rochling F contributed to data analysis and manuscript preparation; Olivera-Martinez M contributed to study formulation, study design, data analysis, and manuscript preparation.

**Institutional review board statement:** This study was reviewed and approved by the University of Nebraska Institutional Review Board No. 304-17-EP.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.



**Neil Bhogal, Amaninder Dhaliwal, Fedja Rochling, Marco Olivera-Martinez,** Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Nebraska Medical Center, Omaha, NE 68198, United States

**Elizabeth Lyden,** Department of Biostatistics, Division of Public Health, University of Nebraska Medical Center, Omaha, NE 68198, United States

**Corresponding author:** Neil Bhogal, Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of Nebraska Medical Center, 982000 NMC, Omaha, NE 68198, United States. neil.bhogal@unmc.edu
**Telephone:** +1-661-4969737

## Abstract

*BACKGROUND*

Liver transplantation is the accepted standard of care for end-stage liver disease due to a variety of etiologies including decompensated cirrhosis, fulminant hepatic failure, and primary hepatic malignancy. There are currently over 13000 candidates on the liver transplant waiting list emphasizing the importance of rigorous patient selection. There are few studies regarding the impact of additional psychosocial barriers to liver transplant including financial hardship, lack of caregiver support, polysubstance abuse, and issues with medical non-compliance. We hypothesized that patients with certain psychosocial comorbidities experienced worse outcomes after liver transplantation.

*AIM*

To assess the impact of certain pre-transplant psychosocial comorbidities on outcomes after liver transplantation.

*METHODS*

A retrospective analysis was performed on all adult patients from 2012-2016. Psychosocial comorbidities including documented medical non-compliance, polysubstance abuse, financial issues, and lack of caregiver support were collected. The primary outcome assessed post-transplantation was survival. Secondary outcomes measured included graft failure, episodes of acute rejection, psychiatric decompensation, number of readmissions, presence of infection, recidivism for alcohol and other substances, and documented caregiver support failure.

*RESULTS*

For the primary outcome, there were no differences in survival. Patients with a

**Manuscript source**: Unsolicited manuscript

**Received:** March 20, 2019
**Peer-review started:** March 20, 2019
**First decision:** April 23, 2019
**Revised:** May 23, 2019
**Accepted:** July 16, 2019
**Article in press:** July 17, 2019
**Published online:** August 27, 2019

**P-Reviewer:** Bramhall S, Chiu KW, Rubbini M, Tsoulfas G
**S-Editor:** Cui LJ
**L-Editor:** Filipodia
**E-Editor:** Ma YJ

history of psychiatric disease had a higher incidence of psychiatric decompensation after liver transplantation (19% *vs* 10%, $P = 0.013$). Treatment of psychiatric disorders resulted in a reduction of the incidence of psychiatric decompensation (21% *vs* 11%, $P = 0.022$). Patients with a history of polysubstance abuse in the transplant evaluation had a higher incidence of substance abuse after transplantation (5.8% *vs* 1.2%, $P = 0.05$). In this cohort, 15 patients (3.8%) were found to have medical compliance issues in the transplant evaluation. Of these specific patients, 13.3% were found to have substance abuse after transplantation as opposed to 1.3% in patients without documented compliance issues ($P = 0.03$).

*CONCLUSION*
Patients with certain psychosocial comorbidities had worse outcomes following liver transplantation. Further prospective and multi-center studies are warranted to properly determine guidelines for liver transplantation regarding this high-risk population.

**Key words:** Liver transplantation; Recidivism; Psychosocial decompensation; Non-compliance; Transplant psychiatry



**Core tip:** As there is a limited number of organs available for transplantation, a successful outcome depends upon a complete evaluation. There is a paucity of data regarding the impact of psychosocial comorbidities and their impact after liver transplantation. Our study found that patients with certain psychosocial comorbidities had worse outcomes after liver transplantation. This work adds to the growing literature that this represents a high-risk population.

**Citation:** Bhogal N, Dhaliwal A, Lyden E, Rochling F, Olivera-Martinez M. Impact of psychosocial comorbidities on clinical outcomes after liver transplantation: Stratification of a high-risk population. *World J Hepatol* 2019; 11(8): 638-645
**URL**: https://www.wjgnet.com/1948-5182/full/v11/i8/638.htm
**DOI**: https://dx.doi.org/10.4254/wjh.v11.i8.638

## INTRODUCTION

Liver transplantation is the accepted standard of care for end-stage liver disease due to a variety of etiologies including decompensated cirrhosis, fulminant hepatic failure, and primary hepatic malignancy[1]. The number of liver transplantations is increasing in the United States with over 33000 procedures performed in the last five years[2]. In addition, more organs are available for transplantation with practice changes such as human immunodeficiency virus, hepatitis B and C infected donor grafts, and living donor donations[3-5]. Also, the landscape is changing regarding liver transplantation in severe alcoholic hepatitis given studies showing favorable outcomes[6,7]. Despite these advances, as of January 2019 there are currently over 13000 candidates on the liver transplant waiting list emphasizing the importance of rigorous patient selection[8].

The liver transplantation process requires a multi-disciplinary approach that facilitates collaboration between different specialties including hepatologists, transplant surgeons, neuropsychologists, psychiatrists, social workers, and ethics committees. There are evidence-based criteria involving the degree of end-organ damage and decompensation required prior to being listed for transplant as organ allocation is based on the model for end-state liver disease score[9]. However, a growing emphasis has been placed on the psychosocial status of patients prior to liver transplantation. The psychosocial assessment of a patient is a critical aspect of the evaluation process prior to being listed for liver transplantation. There is minimal data assessing post-transplant outcomes in patients after they have received a liver transplant that were characterized as having psychosocial problems prior to being evaluated and listed for transplantation.

Despite the aforementioned practice changes, there are still a limited number of organs available for transplantation. Thus, a successful outcome depends upon a complete evaluation[10]. Liver transplant centers have various predefined criteria that

must be met prior to being listed including minimum time of documented sobriety in the case of alcoholic liver disease and range of body-mass index for obese patients[11,12]. In contrast, there are no standardized criteria for selection for transplant in respect to psychosocial comorbidities, and there are no evidence-based guidelines for screening or treatment prior to listing[13]. Psychiatric disorders and polysubstance abuse are common amongst transplant candidates[14]. Previous studies have indicated that several psychosocial morbidities can lead to adverse outcomes after liver transplantation[15]. Not only can there be an impact on morbidity and mortality but also a decrease in the quality of life[16]. Most of the previous studies have focused on psychiatric comorbidities, specifically major depressive disorder and generalized anxiety[17-19]. There are few studies regarding the impact of additional psychosocial barriers to liver transplant including financial hardship, lack of caregiver support, polysubstance abuse, and issues with medical non-compliance. The purpose of our study was to assess the impact of certain pre-transplant psychosocial comorbidities on outcomes after liver transplantation.

## MATERIALS AND METHODS

A retrospective analysis was performed on all patients older than 18 years of age at our institution who underwent liver transplantation over a five-year span from 2012-2016. Approval for the project was obtained by The University of Nebraska institutional review board (protocol 304-17-EP). Demographic data, etiology of liver disease, psychosocial, and psychiatric history were collected on all patients. Patients determined to have psychiatric disease were individuals with a known psychiatric diagnosis who followed with a mental health professional or utilized a psychiatric medication within 12 mo of organ transplant.

Additional data was gathered regarding specific treatment of psychiatric disorders prior to organ transplantation including medications and psychotherapy. Finally, psychosocial comorbidities including documented medical non-compliance, polysubstance abuse, financial issues, and lack of caregiver support were collected. These parameters were ascertained by reviewing the pre-transplant evaluation records including outpatient multi-disciplinary transplant clinic visits, inpatient hospitalization notes, and documentation from patient selection meetings. Medical non-compliance was determined by transplant committee review documenting concerns for adherence to pre-transplant recommendations. For polysubstance abuse-specific agents documented included alcohol, marijuana, cocaine, methamphetamines, heroin, prescription opioids, and prescription benzodiazepines. The methods of detection were serum for ethanol and urine drug screen for the other substances.

The primary outcome assessed post-transplantation was survival. Secondary outcomes measured include graft failure, biopsy proven episodes of acute rejection, psychiatric decompensation defined as the requirement of a psychiatric consultation or new medication, number of readmissions, presence of infection necessitating contact with a healthcare professional, recidivism for alcohol and other substances, and documented caregiver support failure. These outcomes were obtained after reviewing transplant specific electronic medical documentation in the post-transplant period including inpatient records, clinic documentation, and telephone encounters. The databases reviewed that are in use by our institution include Epic electronic medical record and OTTR transplant management system.

### *Statistical analysis*

Descriptive statistics (counts and percentages, means, standard deviations, medians, minimums, and maximums) were used to summarize the data. Graft failure, psychiatric decompensation, presence of infection, and recidivism were measured in a binary fashion. Acute rejection episodes and readmissions were measured as continuous variables. Fisher's exact test was used to look at associations of categorical variables. The Wilcoxon rank sum test was used to compare the number of hospitalizations between the groups. This nonparametric equivalent of the two-sample *t*-test was used due to the skewness of the data. A $P$-value $< 0.05$ was considered statistically significant. All statistical analysis was performed by Elizabeth Lyden, a professional statistician in the department of biostatistics in The College of Public Health at The University of Nebraska Medical Center.

## RESULTS

Our analysis included 391 patients. Demographic data including age, sex, and

etiology of liver disease are summarized in Table 1. The median age of patients undergoing liver transplant was 59 years old (standard deviation: 12.44). Our study included 243 males (62.2%) and 148 females (37.8%). The most common indications for liver transplantation were decompensated cirrhosis secondary to chronic hepatitis C virus ($n$ = 48, 12.3%) followed by non-alcoholic fatty liver disease ($n$ = 47, 12.1%) and alcoholic liver disease ($n$ = 45, 11.6%). There were 141 patients (36.1%) with a history of psychiatric disease. Data regarding psychiatric history is summarized in Table 2. The most common psychiatric diagnosis was major depressive disorder ($n$ = 78, 20%) followed by generalized anxiety disorder ($n$ = 12, 3.1%). There were 92 patients (23.5%) who received therapy for their psychiatric disorders prior to transplant.

There were additional psychosocial comorbidities evaluated in this patient population. These included documented history of financial hardship in the pre-transplant evaluation, history of polysubstance abuse, history of documented non-compliance in the pre-transplant evaluation, and lack of caregiver support. The primary outcome evaluated was overall, 30-d, and 1-year survival post-liver transplant. Secondary outcomes evaluated post-transplant included graft failure, episodes of graft rejection, psychosocial decompensation as previously defined, caregiver support failure, recidivism of alcohol and drug use, number of hospitalizations, and infections necessitating medical care.

For the primary outcome, there were no differences in survival found in the variables compared including history of psychiatric disease, financial hardship, polysubstance abuse, documented compliance issues, or lack of caregiver support. In evaluation of the secondary outcomes, patients with a history of psychiatric disease had a higher incidence of psychiatric decompensation after liver transplantation (19% *vs* 10%, $P$ = 0.013). Treatment of psychiatric disorders resulted in a reduction of the incidence of psychiatric decompensation (21% *vs* 11%, $P$ = 0.022). These results are summarized in Table 3.

Patients with a history of polysubstance abuse in the transplant evaluation had a higher incidence of substance abuse after transplantation (5.8% *vs* 1.2%, $P$ = 0.05). In this specific cohort, 15 patients (3.8%) were found to have medical compliance issues in the transplant evaluation. Of this group, 13.3% were found to have substance abuse after transplantation as opposed to 1.3% in patients without documented compliance issues ($P$ = 0.03). These results are summarized in Table 4. Finally, patients with a history of documented medical non-compliance in the pre-transplantation evaluation had a higher rate of graft failure as opposed to patients without compliance issues (60% *vs* 32%, $P$ = 0.047). There were no other significant differences found in the other secondary outcomes evaluated. These results are summarized in Table 5.

