ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2018 December 27; 10(12): 892-965

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

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ABOUT COVER

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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.

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

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Wen-Wen Tan* **Proofing Editorial Office Director:** *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Koo Jeong Kang, Nikolaos Pyrsopoulos

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

EDITORIAL OFFICE
Jin-Lei Wang, Director

PUBLICATION DATE
December 27, 2018

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World Journal of Hepatology

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World J Hepatol 2018 December 27; 10(12): 892-897

DOI: 10.4254/wjh.v10.i12.892

ISSN 1948-5182 (online)

EDITORIAL

Unresolved issues in the prophylaxis of bacterial infections in patients with cirrhosis

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Author contributions: Dirchwolf M, Marciano S, Martínez J and Ruf AE conceived the study and drafted the manuscript; all authors approved the final version of the article.

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

Manuscript source: Invited manuscript

Received: August 6, 2018
Peer-review started: August 7, 2018
First decision: August 24, 2018
Revised: September 4, 2018
Accepted: October 11, 2018
Article in press: October 11, 2018
Published online: December 27,

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Abstract

Bacterial infections are highly prevalent and a frequent cause of hospitalization and short-term mortality in patients with cirrhosis. Due to their negative impact on survival, antibiotic prophylaxis for bacterial infections in high-risk subgroups of patients with cirrhosis has been the standard of care for decades. Patients with prophylaxis indications include those at risk for a first episode of spontaneous bacterial peritonitis (SBP) due to a low ascitic fluid protein count and impaired liver and kidney function, patients with a prior episode of SBP and those with an episode of gastrointestinal bleeding. Only prophylaxis due to gastrointestinal bleeding has a known and short-time duration. All other indications imply long-lasting exposure to antibiotics - once the threshold requirement for initiating prophylaxis is met - without standardized criteria for re-assessing antibiotic interruption. Despite the fact that the benefit of antibiotic prophylaxis in reducing bacterial infections episodes and mortality has been thoroughly reported, the extended use of antibiotics in patients with cirrhosis has also had negative consequences, including the emergence of multi-drug resistant bacteria. Currently, it is not clear whether restricting the use of broad and fixed antibiotic regimens, tailoring the choice of antibiotics to local bacterial epidemiology or selecting non-antibiotic strategies will be the preferred antibiotic prophylaxis strategy for patients with cirrhosis in the future.

Key words: Cirrhosis; Antibiotic Prophylaxis; Multi-drug resistant bacteria; Spontaneous bacterial peritonitis; Bacterial infections

Core tip: Antibiotic prophylaxis in patients with cirrhosis has proven to be effective in preventing new episodes of bacterial infections and reducing mortality. However, the

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broad and fixed indication of long-term antibiotic therapy in these patients has led to an increase in the emergence of multi-drug resistant bacteria. The development of new strategies for bacterial infection prevention is currently under debate, thus reflecting the need for randomized controlled trials and local epidemiological studies to improve prophylactic antibiotic choice in patients with cirrhosis.

Dirchwolf M, Marciano S, Martínez J, Ruf AE. Unresolved issues in the prophylaxis of bacterial infections in patients with cirrhosis. *World J Hepatol* 2018; 10(12): 892-897
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/892.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.892

INTRODUCTION

During the natural history of cirrhosis, patients may suffer from complications that significantly increase their risk of short-term mortality. One of the main culprits of this outcome is bacterial infections[1], which are highly prevalent. One-third of patients with decompensated cirrhosis will develop an episode of bacterial infection during a one-year period[2], and 24% of patients admitted for an infection will develop a second episode during the same hospital stay[3]. Furthermore, bacterial infections are associated with a grim prognosis: patients with end-stage liver disease listed for liver transplantation who suffer an infection have a 42% risk of de-listing or death within 6 mo from admission[4].

For these reasons, antibiotic prophylaxis has been the standard of care in high-risk patients with cirrhosis for decades[5,6]. The rationale is accomplishing selective intestinal decontamination to decrease bacterial translocation, which is considered the trigger event for bacterial infections in cirrhosis. There is also evidence of an immunomodulatory effect caused by certain antibiotics such as fluoroquinolones that might have an additional beneficial impact on patients with cirrhosis survival[7,8].