## DISCUSSION

A liver transplantation is a high-risk endeavor and thus requires a thorough medical and psychosocial evaluation. Although we did not find any difference in regard to the primary outcome of survival, we believe this study adds to the literature that psychosocial comorbidities have a significant impact on outcomes in liver transplantation as certain psychosocial entities were associated with worse outcomes after liver transplantation. Patients with documented non-compliance in the pre-transplant evaluation had worse outcomes after organ transplantation including a higher incidence of graft failure and recidivism. These findings have been previously seen in relation to renal transplantation but have not been previously documented in respect to liver transplantation to our knowledge[20]. This represents a very high-risk population and further prospective and potentially multi-center work is required to create compliance guidelines prior to listing for transplantation.

Psychiatric disease commonly coexists with chronic liver disease with some estimates of up to 50% of patients with cirrhosis suffer from psychiatric disorders[21]. Although previous studies have shown that major depression is associated with a decreased survival, our study did not corroborate these findings[14,16]. However, our study did find that patients with psychiatric disorders are at higher risk of psychiatric decompensation after liver transplant regardless of how compensated their psychiatric disease status was prior to evaluation. In addition, our findings show that treatment of psychiatric disease was shown to decrease the incidence of psychiatric decompensation after transplantation and thus improve outcomes. This study substantiates several previous studies that psychiatric disorders affect outcomes after liver transplantation. As psychiatric disorders are commonly encountered in patients with end-stage liver disease, this represents a high-risk population. Further prospective studies are required to best optimally manage this group in the pre-

**Table 1 Demographic data**

| | Value | % |
|---|---|---|
| Median age | 59.30 | 12.44 |
| Sex: Male (%): Female (%) | 243 (62.15) | 148 (37.85) |
| Etiology of liver disease | | |
| Alcohol | 45 | 11.54 |
| Nonalcoholic steatohepatitis | 47 | 12.05 |
| Hepatitis C | 48 | 12.31 |
| Hepatitis B | 1 | 0.26 |
| Hepatocellular carcinoma | 1 | 0.26 |
| Autoimmune hepatitis | 20 | 5.13 |
| Cryptogenic | 1 | 0.26 |
| Multi-visceral | 14 | 3.59 |
| Primary sclerosing cholangitis | 25 | 6.41 |
| Primary biliary cholangitis | 12 | 3.08 |
| Alpha-1-antitrypsin-Deficiency | 5 | 1.28 |
| Hepatic vein thrombosis | 2 | 0.51 |
| Hemochromatosis | 1 | 0.26 |
| Total parental nutrition related | 1 | 0.26 |
| Polycystic liver disease | 1 | 0.26 |
| Familial intrahepatic cholestasis | 1 | 0.26 |
| Cholangiocarcinoma | 2 | 0.51 |
| Primary oxaluria | 3 | 0.77 |
| Multiple | 161 | 41.0 |

transplant setting in order to improve outcomes after liver transplantation.

Our study has limitations associated with any retrospective analysis and is subject to confounding factors that were unable to be measured. In addition, the majority of our data was gathered from pre- and post-transplantation documentation thus imparting a level of provider subjectivity in determining certain variables such as psychiatric diagnoses, medical non-compliance, and issues with caregiver support. There is an inherent limitation in ascertaining data retrospectively. However, this is common practice during the transplantation evaluation thus emulating traditional practice. Regardless, this analysis adds valuable information to the growing literature regarding the importance of focusing on psychosocial comorbidities prior to liver transplantation confirming available data that this represents a high-risk population. Further prospective and potentially multi-center studies are warranted to properly determine appropriate guidelines for liver transplantation specifically regarding psychiatric disease, documented medical non-compliance, financial issues, and substance abuse.

**Table 2 Psychiatric history**

| Psychiatric diagnosis | Value | % |
|---|---|---|
| None | 250 | 63.94 |
| Major depression | 78 | 19.95 |
| Generalized anxiety | 12 | 3.07 |
| Bipolar disorder | 5 | 1.28 |
| Attention deficit disorder | 1 | 0.26 |
| Panic disorder | 1 | 0.26 |
| Conversion disorder | 1 | 0.26 |
| Multiple | 43 | 10.98 |

**Table 3 Psychiatric decompensation**

| | | Psychosocial decompensation | | |
|---|---|---|---|---|
| | | No | Yes | Total |
| Psychiatric history | No | 225 (90.0) | 25 (10.0) | 250 |
| | Yes | 114 (80.85) | 27 (19.15) | 141 |
| | Total | 339 | 52 | 391 |
| $P = 0.013$ | | | | |
| Psychiatric treatment | No | 266 (88.96) | 33 (11.04) | 299 |
| | Yes | 73 (79.35) | 19 (20.65) | 92 |
| | Total | 339 | 52 | 391 |
| $P = 0.022$ | | | | |

**Table 4 Recidivism**

| | | Recidivism | | |
|---|---|---|---|---|
| | | No | Yes | Total |
| Substance use | No | 334 (98.82) | 4 (1.18) | 338 |
| | Yes | 49 (94.23) | 3 (5.77) | 52 |
| | Total | 383 | 7 | 390 |
| $P = 0.05$ | | | | |
| Non-compliance | No | 371 (98.67) | 5 (1.33) | 376 |
| | | 13 (86.67) | 2 (13.33) | 15 |
| | | 384 | 7 | 391 |
| $P = 0.03$ | | | | |

**Table 5 Graft failure**

| | | Graft failure | | |
|---|---|---|---|---|
| | | No | Yes | Total |
| Non-compliance | No | 253 (67.29) | 123 (32.71) | 376 |
| | Yes | 6 (40.0) | 9 (60.0) | 15 |
| | Total | 259 | 132 | 391 |
| $P = 0.047$ | | | | |

## ARTICLE HIGHLIGHTS

### Research background

Liver transplantation is the accepted standard of care for end-stage liver disease due to a variety of etiologies including decompensated cirrhosis, fulminant hepatic failure, and primary hepatic malignancy. There are currently over 13000 candidates on the liver transplant waiting list emphasizing the importance of rigorous patient selection. There are few studies regarding the impact of additional psychosocial barriers to liver transplant including financial hardship, lack of caregiver support, polysubstance abuse, and issues with medical non-compliance. We hypothesized that patients with certain psychosocial comorbidities experienced worse outcomes after liver transplantation.

### Research motivation

There are certain accepted criteria to list patients for liver transplantation such as model for end-state liver disease score, age, and body-mass-index. Many patients with liver disease have significant psychosocial comorbidities that may impact outcomes after liver transplantation. There are no evidence-based guidelines regarding psychosocial aspects of the liver transplant evaluation.

### Research objectives

The main objective of this study was to assess the impact of certain pre-transplant psychosocial comorbidities on outcomes after liver transplantation. We found that certain psychosocial comorbidities led to worse outcomes after transplantation.

### Research results

For the primary outcome, there were no differences in survival. Patients with a history of psychiatric disease had a higher incidence of psychiatric decompensation after liver transplantation (19% *vs* 10%, $P$ = 0.013). Treatment of psychiatric disorders resulted in a reduction of the incidence of psychiatric decompensation (21% *vs* 11%, $P$ = 0.022). Patients with a history of polysubstance abuse in the transplant evaluation had a higher incidence of substance abuse after transplantation (5.8% *vs* 1.2%, $P$ = 0.05). In this cohort 15 patients (3.8%) were found to have medical compliance issues in the transplant evaluation. Of these specific patients, 13.3% were found to have substance abuse after transplantation as opposed to 1.3% in patients without documented compliance issues ($P$ = 0.03).

### Research conclusions

Patients with a history of psychiatric disease had a higher incidence of psychiatric decompensation. Treatment of psychiatric disorders led to a reduction of the incidence of psychiatric decompensation after liver transplantation. Patients with a history of polysubstance abuse and medical non-compliance had a higher incidence of substance use after liver transplantation. This study adds to the literature that this represents a high-risk population. Further multi-center and prospective studies are warranted to formulate evidence-based guidelines to assist in evaluating patients undergoing evaluation for liver transplantation.

### Research perspectives

This study highlights the importance of the psychosocial evaluation in the liver transplantation process.

## REFERENCES


1 **Varma V**, Mehta N, Kumaran V, Nundy S. Indications and contraindications for liver transplantation. *Int J Hepatol* 2011; **2011**: 121862 [PMID: 22007310 DOI: 10.4061/2011/121862]

2 **United Network for Organ Sharing (UNOS)**. Transplant trends: transplants by organ type 2012-2016. [Accessed 2019 May]. Available from: URL: https://www.unos.org/data/transplanttrends/#transplants_by_organ_type+year+2016

3 **Joshi D**, O'Grady J, Taylor C, Heaton N, Agarwal K. Liver transplantation in human immunodeficiency virus-positive patients. *Liver Transpl* 2011; **17**: 881-890 [PMID: 21563295 DOI: 10.1002/lt.22329]

4 **Stepanova M**, Sayiner M, de Avila L, Younoszai Z, Racila A, Younossi ZM. Long-term outcomes of liver transplantation in patients with hepatitis C infection are not affected by HCV positivity of a donor. *BMC Gastroenterol* 2016; **16**: 137 [PMID: 27846801 DOI: 10.1186/s12876-016-0551-z]

5 **Song GW**, Lee SG. Living donor liver transplantation. *Curr Opin Organ Transplant* 2014; **19**: 217-222 [PMID: 24807211 DOI: 10.1097/MOT.0000000000000088]

6 **Mathurin P**, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, Castel H, Duhamel A, Pageaux GP, Leroy V, Dharancy S, Louvet A, Boleslawski E, Lucidi V, Gustot T, Francoz C, Letoublon C, Castaing D, Belghiti J, Donckier V, Pruvot FR, Duclos-Vallée JC. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med* 2011; **365**: 1790-1800 [PMID: 22070476 DOI: 10.1056/NEJMoa1105703]

7 **Im GY**, Kim-Schluger L, Shenoy A, Schubert E, Goel A, Friedman SL, Florman S, Schiano TD. Early Liver Transplantation for Severe Alcoholic Hepatitis in the United States--A Single-Center Experience. *Am J Transplant* 2016; **16**: 841-849 [PMID: 26710309 DOI: 10.1111/ajt.13586]

8 **United Network for Organ Sharing (UNOS)**. Organ Candidate Waiting list. [Accessed 2019; May] Available from: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/#

9 **Wiesner R**, Edwards E, Freeman R, Harper A, Kim R, Kamath P, Kremers W, Lake J, Howard T, Merion RM, Wolfe RA, Krom R; United Network for Organ Sharing Liver Disease Severity Score Committee. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; **124**: 91-96 [PMID: 12512033 DOI: 10.1053/gast.2003.50016]

10 **Maldonado JR**, Dubois HC, David EE, Sher Y, Lolak S, Dyal J, Witten D. The Stanford Integrated Psychosocial Assessment for Transplantation (SIPAT): a new tool for the psychosocial evaluation of pre-transplant candidates. *Psychosomatics* 2012; **53**: 123-132 [PMID: 22424160 DOI: 10.1016/j.psym.2011.12.012]

11 **Tandon P**, Goodman KJ, Ma MM, Wong WW, Mason AL, Meeberg G, Bergsten D, Carbonneau M, Bain VG. A shorter duration of pre-transplant abstinence predicts problem drinking after liver transplantation. *Am J Gastroenterol* 2009; **104**: 1700-1706 [PMID: 19471253 DOI: 10.1038/ajg.2009.226]

12 **Nair S**, Verma S, Thuluvath PJ. Obesity and its effect on survival in patients undergoing orthotopic liver transplantation in the United States. *Hepatology* 2002; **35**: 105-109 [PMID: 11786965 DOI: 10.1053/jhep.2002.30318]

13 **Rosenberger EM**, Dew MA, Crone C, DiMartini AF. Psychiatric disorders as risk factors for adverse medical outcomes after solid organ transplantation. *Curr Opin Organ Transplant* 2012; **17**: 188-192 [PMID: 22277955 DOI: 10.1097/MOT.0b013e3283510928]

14 **Guimaro MS**, Lacerda SS, Karam CH, Ferraz-Neto BH, Andreoli PB. Psychosocial profile of patients on the liver transplant list. *Transplant Proc* 2008; **40**: 782-784 [PMID: 18455016 DOI: 10.1016/j.transproceed.2008.02.040]

15 **O'Carroll RE**, Couston M, Cossar J, Masterton G, Hayes PC. Psychological outcome and quality of life following liver transplantation: a prospective, national, single-center study. *Liver Transpl* 2003; **9**: 712-720 [PMID: 12827558 DOI: 10.1053/jlts.2003.50138]

16 **Rogal SS**, Mankaney G, Udawatta V, Chinman M, Good CB, Zickmund S, Bielefeldt K, Chidi A, Jonassaint N, Jazwinski A, Shaikh O, Hughes C, Fontes P, Humar A, DiMartini A. Pre-Transplant Depression Is Associated with Length of Hospitalization, Discharge Disposition, and Survival after Liver Transplantation. *PLoS One* 2016; **11**: e0165517 [PMID: 27820828 DOI: 10.1371/journal.pone.0165517]

17 **Gazdag G**, Horváth GG, Makara M, Ungvari GS, Gerlei Z. Predictive value of psychosocial assessment for the mortality of patients waiting for liver transplantation. *Psychol Health Med* 2016; **21**: 525-529 [PMID: 26549304 DOI: 10.1080/13548506.2015.1109670]

18 **Rogal SS**, Landsittel D, Surman O, Chung RT, Rutherford A. Pretransplant depression, antidepressant use, and outcomes of orthotopic liver transplantation. *Liver Transpl* 2011; **17**: 251-260 [PMID: 21384507 DOI: 10.1002/lt.22231]

19 **Jowsey SG**, Taylor ML, Schneekloth TD, Clark MM. Psychosocial challenges in transplantation. *J Psychiatr Pract* 2001; **7**: 404-414 [PMID: 15990553]

20 **Schweizer RT**, Rovelli M, Palmeri D, Vossler E, Hull D, Bartus S. Noncompliance in organ transplant recipients. *Transplantation* 1990; **49**: 374-377 [PMID: 2305467]

21 **Burra P**, Germani G, Gnoato F, Lazzaro S, Russo FP, Cillo U, Senzolo M. Adherence in liver transplant recipients. *Liver Transpl* 2011; **17**: 760-770 [PMID: 21384527 DOI: 10.1002/lt.22294]

World Journal of Hepatology

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

*World J Hepatol* 2019 August 27; 11(8): 646-655

DOI: 10.4254/wjh.v11.i8.646

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

# Outpatient telephonic transitional care after hospital discharge improves survival in cirrhotic patients

Bhavana Bhagya Rao, Anastasia Sobotka, Rocio Lopez, Carlos Romero-Marrero, William Carey

**ORCID number:** Bhavana Bhagya Rao (0000-0002-5120-4551); Anastasia Sobotka (0000-0002-4493-8215); Rocio Lopez (0000-0002-4319-420X); Carlos Romero-Marrero (0000-0001-8599-9906); William Carey (0000-0002-0409-3748).

**Author contributions:** Rao BB contributed to study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, and drafting of the manuscript; Sobotka A contributed to implementation of intervention, acquisition of data, and critical revision of the manuscript for important intellectual content; Lopez R contributed to statistical analysis; Romero-Marrero C contributed to critical revision of the manuscript for important intellectual content and study supervision; Carey W contributed to study concept and design, critical revision of the manuscript for important intellectual content, and study supervision.

**Institutional review board statement:** The study was reviewed and approved by the Cleveland Clinic Foundation Institutional Review Board.

**Informed consent statement:** After review of the study characteristics, a waiver of informed consent was approved by the Cleveland Clinic Foundation Institutional Review Board.

**Conflict-of-interest statement:** No potential conflicts of interest relevant to this article were reported.

**Bhavana Bhagya Rao, Anastasia Sobotka, Rocio Lopez, Carlos Romero-Marrero, William Carey,** Department of Gastroenterology Hepatology and Nutrition, Cleveland Clinic, Cleveland, OH 44195, United States

**Corresponding author:** William Carey, MD, Doctor, Department of Gastroenterology Hepatology and Nutrition, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, United States. careyw@ccf.org
**Telephone:** +1-216-4446521
**Fax:** +1-216-4455477

## Abstract

### *BACKGROUND*

Intervention to improve outcomes in cirrhotic patients (CP) after hospital discharge often focus on 30 d readmission rate (RR). However, recent studies suggest dissociation between RR and survival. At our center, CP are now offered outpatient telephonic transitional care (OTTC) by a care coordinator for 30 d after hospital discharge.

### *AIM*

To determine the effect of OTTC on survival in CP.

### *METHODS*

In this cohort study from a tertiary center, CP who received OTTC formed the intervention group. They were compared with a control group discharged during the same period. Mortality and RR were compared between the groups.

### *RESULTS*

After OTTC introduction, 194 CP were discharged. After applying exclusion criteria, 169 CP (51% male, mean age 58 years ± 12 years) were included. OTTC group comprised 76 patients and was compared with 93 controls. Baseline disease and index admission related characteristics were not significantly different between the groups. The intervention group showed significantly higher 6 mo survival compared to controls (84.2% *vs* 68.8%; $P = 0.03$), while RR at 1, 3, and 6 mo were comparable. On multivariable analysis, the intervention group showed lower odds for mortality compared to the controls (hazard ratio: 0.4; 95% confidence interval: 0.2-0.82; $P = 0.012$), while higher model for end-stage liver disease scores were associated with higher mortality (hazard ratio: 1.05; 95% confidence interval: 1.01-1.1; $P = 0.024$).

### *CONCLUSION*

**Manuscript source**: Unsolicited manuscript

**Received:** January 29, 2019
**Peer-review started:** January 29, 2019
**First decision:** March 3, 2019
**Revised:** June 12, 2019
**Accepted:** July 4, 2019
**Article in press:** July 5, 2019
**Published online:** August 27, 2019

**P-Reviewer:** Jamali R, Ong J, Zhu YY
**S-Editor:** Cui LJ
**L-Editor:** Filipodia
**E-Editor:** MaYJ

CP provided OTTC had higher 6 mo survival compared to controls without a difference in RR. Use of RR to gauge quality of care provided during hospitalization or subsequent transitional care programs should be revisited.

**Key words:** Quality improvement; Transitional care; Outpatient monitoring; Outcomes assessment



**Core tip:** We share results of a novel intervention that provides transitional care *via* telephone to cirrhotic patients after hospital discharge. Over a 6 mo follow-up, the intervention group experienced 60% lower odds for mortality compared to controls with similar readmission rates. Our manuscript not only describes an effective transitional care program to improve post-discharge outcomes in cirrhotic patients but also highlights the need to acknowledge the dissociation between readmissions and survival in this population.

**Citation:** Rao BB, Sobotka A, Lopez R, Romero-Marrero C, Carey W. Outpatient telephonic transitional care after hospital discharge improves survival in cirrhotic patients. *World J Hepatol* 2019; 11(8): 646-655
**URL**: https://www.wjgnet.com/1948-5182/full/v11/i8/646.htm
**DOI**: https://dx.doi.org/10.4254/wjh.v11.i8.646

## INTRODUCTION

Cirrhosis leads to over 150000 hospitalizations at an annual cost of nearly $4 billion in the United States[1,2]. There is growing awareness and concern regarding the high rates of readmission, which constitutes a significant medical, psychosocial, and financial burden[3-5]. A large prospective study involving 14 tertiary-care hepatology centers in the United States and Canada noted that 53% of cirrhotic patients (CP) experience at least one readmission within 3 mo of hospital discharge (HD)[6]. Readmission rate (RR) has been proposed as a national quality indicator and a factor that could gauge organizational performance and determine rates of reimbursement[3]. However, limiting readmissions in patients with advanced disease and complex medical conditions is challenging and not always in their best interest. Indeed, some have suggested that a reduction in readmissions may prejudice survival[7,8].

A few have tested the utility of adopting specialized interventions for reducing RR in CP. These include use of electronic checklists for discharge[9], intensive monitoring by a nurse practitioners after discharge[8], providing early outpatient follow-up[7], or creation of a dedicated outpatient hepatology caregiver team along with setting up of an outpatient paracentesis clinic[10]. While all the studies noted an improvement in adherence to medications and follow-up clinic visits with the interventions, the rate of readmissions remained unchanged[8] or even increased[7] despite a reduction in mortality. These findings reflect both efficacy of the intervention and dissociation between RR and survival.

At our center, outpatient telephonic transitional care (OTTC) was introduced with the goal of improving post hospitalization outcomes in CP. The primary objective of this study was to determine the effect of OTTC on survival at 1, 3, and 6 mo after HD in CP. The secondary objective was to determine the effect of OTTC on RR at 1, 3, and 6 mo after HD and explore the relationship of RR to survival.

## MATERIALS AND METHODS

### *Study design*

At our tertiary care center, the OTTC program was introduced on March 1, 2016. It is delivered by a dedicated nurse care coordinator. The program is offered to CP for a period of 30 d after HD, provided the patients are not being discharged to hospice care. The OTTC program involves telephone based follow-up, active monitoring of diagnostic tests, coordination of outpatient care, and disease and medication related counseling. In the pilot phase of this program due to limited manpower, the OTTC

program was only offered to CP who were deemed at high risk for readmission. This determination was made by the multi-disciplinary inpatient hepatology team prior to discharge. A registry of all the patients who received OTTC care was maintained. Standard of care treatment was continued for all study patients during their inpatient and transitional care period and the OTTC program was offered as an additional intervention to selected patients.

### Patient selection

Hospital administrative data was surveyed to obtain a list of all the CP discharged from the inpatient hepatology service on our main campus facility between March 1 and December 31, 2016. All patients discharged within 2 mo since OTTC initiation were excluded from analysis because the tenets of the program were being actively modified and improved during this preliminary period, after which all the protocols were finalized. All patients were followed up for a 6 mo period after index hospitalization. Patients who had readmissions to the hospital for liver transplantation or readmission for reasons unrelated to underlying liver disease during the follow-up were excluded. Patients who were lost to all healthcare contact with any of our facilities in the follow-up period were excluded because no determination of their readmission or survival status could be reliably made. Among all the CP, those who received OTTC formed the intervention group and those who were discharged during the same period without the OTTC intervention formed the control group.

### Data collection

Chart review was done to obtain demographic data (gender, sex, insurance coverage), details regarding liver disease [etiology, related complications, model for end-stage liver disease (MELD) score], medications, laboratory, imaging, and endoscopic data for all study patients. Characteristics of index and subsequent hospitalizations including reason for admission, medical problems addressed during hospitalization, length of stay, and destination at discharge were recorded. While the OTTC program was provided to only the CP being discharged from our main campus, readmissions were tracked both to our main campus and satellite facilities. Details regarding scheduling, timing, and adherence to post discharge follow-up appointments in the hepatology clinic and at the paracentesis procedure unit were obtained.

Rates of actuarial survival at 1, 3, and 6 mo after index HD was compared between the intervention and control group. In addition, unplanned RR at 1, 3, and 6 mo after index hospitalization were also compared between the groups.