Currently, there are two clinical scenarios for antibiotic prophylaxis in cirrhosis: prevention of spontaneous bacterial peritonitis (SBP) and prevention of bacterial infections after upper gastrointestinal bleeding.

Antibiotic prophylaxis for the prevention of SBP is currently recommended in most practice guidelines[1,9]. Primary prophylaxis with fluoroquinolones is indicated in patients with poor liver or renal function and a low ascitic fluid protein count, since these patients showed higher short-term survival under prophylaxis in randomized trials[10]. However, a meta-analysis of studies addressing primary prophylaxis in patients with SBP failed to confirm a benefit in survival[5].

The use of norfloxacin after a first episode of SBP to prevent its recurrence is the most robust and widely recognized indication of antibiotic prophylaxis in cirrhosis. Data to sustain secondary prophylaxis for SBP arose from two clinical trials performed in the 1990s and early 2000s. These studies showed that the cumulative incidence of SBP recurrence at one year reached 20%-26% in patients receiving norfloxacin compared to 68% in patients in the placebo group[11,12]. Notably, these randomized controlled trials included fluoroquinolones that were effective in treating Gram-negative bacilli, the predominant etiology of SBP at that time. Whether these antibiotics are useful in today's changing bacteria epidemiology has not been re-assessed; to our knowledge, no other studies evaluating secondary prophylaxis for SBP have been published.

Patients with cirrhosis undergoing an episode of acute gastrointestinal bleeding have also proven to benefit from antibiotic prophylaxis. In a robust meta-analysis conducted by Chavez-Tapia *et al*[13], treatment with antibiotics reduced bacterial infections, all-cause mortality, re-bleeding events and hospitalization length in patients with acute gastrointestinal bleeding.

PITFALLS OF ANTIBIOTIC PROPHYLAXIS IN CIRRHOSIS: EMERGENCE OF RESISTANT BACTERIA AND DRUG TOXICITY

Only prophylaxis due to gastrointestinal bleeding has a known and short-time duration. All other indications imply long-lasting exposure to antibiotics; once the

threshold requirement for initiating prophylaxis is met, without standardized criteria for re-assessing antibiotic interruption, the patient usually maintained antibiotic use until liver transplantation or death. Another usual scenario in clinical practice is the concomitant use of two types of antibiotics as prophylaxis in a single patient (*i.e.*, rifaximin for hepatic encephalopathy and norfloxacin for SBP prevention), with conflicting conclusions as to the efficacy of using only one drug for both objectives[14-16].

Consequences of long-term antibiotic use in patients with cirrhosis are now increasingly being reported. There has been a shift in the type of responsible microorganisms: initially, in the 1990s, Gram-negative bacilli caused two thirds or more of bacterial infections in patients with cirrhosis, whereas in the last 20 years, Gram-positive cocci have been identified in almost one-half of infections in this population[17-20].

Most importantly, the prevalence of resistant and multi-drug resistant bacteria (MDR: bacteria with acquired non-susceptibility to at least one agent in three main antibiotic families[21]) has significantly scaled. The prevalence of MDR bacteria increased by 100% when comparing two studies performed in 2002 and 2007-2011 that analyzed bacterial infections in patients with cirrhosis during hospitalization[19]. In the latter study, MDR infections accounted for 18%-23% of all identified bacteria[22]. Several authors have reported similar or even higher rates of MDR bacteria in different geographies and settings (up to one-half of bacterial infections in health-care acquired settings were caused by MDR bacteria)[23,24]. The main risk factors identified for the development of MDR infections were prior contact with the health-care system, a nosocomial or health-associated origin of infection, the use of norfloxacin prophylaxis, recent use of other antibiotics (cephalosporins or beta-lactams) or recent infection by MDR bacteria[22,23,25].

Higher rates of MDR bacterial infections are parallel with higher rates of inadequate initial empirical therapy. Initial empirical therapy has proven to be insufficient in as much as 90% of bacterial infections, but these rates are dependent on the origin of the infection and susceptibility pattern of the responsible bacteria. As expected, the extension of antibiotic resistance and failure of empirical therapy are an independent predictor of morbidity and mortality[23,26-28]. Currently, it is suggested that empirical antibiotic therapy should be based on the origin and type of infection, its severity, recent antibiotic use and the prevalence of MDR bacteria[29]. Thus, there is a growing need for conducting local studies to identify the epidemiology of MDR bacteria in patients with cirrhosis in each geography (for instance, with microbiological surveillance[29]), as well as exploring other prophylactic strategies for bacterial infections other than extended antimicrobial use.