### Statistical analysis

Data are presented as mean ± standard deviation, median ($25^{th}$, $75^{th}$ percentiles), or frequency (percent). A univariable analysis was performed to assess differences between the two groups. Non-parametric Kruskal-Wallis tests were used to compare continuous or ordinal variables, and Pearson's chi-square tests were used for categorical factors. Follow-up time was defined as months since initial discharge to the first of readmission or death, and subjects were censored at 6 mo if still alive without readmission. Readmission and death were treated as competing events and cumulative incidence of readmission was estimated using the Fine and Gray competing risks model. In addition, multivariable Cox regression analysis was performed to assess factors associated with mortality. An automated stepwise variable selection was used to choose the final models. Survival analysis was done to assess differences in overall survival between the groups. All analyses were performed using SAS (version 9.4, The SAS Institute, Cary, NC, United States), and a $P < 0.05$ was considered as statistically significant.

## RESULTS

Between May 1 and December 31, 2016, 194 CP were discharged from the inpatient hepatology service. A total of 169 CP (51% male, mean age 58 ± 12 years) formed the study cohort with the intervention and control groups having 76 (45%) and 93 (55%) patients, respectively. Flowchart describing study cohort selection is depicted in Figure 1.

Common etiologies for cirrhosis in the cohort were alcoholic (32.5%) and non-alcoholic fatty liver disease (23.7%) with average MELD score during index hospitalization being 18. Medical complications including hepatic encephalopathy, infections, acute kidney injury, and gastrointestinal bleeding were each addressed in approximately a third of the cohort during index hospitalization, which spanned a median 5 d. The intervention and control groups showed no significant difference

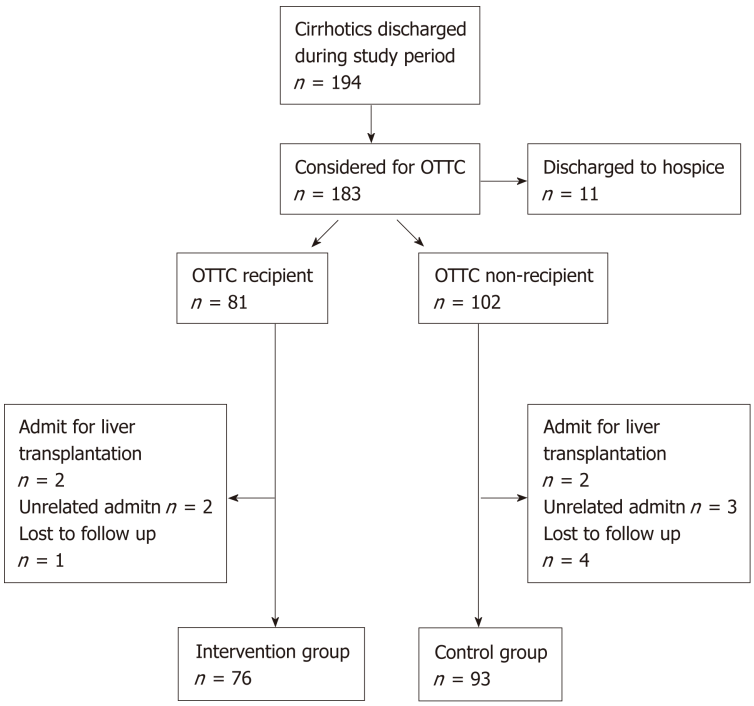


**Figure 1 Flowchart showing study cohort selection.** OTTC: Outpatient telephonic transitional care program.

with regards to baseline disease or index hospitalization related characteristics (Table 1).

A follow-up appointment in the outpatient hepatology clinic was provided prior to HD to 83% of the cohort. Median duration to appointment was 14 d and adherence was noted in 59 (35%) patients. The proportion of patients with follow-up scheduled at discharge and those who showed adherence to it were comparable in the intervention and control groups.

Unplanned hospital readmissions were noted in 37%, 55%, and 63% of the cohort at 1, 3, and 6 mo after index HD, respectively. The median length of re-hospitalization was 6 d. Rates of readmission at each of the intervals were comparable between the intervention and control groups. Median time to readmit was 24 d for the cohort, which was also similar between the two groups. Kaplan-Meier curves comparing RR ($P$ = 0.82) between the two groups are depicted in Figure 2A.

Survival at 1, 3, and 6 mo for the cohort was 92%, 81%, and 76%, respectively. The causes of death in the cohort were septic shock ($n$ = 26), acute renal failure and dyselectrolytemia ($n$ = 4), acute respiratory failure ($n$ = 4), gastrointestinal bleeding ($n$ = 2), cardiac arrhythmia ($n$ = 2), intra-cranial hemorrhage ($n$ = 2), and pulmonary embolism ($n$ = 1). The intervention group showed a tendency towards greater survival compared to the controls at 1 mo (95% *vs* 90%; $P$ = 0.39) and 3 mo (87% *vs* 76%; $P$ = 0.11). This difference met statistical significance at 6 mo (84% *vs* 69%; $P$ = 0.03). Kaplan-Meier curves comparing survival ($P$ = 0.03) for the two groups are depicted in Figure 2B.

On multivariable analysis of demographic, disease, and hospitalization related characteristics only two factors showed a significant association with mortality (Table 2). Patients in the intervention group showed a hazard ratio of 0.4 (95% confidence interval: 0.2-0.82) for mortality when compared to the control group ($P$ = 0.012). Also, with every 1 unit increase in MELD score the hazard for mortality increased 1.05 times (95% confidence interval: 1.01-1.1; $P$ = 0.024). None of the factors showed any significant association with readmissions on multivariate analysis (Table 3).

## DISCUSSION

We demonstrate the value of an outpatient telephone based transitional care program in improving post HD survival in CP. CP who received the intervention were 60% less likely to die than patients in the control group during the 6 mo follow-up. This survival benefit was independent of an effect on RR demonstrating dissociation between these outcomes and raising awareness on the need to reconsider the parameters in use for gauging quality of care provided during hospitalization and

**Table 1 Comparison of baseline demographic and disease related characteristics between the groups**

| Factor | Total, *n* = 169 | Intervention, *n* = 76 | Control, *n* = 93 | *P* value |
|---|---|---|---|---|
| Male | 86 (50.9) | 41 (53.9) | 45 (48.4) | 0.54 |
| Age | 58.2 ± 12.0 | 58.6 ± 11.4 | 57.9 ± 12.6 | 0.75 |
| Etiology of cirrhosis | | | | |
| Alcohol | 55 (32.5) | 27 (35.5) | 28 (30.1) | |
| NAFLD | 40 (23.7) | 18 (23.7) | 22 (23.7) | |
| HCV | 27 (16.0) | 11 (14.5) | 16 (17.2) | |
| HBV | 2 (1.2) | 0 | 2 (2.2) | 0.49 |
| Combination of above | 5 (3.0) | 1 (1.3) | 4 (4.3) | |
| Others | 40 (24.9) | 19 (25.0) | 21 (22.6) | |
| MELD score during index admission | 17.7 ± 7.9 | 17.8 ± 8.6 | 17.5 ± 7.3 | 0.88 |
| Problems during initial hospitalization | | | | |
| HE | 64 (37.9) | 34 (44.7) | 30 (32.3) | 0.11 |
| Infection | 51 (30.2) | 23 (30.3) | 28 (30.1) | 1 |
| AKI | 64 (37.9) | 30 (39.5) | 34 (36.6) | 0.75 |
| GIB | 57 (33.7) | 28 (36.8) | 29 (31.2) | 0.51 |
| Index admission LOS, d | 5 (3.0, 9.0) | 6 (3.0, 10.5) | 5 (4.0, 9.0) | 0.95 |
| Discharge destination | | | | 0.43 |
| Home | 100 (59.2) | 41 (53.9) | 59 (63.4) | |
| Home with care | 35 (20.7) | 17 (22.4) | 18 (19.4) | |
| Skilled nursing facility | 34 (20.1) | 18 (23.7) | 16 (17.2) | |
| Follow up appointment provided prior to discharge | 138 (82.9) | 63 (82.9) | 75 (80.6) | 0.53 |
| Came for appointment | | | | |
| Yes | 59 (34.9) | 27 (35.5) | 32 (34.4) | 0.98 |
| No | 63 (37.3) | 29 (38.2) | 34 (36.6) | |
| No appointment provided | 30 (17.8) | 13 (17.1) | 17 (18.3) | |
| Admitted before appointment | 16 (9.5) | 7 (9.2) | 9 (9.7) | |
| Died before appointment | 1 (0.6) | 0 | 1 (1.1) | |
| Duration between discharge and appointment | 14 (7, 28) | 14 (7, 28) | 13 (9, 23) | 0.79 |
| Readmissions within | | | | |
| 1 mo | 63 (37.3) | 28 (36.8) | 35 (37.6) | 1 |
| 3 mo | 93 (55.0) | 43 (56.6) | 51 (54.8) | 0.76 |
| 6 mo | 107 (63.3) | 49 (64.5) | 58 (62.4) | 0.87 |
| Problems during initial readmission | | | | |
| HE | 36 (21.3) | 21 (42.9) | 15 (26.0) | 0.11 |
| Infection | 28 (16.6) | 14 (28.6) | 14 (24.1) | 1 |
| AKI | 47 (27.8) | 22 (44.9) | 25 (43.1) | 1 |
| GIB | 17 (10.1) | 6 (12.2) | 11 (19.0) | 0.3 |
| Time between discharge and readmit, d | 24 (11, 66) | 22 (11, 59) | 23.5 (12, 57) | 0.63 |
| Readmission LOS, d | 6 (3, 10) | 5 (3, 8) | 6 (4, 12) | 0.06 |
| Alive at | | | | |
| 1 mo | 156 (92.3) | 72 (94.7) | 84 (90.3) | 0.39 |
| 3 mo | 137 (81.1) | 66 (86.8) | 71 (76.3) | 0.11 |
| 6 mo | 128 (75.7) | 64 (84.2) | 64 (68.8) | 0.03[1] |

Statistics presented as median (P25, P75) or *n* (column %).
[1]*P* < 0.05 considered significant. HCV: Hepatitis C virus; HBV: Hepatitis B virus; NAFLD: Non-alcoholic fatty liver disease; MELD: Model for end-stage liver disease; HE: Hepatic encephalopathy; AKI: Acute kidney injury; GIB: Gastrointestinal bleeding; LOS: Length of stay.

subsequent transitional care programs.