Last but not least, the prolonged use of fluoroquinolones as prophylaxis may cause significant adverse events. This type of antibiotics has had prior warnings issued by the United States Food and Drug Admnistration referring to disabling and potentially permanent side effects involving tendons, muscles, joints, nerves and the central nervous system. Recently, this agency has strengthened its black box warning for fluoroquinolones, including a separate notice about the drug's potential mental side effects (disturbances in attention, disorientation, agitation, nervousness, memory impairment and delirium) and the risk of coma with hypoglycemia[30].

DIVERGENCE FROM ANTIBIOTIC PROPHYLAXIS TO PREVENT BACTERIAL INFECTIONS

Different alternatives to antibiotic prophylaxis have been suggested - whether replacing or complementing the use of antibiotics - such as the use of probiotics, fecal microbiota transplantation, statins, prokinetics and granulocyte colony-stimulating factor. Other suggested measures include restricting or suspending the use of other types of drugs, such as proton pump inhibitors or beta-blockers, that may influence bacterial infection incidence or outcome[31-39]. However, data regarding the efficacy of these strategies are contradictory or insufficient at the present time. Thus, non-antibiotic strategies are yet to be included in the standard of care practice guidelines.

UNANSWERED QUESTIONS ABOUT ANTIBIOTIC PROPHYLAXIS

The problem that may arise in the near future is the following: if current antibiotic prophylaxis regimens are sustained as the only strategy to prevent infections, and

physicians continue to choose wide-spectrum empiric antibiotics due to the increasing prevalence of MDR bacteria in patients with cirrhosis, what will happen when the available choices for antimicrobials run out? Another concern refers to the maintenance of standard antibiotic prophylaxis in a patient who suffered from an infection caused by quinolone-resistant MDR bacteria. In these cases, whether prophylaxis with the standard antibiotic choice would prevent new episodes of infection is uncertain. Perhaps these patients would benefit from another type of prophylaxis or even with the definite suspension of antibiotic prophylaxis?

CONCLUSION

Broad use of uninterrupted antibiotic prophylaxis in patients with cirrhosis has contributed to a shift in bacterial epidemiology and antibiotic resistance patterns. The emergence of MDR bacteria has negatively impacted the effectiveness of bacterial infection treatment. The need to conduct regional studies to detect the type and antibiotic susceptibility of bacteria causing infections appears to be clear. Perhaps antibiotic prophylaxis could also be tailored to local bacterial epidemiology in the future, to increase its effectiveness and decrease its deleterious effects. Thus, future studies are needed to better understand the role of antibiotic prophylaxis and to put in perspective the actual risks and benefits of current recommendations. If more rigorous and personalized use of antibiotic prophylaxis is not advocated, there may be a time in the near future when physicians run out of options to treat resistant bacterial infections in cirrhosis.

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10.1111/apt.12430]

P- Reviewer: Rezaee-Zavareh MS, Kaya M, Namisaki T, Tijera FHDL
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World Journal of Hepatology

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World J Hepatol 2018 December 27; 10(12): 898-906

DOI: 10.4254/wjh.v10.i12.898

ISSN 1948-5182 (online)

EDITORIAL

Hepatitis C virus-associated hepatocellular carcinoma after sustained virologic response

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Author contributions: All authors contributed to all aspects of this paper.

Conflict-of-interest statement: Sasaki R, Yokosuka O and Moriyama M declare no conflict of interest related to this publication. Kanda T reports grants and other from Abbvie, grants and other from MSD K.K., grants and other from Chugai Pharmaceutical CO., LTD., grants from Sysmex, other from Gilead Sciences, other from Bristol-Myers Squibb, other from Otsuka Pharmaceutical, grants from Daiichi-Sankyo, grants from Shionogi, other from PharmaEssentia, outside the submitted work. Kato N reports grants and other from Abbvie, grants and other from MSD K.K., grants and other from Chugai Pharmaceutical CO., LTD., grants and other from Mitsubishi Tanabe Pharma, grants and other from Gilead Sciences, grants and other from Bristol-Myers Squibb, outside the submitted work.