Multiple studies demonstrate high RR among CP, which not only levy a financial burden but also negatively impact patient satisfaction, quality of life, and access to liver transplantation[5,11-16]. The most frequent reasons for readmissions such as recurrent hepatic encephalopathy, renal injury, symptomatic ascites, or nosocomial infections are potentially modifiable[4,6,11,13,15-19]. Data from the North American

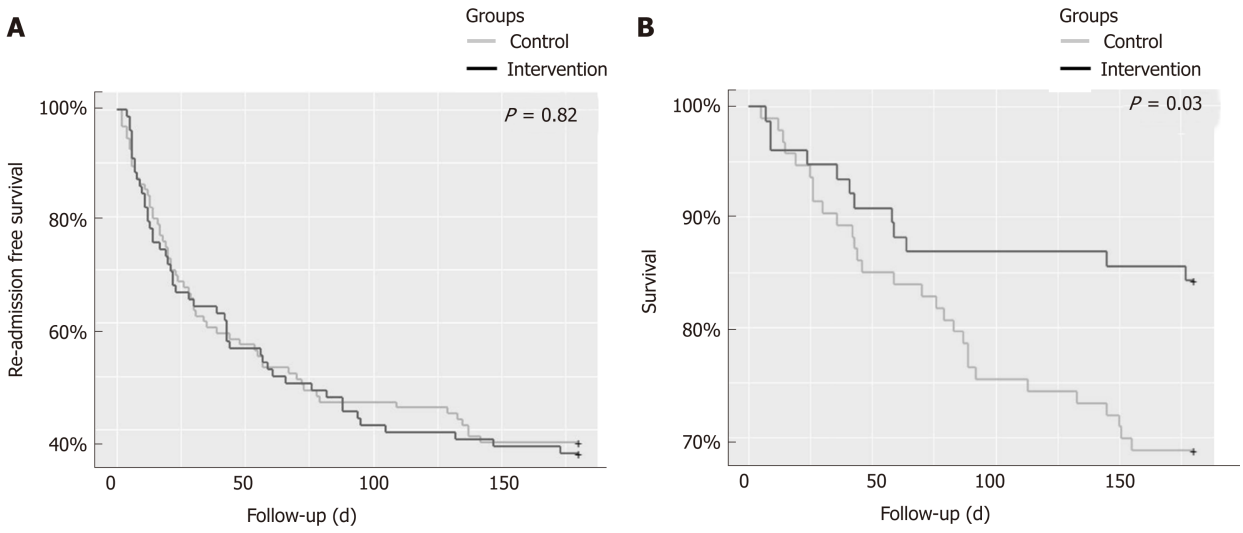


**Figure 2 Kaplan-Meier plot comparing (A) readmission rates and (B) survival between the intervention and control group.**

Consortium for the Study of End Stage Liver Diseases showed that more than half of the 1013 study patients were readmitted within 3 mo[6]. Overall 31% had one readmit while 22% patients had two or more. A model based on MELD score, proton pump inhibitor used, and length of stay was developed to try to predict the risk of readmission, but it was not effective in 30% of cases. This suggests that new unexpected changes that developed in the early post discharge period influence patient outcomes. These results make a strong argument for close monitoring of patients in the post discharge period and facilitation of post discharge communication between the patients and healthcare professionals[6,16,20].

RR has been adopted as a key quality measure and reimbursement determinant in some chronic medical conditions (*e.g.*, heart failure and chronic obstructive pulmonary disease) with a suggestion to include cirrhosis as well in this realm[6]. However, in a large nationwide study that assessed the impact of the Hospital Readmissions Reduction program[21] on outcomes in 115245 patients admitted with heart failure, the rates of both 30 d and 1 yr risk adjusted mortality were found to be markedly increased despite a reduction in readmissions[22]. Thus, there is serious concern over the focus on RR and its reduction and the possible unintended consequences on patient survival in patients with complex disease states[23,24].

Kanwal *et al*[5] reported results from 122 Veteran Affairs hospitals where CP were offered a follow-up appointment in the hepatology clinic within 7 d of HD. In a 30 d follow-up period, the intervention group was noted to have 1.1 times higher odds for readmission when compared to controls. However, the intervention group showed 40% lower risk for 30 d mortality. This survival benefit has been hypothesized to be secondary to improved coordination of care, better communication with patients, timely adjustment of medications, follow up of outstanding tests, and enabling early readmission when warranted. These factors and efforts are common to our OTTC program and may serve as rationale for the survival benefit noted with our intervention as well.

Tapper *et al*[9] studied the impact of using checklists at discharge to address appropriate medication use in CP. They noted a 40% reduction in 30 d readmissions; however, 90 d mortality rates were unchanged. It is hypothesized that while improvements in care provided during the hospitalization and at the time of discharge can reduce short term readmits, a more long lasting favorable impact on survival cannot be obtained without close outpatient transitional care. Yet other studies, which focused on setting up robust outpatient caregiver teams for monitoring CP after discharge showed conflicting outcomes. However, their results may have been limited by small sample size[8,10]. A comparison of these studies with ours is offered in Table 4.

At our center, the OTTC was designed to provide individualized, patient specific care and monitor them closely for an additional 30 d after HD. CP often have complex medical needs with rapidly fluctuating parameters and are at high risk for developing multiple complications including infections, renal injury, dyselectroylytemia, or gastrointestinal bleeding. Recurrent hepatic encephalopathy is easily precipitated by any of the above complications or non-adherence to lactulose. After discharge, monitoring these sick patients closely and coordinating their outpatient care,

**Table 2 Multivariate analysis of factors associated with mortality**

| | Multivariate analysis | |
|---|---|---|
| **Factor** | **HR (95%CI)** | ***P* value** |
| Intervention *vs* controls | 0.4 (0.2-0.82) | 0.012 |
| Age | 1.01 (0.99-1.04) | 0.503 |
| Female gender | 1.2 (0.6-2.4) | 0.605 |
| Etiology of cirrhosis | | |
| EtOH *vs* HCV | 0.72 (0.29-1.8) | 0.48 |
| NAFLD *vs* HCV | 0.41 (0.24-2.1) | 0.088 |
| MELD score (for every 1 unit increment) | 1.05 (1.01-1.1) | 0.024 |
| Index hospitalization length of stay (for every 1 d increment) | 0.99 (0.95-1.02) | 0.47 |
| Discharge to home with home care *vs* home | 1.65 (0.75-3.64) | 0.212 |
| Discharge to SNF *vs* home | 1.4 (0.56-3.48) | 0.47 |

HR: Hazard ratio; CI: Confidence interval; EtOH: Alcohol; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; MELD: Model for end-stage liver disease; SNF: Skilled nursing facility.

especially for patients who live at great distances from our tertiary referral center, can be challenging for the primary hepatologists. In this regard, having a care coordinator to actively follow up and order additional outpatient diagnostic tests, arrange follow-up visits or timely referrals to specialists, facilitate readmissions when complications arise, and provide medication and disease related counselling to the patients serves as a great source of support for patients, primary hepatologists, and local physicians alike. While these interventions are similar to that suggested in the study by Wigg *et al*[8], with our larger cohort size and tracking of long term outcomes, a clear survival benefit could be discerned. We hypothesize that the OTTC has no appreciable effect on RR because often the medical complications that develop in decompensated CP cannot be safely managed in an ambulatory setting, and hence readmissions are unavoidable and even beneficial in the care of these ill patients. Early identification of development of complications by the care coordinator may have prompted readmissions, and this in turn may have played a role in mediating the survival benefit. Hence, we argue that the focus of judging quality of CP care should shift away from RR.

Despite its several strengths our study is not without its limitations. This is a single center, retrospective analysis. There is a degree of selection bias because only the CP deemed high risk for readmission were offered OTTC. This determination may have been subjective; however, it was made by the multi-disciplinary inpatient care team after careful consideration of a wide variety of medico-social conditions. One could argue that despite being a higher risk patient group, the intervention improved survival. Expanding the OTTC to include all CP would be the ideal next step in assessing this intervention. Also, because the OTTC interventions were individualized to each patient's specific needs, the individual interventions were not quantified and compared during the analysis.

In conclusion, CP provided OTTC had a higher 6 mo survival compared to controls despite RR being comparable to controls. Tenets of OTTC that mediate this benefit should be studied, and the potential expansion of OTTC merits explored. The varied impact of the different interventions of OTTC would need to be studied further. RR may not be an appropriate end point to gauge the quality of care provided during hospitalization or subsequent transitional care programs, and hence a focus on post discharge survival should be maintained while adopting and gauging transitional care interventions.

**Table 3 Multivariate fine and gray competing risk analysis of factors associated with readmission**

| Factor | Multivariate analysis | |
|---|---|---|
| | HR (95%CI) | *P* value |
| Intervention *vs* controls | 0.99 (0.66-1.48) | 0.95 |
| Age | 0.99 (0.97-1.01) | 0.39 |
| Female gender | 1.36 (0.87-2.1) | 0.18 |
| Etiology of cirrhosis | | |
| EtOH *vs* HCV | 0.99 (0.13-7.77) | 0.99 |
| NAFLD *vs* HCV | 1.03 (0.51-2.54) | 0.93 |
| MELD score (for every 1 unit increment) | 1.03 (0.99-1.05) | 0.09 |
| Index hospitalization length of stay (for every 1 d increment) | 0.99 (0.97-1.02) | 0.59 |
| Discharge to home with home care *vs* home | 1.12 (0.69-2.01) | 0.55 |
| Discharge to SNF *vs* home | 0.96 (0.51-1.83) | 0.91 |

HR: Hazard ratio; CI: Confidence interval; EtOH: Alcohol; HCV: Hepatitis C virus; NAFLD: Non-alcoholic fatty liver disease; MELD: Model for end-stage liver disease; SNF: Skilled nursing facility.

**Table 4 Comparison of studies describing various interventions targeted to improve outcomes after hospital discharge in cirrhotic patients**

| Ref. | Interven -tion type | Number of patients | Timing of interven -tion | Unplanned readmission rate | | | Mortality | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 mo | 3 mo | 6 mo | 1 mo | 3 mo | 6 mo |
| Wigg *et al*[8], 2013 | Chronic disease manageme -nt program | C:20 T:40 | 12 mo after discharge | C: 0.4/person/yr T: 1/person/yr | | | [1]C: 15% [1]T: 10% | | |
| Morando *et al*[10], 2013 | Care manageme -nt checkup model | C: 60 T: 40 | 12 mo after discharge | C: 42% T: 15% | NA | NA | [1]C: 46% [1]T: 23% | | |
| Tapper *et al*[9], 2016 | Handheld (1st phase) and electronic (2nd phase) checklists | C: 626 T: 1st: 470 2nd: 624 | Inpatient stay | C: 38% T: 1st: 35% 2nd: 27% | NA | NA | NA | C: 20% T: 1st: 15% 2nd: 21% | NA |
| Kanwal *et al*[7], 2016 | Early follow up in clinic | C: 17094 T: 8123 | At discharge | C: 14% T: 15% | NA | NA | C: 5% T: 3% | NA | NA |
| Current study | Outpatient telephonic transitional care | C: 93 T: 76 | 30 d after discharge | C: 38% T: 37% | C: 55% T: 57% | C: 62% T: 65% | C: 10% T: 5% | C: 24% T: 13% | C: 31% T: 16% |

[1]Only data on 12 mo mortality rates are available. C: Control arm; T: Intervention arm; NA: Not available.

## ARTICLE HIGHLIGHTS

### *Research background*

Given the increasing concern about the high rates of readmission in cirrhotic patients (CP) after hospital discharge (HD), focus is now being laid on transitional care interventions to try to mediate a reduction. However, prior studies have also demonstrated a possible adverse impact on patient survival with reduced readmissions. Hence additional studies to comprehensively assess post discharge outcomes in CP and to try to improve them are necessary.

### *Research motivation*

It is alarming but true that nearly 53% of CP get readmitted at least once within 3 mo of HD. This implies a tremendous financial and psychosocial burden to our current healthcare system and measures to improve the prognosis of patients after HD warrant attention.

### Research objectives
We developed and evaluated a novel strategy for the care of CP at our center called the outpatient telephonic transitional care program (OTTC). The objectives of this study were to determine the effect of OTTC on survival and readmission rates (RR) at different intervals up to 6 mo after HD in CP and thus further explore the relationship of RR to survival.

### Research methods
In this observational study, CP who were treated in our inpatient hepatology service between March 1 and December 31, 2016 were retrospectively assessed. Those who had received the OTTC program formed the intervention arm, and the rest formed concomitant controls. Survival and RR at 1, 3, and 6 mo after HD were compared between the two groups.

### Research results
In our study, an overall RR of 55% was noted within 3 mo of HD, which correlates with the national average. Interestingly the RR at 1, 3, and 6 mo were comparable between the intervention and control groups. However, the patients who received the OTTC intervention showed markedly better 6 mo survival compared to the controls with a hazard ratio of 0.4 (95% confidence interval: 0.2-0.82; $P = 0.012$).

### Research conclusions
In this study, we demonstrated the beneficial impact of a novel transitional care intervention program that provided a survival benefit to CP after HD. In addition, we highlighted an important dissociation between RR and survival, thus shedding further light on the importance of focusing on survival rather than RR as an outcome while assessing post discharge outcomes in CP. Given the high burden on hospitalizations for CP, our novel and easy to implement intervention may now be adopted at multiple centers to further assess its impact and provide improved care for CP.

### Research perspectives
Our results reaffirm that CP remain at significant risk for readmission and mortality after HD. A focus on providing appropriate transitional care is essential to improve post discharge outcomes. The OTTC program we describe is minimally resource intensive and can afford a survival benefit to CP. The tenets of the OTTC program should be further explored and assessed in other institutions and settings. Continued emphasis on survival rather than RR is warranted because CP demonstrated a dissociation between these parameters.

## REFERENCES


1 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]
2 **Volk ML**, Piette JD, Singal AS, Lok AS. Chronic disease management for patients with cirrhosis. *Gastroenterology* 2010; **139**: 14-16.e1 [PMID: 20553725 DOI: 10.1053/j.gastro.2010.05.017]
3 **Tapper EB**. Challenge accepted: Confronting readmissions for our patients with cirrhosis. *Hepatology* 2016; **64**: 26-28 [PMID: 26806609 DOI: 10.1002/hep.28471]
4 **Fagan KJ**, Zhao EY, Horsfall LU, Ruffin BJ, Kruger MS, McPhail SM, O'Rourke P, Ballard E, Irvine KM, Powell EE. Burden of decompensated cirrhosis and ascites on hospital services in a tertiary care facility: time for change? *Intern Med J* 2014; **44**: 865-872 [PMID: 24893971 DOI: 10.1111/imj.12491]
5 **Kanwal F**, Volk M, Singal A, Angeli P, Talwalkar J. Improving quality of health care for patients with cirrhosis. *Gastroenterology* 2014; **147**: 1204-1207 [PMID: 25449026 DOI: 10.1053/j.gastro.2014.10.029]
6 **Bajaj JS**, Reddy KR, Tandon P, Wong F, Kamath PS, Garcia-Tsao G, Maliakkal B, Biggins SW, Thuluvath PJ, Fallon MB, Subramanian RM, Vargas H, Thacker LR, O'Leary JG; North American Consortium for the Study of End-Stage Liver Disease. The 3-month readmission rate remains unacceptably high in a large North American cohort of patients with cirrhosis. *Hepatology* 2016; **64**: 200-208 [PMID: 26690389 DOI: 10.1002/hep.28414]
7 **Kanwal F**, Asch SM, Kramer JR, Cao Y, Asrani S, El-Serag HB. Early outpatient follow-up and 30-day outcomes in patients hospitalized with cirrhosis. *Hepatology* 2016; **64**: 569-581 [PMID: 26991920 DOI: 10.1002/hep.28558]
8 **Wigg AJ**, McCormick R, Wundke R, Woodman RJ. Efficacy of a chronic disease management model for patients with chronic liver failure. *Clin Gastroenterol Hepatol* 2013; **11**: 850-858.e1-4 [PMID: 23375997 DOI: 10.1016/j.cgh.2013.01.014]
9 **Tapper EB**, Finkelstein D, Mittleman MA, Piatkowski G, Chang M, Lai M. A Quality Improvement Initiative Reduces 30-Day Rate of Readmission for Patients With Cirrhosis. *Clin Gastroenterol Hepatol* 2016; **14**: 753-759 [PMID: 26407750 DOI: 10.1016/j.cgh.2015.08.041]
10 **Morando F**, Maresio G, Piano S, Fasolato S, Cavallin M, Romano A, Rosi S, Gola E, Frigo AC, Stanco M, Destro C, Rupolo G, Mantoan D, Gatta A, Angeli P. How to improve care in outpatients with cirrhosis and ascites: a new model of care coordination by consultant hepatologists. *J Hepatol* 2013; **59**: 257-264 [PMID: 23523582 DOI: 10.1016/j.jhep.2013.03.010]
11 **Ganesh S**, Rogal SS, Yadav D, Humar A, Behari J. Risk factors for frequent readmissions and barriers to transplantation in patients with cirrhosis. *PLoS One* 2013; **8**: e55140 [PMID: 23383085 DOI: 10.1371/journal.pone.0055140]
12 **Wong EL**, Cheung AW, Leung MC, Yam CH, Chan FW, Wong FY, Yeoh EK. Unplanned readmission rates, length of hospital stay, mortality, and medical costs of ten common medical conditions: a retrospective analysis of Hong Kong hospital data. *BMC Health Serv Res* 2011; **11**: 149 [PMID: 21679471 DOI: 10.1186/1472-6963-11-149]
13 **Berman K**, Tandra S, Forssell K, Vuppalanchi R, Burton JR, Nguyen J, Mullis D, Kwo P, Chalasani N. Incidence and predictors of 30-day readmission among patients hospitalized for advanced liver disease.

*Clin Gastroenterol Hepatol* 2011; **9**: 254-259 [PMID: 21092762 DOI: 10.1016/j.cgh.2010.10.035]
14 **Picker D**, Heard K, Bailey TC, Martin NR, LaRossa GN, Kollef MH. The number of discharge medications predicts thirty-day hospital readmission: a cohort study. *BMC Health Serv Res* 2015; **15**: 282 [PMID: 26202163 DOI: 10.1186/s12913-015-0950-9]
15 **Singal AG**, Rahimi RS, Clark C, Ma Y, Cuthbert JA, Rockey DC, Amarasingham R. An automated model using electronic medical record data identifies patients with cirrhosis at high risk for readmission. *Clin Gastroenterol Hepatol* 2013; **11**: 1335-1341.e1 [PMID: 23591286 DOI: 10.1016/j.cgh.2013.03.022]
16 **Volk ML**, Tocco RS, Bazick J, Rakoski MO, Lok AS. Hospital readmissions among patients with decompensated cirrhosis. *Am J Gastroenterol* 2012; **107**: 247-252 [PMID: 21931378 DOI: 10.1038/ajg.2011.314]
17 **Merli M**, Lucidi C, Giannelli V, Giusto M, Riggio O, Falcone M, Ridola L, Attili AF, Venditti M. Cirrhotic patients are at risk for health care-associated bacterial infections. *Clin Gastroenterol Hepatol* 2010; **8**: 979-985 [PMID: 20621200 DOI: 10.1016/j.cgh.2010.06.024]
18 **Agrawal K**, Kumar P, Markert R, Agrawal S. Risk Factors for 30-Day Readmissions of Individuals with Decompensated Cirrhosis. *South Med J* 2015; **108**: 682-687 [PMID: 26539950 DOI: 10.14423/SMJ.0000000000000371]
19 **Saab S**. Evaluation of the impact of rehospitalization in the management of hepatic encephalopathy. *Int J Gen Med* 2015; **8**: 165-173 [PMID: 25999756 DOI: 10.2147/IJGM.S81878]
20 **Mellinger JL**, Volk ML. Multidisciplinary management of patients with cirrhosis: a need for care coordination. *Clin Gastroenterol Hepatol* 2013; **11**: 217-223 [PMID: 23142204 DOI: 10.1016/j.cgh.2012.10.040]
21 **Centers for Medicare and Medicaid Services (CMS), HHS**. Medicare program; hospital inpatient value-based purchasing program. Final rule. *Fed Regist* 2011; **76**: 26490-26547 [PMID: 21548401]
22 **Gupta A**, Allen LA, Bhatt DL, Cox M, DeVore AD, Heidenreich PA, Hernandez AF, Peterson ED, Matsouaka RA, Yancy CW, Fonarow GC. Association of the Hospital Readmissions Reduction Program Implementation With Readmission and Mortality Outcomes in Heart Failure. *JAMA Cardiol* 2018; **3**: 44-53 [PMID: 29128869 DOI: 10.1001/jamacardio.2017.4265]
23 **Gorodeski EZ**, Starling RC, Blackstone EH. Are all readmissions bad readmissions? *N Engl J Med* 2010; **363**: 297-298 [PMID: 20647209 DOI: 10.1056/NEJMc1001882]
24 **Press MJ**, Scanlon DP, Ryan AM, Zhu J, Navathe AS, Mittler JN, Volpp KG. Limits of readmission rates in measuring hospital quality suggest the need for added metrics. *Health Aff (Millwood)* 2013; **32**: 1083-1091 [PMID: 23733983 DOI: 10.1377/hlthaff.2012.0518]

World Journal of Hepatology

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

*World J Hepatol* 2019 August 27; 11(8): 656-662

DOI: 10.4254/wjh.v11.i8.656

ISSN 1948-5182 (online)

CASE REPORT

# Fascioliasis presenting as colon cancer liver metastasis on $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography: A case report

Sami Akbulut, Egemen Ozdemir, Emine Samdanci, Selver Unsal, Murat Harputluoglu, Sezai Yilmaz

**ORCID number:** Sami Akbulut (0000-0002-6864-7711); Egemen Ozdemir (0000-0002-8022-8245); Emine Samdanci (0000-0002-0034-5186); Selver Unsal (0000-0001-8316-5043); Murat Harputluoglu (0000-0002-9415-147X); Sezai Yilmaz (0000-0002-8044-0297).

**Author contributions:** Akbulut S, and Ozdemir E designed the report; Akbulut S and Unsal S collected the patient's clinical data; Samdanci E provided histopathological information; Akbulut S and Yilmaz S performed surgical procedure; Harputluoglu M performed endoscopic procedure; Akbulut S, Ozdemir E and Yilmaz S analyzed the data and wrote the paper.

**Informed consent statement:** All study participants provided informed written consent prior to study enrollment.

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

**CARE Checklist (2016) statement:** The authors have read the CARE Checklist (2016), and the manuscript was prepared and revised according to the CARE Checklist (2016).



**Sami Akbulut, Sezai Yilmaz,** Liver Transplant Institute, Inonu University, Malatya 44280, Turkey

**Egemen Ozdemir,** Department of Surgery, Inonu University Faculty of Medicine, Malatya 44280, Turkey

**Emine Samdanci,** Department of Pathology, Inonu University Faculty of Medicine, Malatya 44280, Turkey

**Selver Unsal,** Department of Nursing Service, Liver Transplant Institute, Inonu University, Malatya 44280, Turkey

**Murat Harputluoglu,** Department of Gastroenterology, Inonu University Faculty of Medicine, Malatya 44280, Turkey

**Corresponding author:** Sami Akbulut, MD, Associate Professor, Liver Transplant Institute, Inonu University, Elazig Yolu 10. Km, Malatya 44280, Turkey. akbulutsami@gmail.com
**Telephone:** +90-422-3410660
**Fax:** +90-422-3410036

## Abstract

### *BACKGROUND*

Fascioliasis is caused by watercress and similar freshwater plants or drinking water or beverages contaminated with metacercariae. Fascioliasis can radiologically mimic many primary or metastatic liver tumors. Herein, we aimed to present the treatment process of a patient with fascioliasis mimicking colon cancer liver metastasis.

### *CASE SUMMARY*

A 35-year-old woman who underwent right hemicolectomy due to cecum cancer was referred to our clinic for management of colon cancer liver metastasis. Both computed tomography and $^{18}$F-fluorodeoxyglucose positron emission tomography revealed several tumoral lesions localized in the right lobe of the liver. After a 6-course FOLFOX (folinic acid, fluorouracil, oxaliplatin) and bevacizumab regimen, the hypermetabolic state on both liver and abdominal lymph nodes continued, and chemotherapy was extended to a 12-course regimen. The patient was referred to our institute when the liver lesions were detected to be larger on dynamic liver magnetic resonance imaging 6 weeks after completion of chemotherapy. Right hepatectomy was performed, and

**Manuscript source**: Unsolicited manuscript

**Received:** April 29, 2019
**Peer-review started:** April 29, 2019
**First decision:** June 3, 2019
**Revised:** June 14, 2019
**Accepted:** August 20, 2019
**Article in press:** August 20, 2019
**Published online:** August 27, 2019

**P-Reviewer:** Mas-Coma S, Suzuki H, Sun X
**S-Editor:** Cui LJ
**L-Editor:** A
**E-Editor:** Zhang YL

histopathological examination was compatible with fascioliasis. *Fasciola hepatica* IgG enzyme-linked immunosorbent assay was positive. The patient was administered two doses of triclabendazole (10 mg/kg/dose) 24 h apart. During the follow-up period, dilatation was detected in the common bile duct, and *Fasciola* parasites were extracted from the common bile duct by endoscopic retrograde cholangiopancreatography (ERCP). Triclabendazole was administered to the patient after ERCP.