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Abstract

The introduction of a direct-acting antiviral (DAA) for patients with hepatitis C virus (HCV) infection, could lead to higher sustained virologic response (SVR) rates with fewer adverse events, and it could shorten the treatment duration relative to the interferon era. Although most recent clinical studies have demonstrated that the occurrence rates of hepatocellular carcinoma (HCC) are decreased by SVR with both interferon-based and interferon-free-regimens, there are several reports about the unexpected observation of high rates of early tumor occurrence and recurrence in patients with HCV-related HCC undergoing interferon-free therapy despite SVR. Several mechanisms of HCC occurrence and rapid immunological changes, including cytokines and chemokines during and after DAA treatment, have also been reported. We focused on the possibilities that HCC occurs or recurs during and after DAA treatment, based on the reported clinical and basic studies. Further studies and observations will be needed to determine the short-term and long-term effects on hepatocarcinogenesis caused by the eradication of HCV with DAAs. New serum biomarkers and a follow-up system for HCV-patients with SVR should be established.

Key words: Hepatitis C virus; Hepatocellular carcinoma; Sustained virologic response; Direct-acting antiviral agents

Core tip: The incidence of hepatocellular carcinoma (HCC) in hepatitis C virus (HCV) patients with sustained virologic response (SVR) after direct-acting antiviral (DAA)

Manuscript source: Invited manuscript

Received: September 13, 2018
Peer-review started: September 13, 2018
First decision: October 5, 2018
Revised: November 8, 2018
Accepted: November 15, 2018
Article in press: November 16, 2018
Published online: December 27, 2018

treatment is a serious health issue. We focused on the role of DAA treatment in hepatocarcinogenesis. DAAs may also lead to rapid changes in immune status through interactions between the host and HCV. Changes in the immune system may play a role in the progression of HCC. Further observations are needed to determine the effects on hepatocarcinogenesis caused by the eradication of HCV with DAAs.

Sasaki R, Kanda T, Kato N, Yokosuka O, Moriyama M. Hepatitis C virus-associated hepatocellular carcinoma after sustained virologic response. *World J Hepatol* 2018; 10(12): 898-906
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/898.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.898

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer type[1] and the third most likely cause of cancer related deaths[2]. HCC is associated with chronic liver disease and cirrhosis in > 90% of cases[3]. In the West and Japan, hepatitis C virus (HCV) infection is one of the leading causes of chronic hepatitis, cirrhosis, and HCC. HCV affects approximately 130-210 million people worldwide, or 2%-3% of the world's population[4]. Approximately 20%-30% of chronically HCV infected patients show liver cirrhosis[5], and 1%-4% of cirrhotic patients develop HCC per year[6]. HCC is characterized by a 5-year survival rate of 10%-12%[7].

Recently, several regimens of direct-acting antiviral (DAA) combinations have been developed for the treatment of chronic HCV infection[8]. The introduction of DAA agents has improved sustained virologic response (SVR) rates to approximately 90% and shortened treatment duration[9]. DAAs also help to overcome interferon non-responsiveness[10]. SVR is associated with improved overall survival in HCV infected patients. Recurrence-free survival in HCV infected patients who have undergone resection or locoregional therapy for HCC is also improved by SVR[11].

According to studies from the interferon era, the survival benefit in HCC patients infected with HCV has been postulated to occur through anti-inflammatory, antiangiogenic, and antiviral properties, and interferon-based antiviral therapies were associated with improved outcomes in HCC patients who were infected with HCV during long-term observation[12,13]. However, treatment with DAA therapy can promptly eradicate serum HCV ribose nucleic acid (RNA), and liver failure, including HCC, may occur after the achievement of SVR[14]. In 2016, two articles suggested an unexpectedly higher rate of early occurrence and recurrence of HCC in HCV-infected patients who were treated with DAAs[15,16]. Both had relatively shorter-term follow-up periods after the end of treatment (EOT). However, several articles presenting the opposite data or the data from longer-term follow-up periods have been published. A conclusion has not been reached in this matter and several studies are still ongoing. Considering these circumstances, this report focuses on hepatocarcinogenesis after DAA treatment, which will be discussed based on clinical points of view.