*CONCLUSION*
Parasitic diseases, such as those caused by *Fasciola hepatica*, should be kept in mind in the differential diagnosis of primary or metastatic liver tumors, such as colorectal cancer liver metastasis, in patients living in endemic areas.

**Key words:** Colon cancer liver metastasis; Fasciola hepatica; Positron emission tomography; Misdiagnosis; Case report



**Core tip:** Human fascioliasis is caused by drinking water or freshwater plants (watercress, *etc*.) contaminated with metacercariae. Fascioliasis may remain asymptomatic for many years and is usually detected incidentally during radiological examinations for other reasons. Radiologically, fascioliasis can be confused with many other benign and malignant hepatobiliary diseases. One of the most common malignant liver diseases mimicking fascioliasis includes colon cancer liver metastasis. We aimed to present the diagnosis and treatment process in a patient with fascioliasis radiologically mimicking colon cancer liver metastasis.

**Citation:** Akbulut S, Ozdemir E, Samdanci E, Unsal S, Harputluoglu M, Yilmaz S. Fascioliasis presenting as colon cancer liver metastasis on $^{18}$F-fluorodeoxyglucose positron emission tomography/computed tomography: A case report. *World J Hepatol* 2019; 11(8): 656-662
**URL**: https://www.wjgnet.com/1948-5182/full/v11/i8/656.htm
**DOI**: https://dx.doi.org/10.4254/wjh.v11.i8.656

## INTRODUCTION

Fascioliasis (liver fluke) is a parasitic disease of the liver caused by a type of trematode of the species *Fasciola hepatica* and less frequently by *F. gigantica*. Humans are infected by ingesting metacercariae-containing watercress and similar freshwater plants or drinking contaminated water. After ingestion of contaminated water or beverages, the metacercariae excyst in the duodenum, and the larvae migrate through the intestinal wall into the peritoneum. After penetrating the liver capsule, the larvae pass through the liver parenchyma and reach the biliary ducts, where they develop into adult flukes[1,2]. During this migration, they cause destruction in the liver parenchyma, which is characterized by necrosis and fibrosis. The larval form matures to a 3-cm long and 1-cm wide leaf-shaped adult form in approximately 12 wk. Parasites in the biliary ducts remain asymptomatic for years in most patients and are usually detected incidentally during radiological examinations for other reasons. The most important steps in the diagnosis of fascioliasis are clinical suspicion, detection of eggs in the stool, serological tests, molecular tests, and endoscopic and radiological examinations. Radiologically, fascioliasis can be confused with many other benign and malignant diseases of the liver and biliary tract[3]. We aimed to present the diagnosis and treatment process of a patient with fascioliasis radiologically mimicking liver metastasis of colon cancer.

## CASE REPORT

### *Chief complaints*

A 35-year-old female patient who underwent right hemicolectomy for cecal cancer

was referred to our Liver Transplant Institute for surgical treatment of colon cancer liver metastasis.

### *Personal History*

Patient stated that she had been eating watercress for a long time.

### *History of past illness*

The patient stated that she underwent colonoscopy at another clinic because of exhaustion, fatigue, and anemia, and right hemicolectomy was performed upon detection of a cecal tumor on colonoscopy. Histopathological examination revealed cecal adenocarcinoma with subserosal fat tissue invasion and five metastatic lymph nodes in 32 lymph nodes. Preoperative contrast-enhanced abdominal computed tomography (CT) revealed hypodense and heterogenous lesions with soft tissue density that were 40 mm x 33 mm in diameter in the right lobe of the liver (Figure 1). At the 3rd postoperative week, multiple hypermetabolic lesions with SUVmax of 5.3 in segments V, VII, and VIII of the liver were detected on performing $^{18}$F-fluorodeoxyglucose positron emission tomography/CT ($^{18}$F-FDG-PET/CT) after 5.5 mCi $^{18}$F-FDG injection (Figure 2). In addition, hypermetabolic lesions were detected in the subhepatic (SUVmax: 7.1), superior mesenteric (SUVmax: 7.1), and aortocaval window (SUVmax: 7.3). Six cycles of adjuvant FOLFOX (folinic acid, fluorouracil, oxaliplatin) and bevacizumab were administered to the patient. In control $^{18}$F-FDG-PET/CT, the SUVmax values of liver lesions decreased to 3.8, and the SUVmax values of the lymph nodes did not regress. Thus, the same chemotherapy regimen was continued, and control $^{18}$F-FDG-PET/CT was performed at the end of 12th chemotherapy course. In the last $^{18}$F-FDG-PET/CT, all previously seen lesions were stable, whereas they regressed significantly both dimensionally and metabolically. Subsequently, the patient was referred to our center. Dynamic liver magnetic resonance imaging (MRI) performed immediately after the 12th chemotherapy course showed regression of the lesions in the liver. After 6 weeks, dynamic MRI at our center revealed that the lesions were more prominent than those on previous magnetic resonance images. Because the patient had colon cancer, the lesions in the liver were hypermetabolic in $^{18}$F-FDG-PET/CT, and the lesions started to regrow after chemotherapy, and the patient planned to undergo surgery. Right hepatectomy was performed to include all lesions with intraoperative ultrasonography. In addition, all lymph nodes in the hepatoduodenal ligament, celiac, and aortocaval window were resected, and a frozen section was sent for analysis, and no tumor was detected in the lymph nodes. In the histopathological examination of the hepatectomy specimen, the lesions had granulomatous foci on a tract, characterized by suppuration enriched with eosinophils (Figure 3-4), which was noted to be compatible with *F. hepatica* infection by a pathologist. Based on this result, preoperative blood tests of the patient were reexamined, and eosinophil count was 0.78 × $10^9$/L (normal range: 0–0.5). *F. hepatica* IgG enzyme-linked immunosorbent assay (ELISA) (Synlab MVZ Leinfelden GmbH, Leinfelden-Echterdingen, Germany) was positive.

## FINAL DIAGNOSIS

The final diagnosis of the presented case is fascioliasis mimicking colon cancer liver metastasis.

## TREATMENT

The patient was administered two doses (10 mg/kg/dose) of triclabendazole (Egaten, Novartis Pharma, Switzerland) 24 h apart.

## OUTCOME AND FOLLOW-UP

At the 4th postoperative month follow-up, the alkaline phosphatase and gamma-glutamyl transferase values were elevated, and the common bile duct was significantly dilated on magnetic resonance cholangiopancreatography (MRCP) images. Several *Fasciola* parasites were extracted from the common bile duct with endoscopic retrograde cholangiopancreatography (ERCP) (Figure 5). Triclabendazole (10 mg/kg/dose) therapy was administered to the patient after ERCP. The patient is still followed up without hepatobiliary complications. Although blood alpha-fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels were normal during the

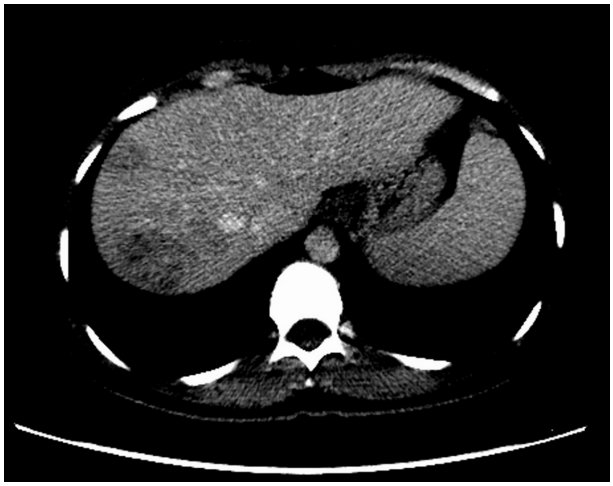

**Figure 1 Computed tomography image after contrast medium injection.** Axial computed tomography image revealed that hypodense and heterogenous lesions with soft tissue density of maximally 40 mm × 33 mm in diameter in the right lobe of the liver.

follow-up period, the CA19-9 level showed changes in the postoperative period (Figure 6). However, the CA19-9 level dramatically decreased after ERCP and returned to normal limits (normal range: 0–35 U/mL). No findings of dead or living parasites were detected in the last control ERCP.

## DISCUSSION

Two phases in the life cycle of *F. hepatica* have been defined: acute (hepatic) and chronic (biliary). The phase where the parasite penetrates through the liver capsule and invades the liver parenchyma is the hepatic phase. In this phase, parasites digest hepatocytes, open tunnels in the parenchyma, and remain in the parenchyma for months[2,4]. Signs and symptoms in the hepatic phase commonly mimic those of liver abscess[2,5]. Patients in this phase usually experience non-specific symptoms, such as loss of appetite, weight loss, right upper quadrant pain, fever, sweating, urticaria, and arthralgia[6]. Hepatomegaly, right upper quadrant pain, and prominent eosinophilia can be seen[4,5]. The phase where the parasite becomes visible in the bile ducts is the biliary phase. In this phase, signs and symptoms, such as biliary colic, epigastric pain, and jaundice, which are related to biliary tract obstruction, develop. The level of cholestatic enzymes and bilirubin may increase[7]. Symptoms of acute pancreatitis may develop in patients with distal bile duct obstruction[8].

The following diagnostic modalities are used in the diagnosis of fascioliasis: clinical suspicion, parasite eggs observed by direct microscopy (stool, bile, or duodenal aspirate), detection of DNA of the parasite with real-time polymerase chain reaction (stool, bile or duodenal aspirate), serological tests (immunoelectrophoresis, counter-immunoelectrophoresis, metacercarial precipitin test, indirect hemagglutination), complement fixation, immunofluorescence assay, radioimmunoassays, enzyme-linked immunofiltration assay (ELIFA), enzyme-linked immuno-electrotransfer blot (EITB) or western blot, Falcon assay screening test–ELISA (FAST-ELISA), micro-ELISA, dot-ELISA], radiological examination of lesions of the liver parenchyma and biliary ducts (CT, MRI, MRCP, and $^{18}$F-FDG-PET/CT), endoscopic examination of parasite directly (ERCP, endo-ultrasonography) and histopathological examination of the parasite on tissue biopsy[3,9,10].

Appearance of parasite eggs in the stool has a diagnostic value only in the biliary phase[5,9]. Eosinophilia and anemia may be seen in biochemical tests[3,11]. ELISA is the leading serological test with high sensitivity and rapid results[6]. However, it should be supported by radiological findings due to its high false positive and negative rates[12]. Appearance of tunnel-like linear and branched hypodense lesions on CT is characteristic[3]. In most patients, iso-hyperintense (T2) and iso-hypointense (T1) lesions can be seen on MR and MRCP images, and these lesions can mimic malignant diseases of the biliary tract. ERCP and endoscopic ultrasonography are very useful in the biliary phase[9,11]. Similar to this case, ERCP is the most appropriate treatment modality both to remove parasites from the bile ducts and to decompress the bile ducts.

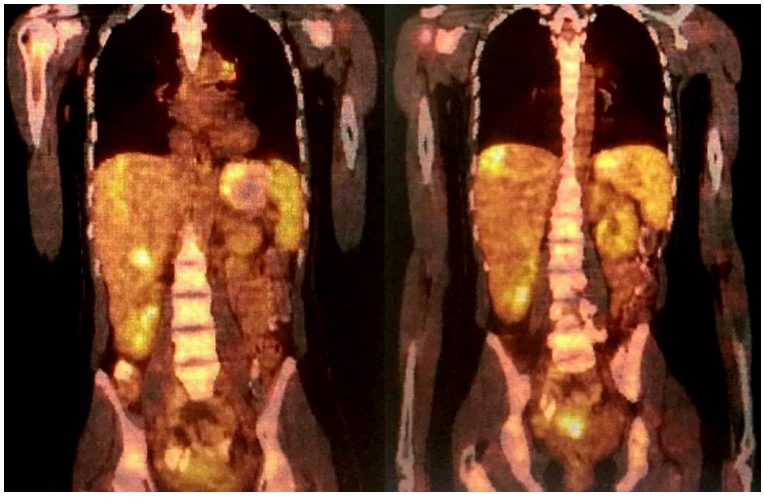

**Figure 2 $^{18}$F-fluorodeoxyglucose positron emission tomography/Computed tomography was performed using 5.5 mCi of $^{18}$F-fluorodeoxyglucose injection.** Maximum intensity projection of images shows that high fluorodeoxyglucose uptakes in different segments of the liver.

Diagnosis, staging, response to treatment, and recurrence of malignant diseases can be determined successfully by $^{18}$F-FDG-PET/CT. However, false-positive results can be obtained in inflammations due to radiotherapy and surgery or in various chronic infections. These can sometimes mimic malignancy[13-15]. Similar to this case, carefully evaluating high FDG uptake in PET/CT is particularly important in a patient with malignancy. Otherwise, because patients can only be cured with anti-helminthic treatment, they may undergo unnecessary major surgical procedures or chemotherapy.

The most common diseases confused with fascioliasis are viral hepatitis, liver abscess, cholecystitis, sclerosing cholangitis, AIDS-associated cholangitis, ruptured hydatid cyst, and ascariasis, clonorchiasis, and primary and metastatic tumors of the liver and biliary tract[2,5,9]. Therefore, misinterpretation of these signs and symptoms in areas where fascioliasis is not endemic is possible, and thus, diagnosis may be delayed[9]. Similar to this case, in a patient with colon cancer, it is expected that liver lesions will be interpreted as a tumor by a radiologist or nuclear medicine specialist without experience in fascioliasis.

The first and best option for the treatment of fascioliasis is triclabendazole, and another alternative drug is nitazoxanide. Triclabendazole is used in two doses (10 mg/kg/dose) 24 h apart and is effective at all stages regardless of the phase of the disease. Nitazoxanide is a good alternative to triclabendazole, and it is administered as 500 mg twice daily for 7 d[16]. Other drugs that were used previously and are no longer recommended due to drug resistance or toxicity, including bithionol, praziquantel, emetine, dehydroemetine, metronidazole, albendazole, niclofolan, and chloroquine. In this case, we had to administer it twice in a few months.

## CONCLUSION

Fascioliasis may mimic many benign and malignant diseases of the liver due to its non-specific signs and symptoms. Differential diagnosis is quite difficult, if there is no clinical suspicion, especially in patients with simultaneous fascioliasis and gastrointestinal malignancy. Therefore, careful evaluation of diagnostic imaging tools in patients living in endemic areas and the use of other diagnostic tools in suspected cases are vital.

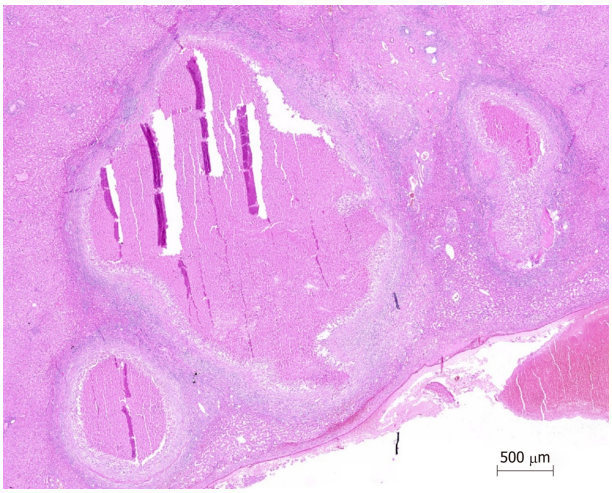


**Figure 3 Granuloma with central necrosis (HE ×100).**

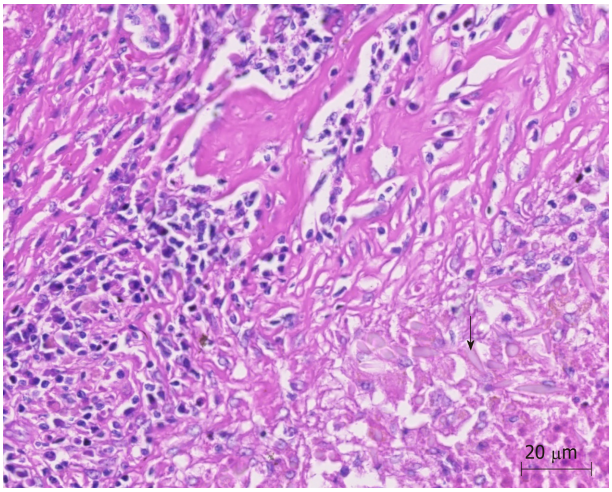


**Figure 4 Charcot–Leyden crystals near granuloma (arrows) (HE ×100).**

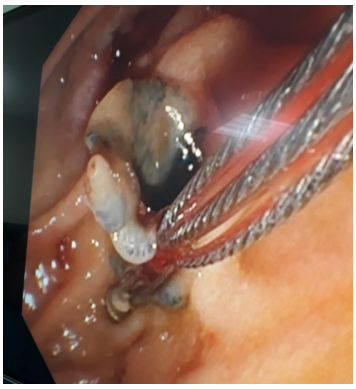

**Figure 5 Endoscopic view of endoscopic retrograde cholangiopancreatography procedure.** Extraction of *Fasciola hepatica* in the common bile duct using a basket catheter.

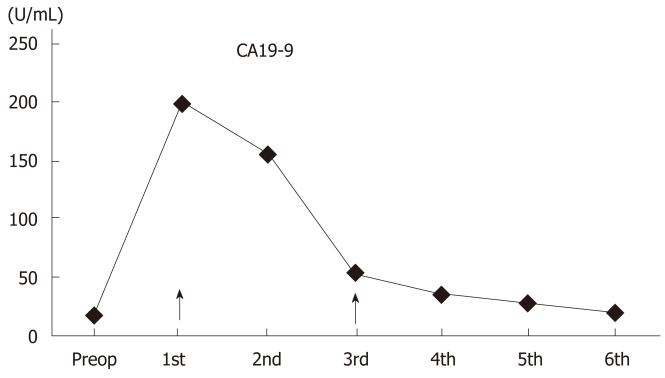


**Figure 6 Course of blood CA19-9 levels.** Black arrows indicate the days of endoscopic retrograde cholangiopancreatography.

## REFERENCES


1 **Mas-Coma S**, Bargues MD, Valero MA. Human fascioliasis infection sources, their diversity, incidence factors, analytical methods and prevention measures. *Parasitology* 2018; **145**: 1665-1699 [PMID: 29991363 DOI: 10.1017/S0031182018000914]
2 **Lim JH**, Mairiang E, Ahn GH. Biliary parasitic diseases including clonorchiasis, opisthorchiasis and fascioliasis. *Abdom Imaging* 2008; **33**: 157-165 [PMID: 17934771 DOI: 10.1007/s00261-007-9326-x]
3 **Kaya M**, Beştaş R, Cetin S. Clinical presentation and management of Fasciola hepatica infection: single-center experience. *World J Gastroenterol* 2011; **17**: 4899-4904 [PMID: 22171131 DOI: 10.3748/wjg.v17.i44.4899]
4 **Teichmann D**, Grobusch MP, Göbels K, Müller HP, Koehler W, Suttorp N. Acute fascioliasis with multiple liver abscesses. *Scand J Infect Dis* 2000; **32**: 558-560 [PMID: 11055665]
5 **Aksoy DY**, Kerimoğlu U, Oto A, Ergüven S, Arslan S, Unal S, Batman F, Bayraktar Y. Fasciola hepatica infection: clinical and computerized tomographic findings of ten patients. *Turk J Gastroenterol* 2006; **17**: 40-45 [PMID: 16830276]
6 **Lin D**, Wang X, Wang Z, Li W, Zang Y, Li N. Diagnosis and management of hepatic fascioliasis mimicking intrahepatic cholangiocarcinoma: a case report. *Int J Clin Exp Med* 2017; **10**: 1385–92
7 **Bektaş M**, Dökmeci A, Cinar K, Halici I, Oztas E, Karayalcin S, Idilman R, Sarioglu M, Ustun Y, Nazligul Y, Ormeci N, Ozkan H, Bozkaya H, Yurdaydin C. Endoscopic management of biliary parasitic diseases. *Dig Dis Sci* 2010; **55**: 1472-1478 [PMID: 19513838 DOI: 10.1007/s10620-009-0850-0]
8 **Echenique-Elizondo M**, Amondarain J, Lirón de Robles C. Fascioliasis: an exceptional cause of acute pancreatitis. *JOP* 2005; **6**: 36-39 [PMID: 15650283]
9 **Kabaalioğlu A**, Cubuk M, Senol U, Cevikol C, Karaali K, Apaydin A, Sindel T, Lüleci E. Fascioliasis: US, CT, and MRI findings with new observations. *Abdom Imaging* 2000; **25**: 400-404 [PMID: 10926194 DOI: 10.1007/s002610000017]
10 **Mas-Coma S**, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. *Parasitology* 2014; **141**: 1918-1946 [PMID: 25077569 DOI: 10.1017/S0031182014000869]
11 **Gulsen MT**, Savas MC, Koruk M, Kadayifci A, Demirci F. Fascioliasis: a report of five cases presenting with common bile duct obstruction. *Neth J Med* 2006; **64**: 17-19 [PMID: 16421437]
12 **Kaya M**, Beştaş R, Çiçek M, Önder A, Kaplan MA. The value of micro-ELISA test in the diagnosis of Fasciola hepatica infection. *Turkiye Parazitol Derg* 2013; **37**: 23-27 [PMID: 23619041 DOI: 10.5152/tpd.2013.06]
13 **Lin KH**, Wang JH, Peng NJ. Disseminated nontuberculous mycobacterial infection mimic metastases on PET/CT scan. *Clin Nucl Med* 2008; **33**: 276-277 [PMID: 18356668 DOI: 10.1097/RLU.0b013e3181662fb2]
14 **Tan GJ**, Berlangieri SU, Lee ST, Scott AM. FDG PET/CT in the liver: lesions mimicking malignancies. *Abdom Imaging* 2014; **39**: 187-195 [PMID: 24233161 DOI: 10.1007/s00261-013-0043-3]
15 **Sürücü E**, Demir Y, Dülger AC, Batur A, Ölmez Ş, Kitapçı MT. Fasciola Hepatica Mimicking Malignancy on 18F-Fluorodeoxyglucose-Positron Emission Tomography/Computed Tomography. *Mol Imaging Radionucl Ther* 2016; **25**: 143-146 [PMID: 27751978 DOI: 10.4274/mirt.97759]
16 **Favennec L**, Jave Ortiz J, Gargala G, Lopez Chegne N, Ayoub A, Rossignol JF. Double-blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. *Aliment Pharmacol Ther* 2003; **17**: 265-270 [PMID: 12534412 DOI: 10.1046/j.1365-2036.2003.01419.x]


Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com