HCC OCCURRENCE AND RECURRENCE AFTER SVR BY DAA TREATMENT

Interferon-free regimens with DAA combination can be used to treat HCV-infected individuals who cannot be treated with interferon-based regimens, such as older patients, patients with comorbidities, patients with cirrhosis, or patients with a history of HCC[17]. HCC recurrence or HCC occurrence, respectively, has been defined as the appearance of HCC in a patient with or without history of HCC [18].

In general, HCV infected patients with advanced liver fibrosis tend to develop HCC, compared to those with mild or moderate liver fibrosis[19]. Patients whose HCC has been curatively treated, also have a much higher risk of recurrence of HCC[20].

In 2016, Conti *et al*[15] reported that DAA therapy induced SVR in 91% of patients. During a 24-wk follow-up, HCC occurrence and recurrence, respectively, were detected in 9 of 285 patients (3.16%) and in 17 of 59 patients (28.8%); a total of 26 patients developped HCC. They also demonstrated that neither HCV genotype nor therapeutic DAA regimen correlated to HCC occurrence or HCC recurrence[15].

Similarly, Reig *et al*[16] reported an unexpectedly high rate and pattern of tumor recurrence coinciding with HCV clearance, suggesting the possible disruption of immune tumor surveillance. In their study[16], 8 (13.8%), 45 (77.6%), 2 (3.4%), 3 (5.2%) patients were HCV genotypes 1a, 1b, 3 and 4, respectively.

Guarino *et al*[18] extensively reviewed the association between DAA and HCC in patients with chronic HCV infection. They reported that, among 11 and 18 studies, the HCC occurrence and recurrence rates ranged from 0 to 7.4% and from 0 to 54.4%, respectively, although their observation periods were relatively shorter.

Li *et al*[21] reported that the short-term incidence of HCC is not increased after the eradication of HCV with DAA and mentioned that the previous reports about higher rates of HCC associated with DAAs may be related to the fact that those patients had a higher risk of developing HCC. Notably, this study also suggests that some patients have a higher risk of developing HCC after achieving SVR with DAA. It is important to elucidate the mechanism of the development of HCC after achieving SVR with DAA and to investigate the patients' characteristics. Thus, the rates of HCC occurrence or recurrence varied from a clinical point of views.

CLINICAL INDICATORS OF HCC OCCURRENCE AND RECURRENCE AFTER SVR BY DAA TREATMENT

HCV-infected patients have a decreased risk of HCC after achieving SVR by interferon treatment[11,22]. Previous studies reported that biomarkers including aspartate aminotransferase (AST), old age, liver cirrhosis and higher posttreatment alpha-fetoprotein (AFP) can predict HCC in patients after interferon therapy[23]. Toyoda *et al*[24] suggested that an elevated indicator of liver fibrosis, the FIB-4 index at SVR24, is also a predictor of HCC development in SVR patients. The FIB-4 index was a prediction of 5-year survival in HCV infected patients in the interferon-era[25].

Nguyen *et al*[26] suggested that AFP decreased significantly from pretreatment (median 7.2 ng/mL) to EOT (4.2 ng/mL) and at 12 wk after treatment (4.2 ng/mL) with DAAs. Liver inflammation increased AFP values in the absence of HCC. Of interest, they suggested that the pattern for normalization of AFP with entecavir showed a shorter period and gradual reduction compared to patients treated with pegylated-interferon.

Similarly, Nagaoki *et al*[27] showed that serum AFP levels decreased to similar levels at SVR24 both in the pegylated-interferon plus ribavirin and the DAAs treatment groups, and similar rates of HCC development existed in these two HCV genotype 1 infected patients groups (the cumulative HCC development rates after 1-, 3- and 5-years were 1.5%, 10% and 19% and 1.5%, 10% and 12%, respectively). These data suggested the possible reduced potential for HCC development by DAA treatment is as same as that of interferon-based treatment.

Moreover, Tag-Adeen *et al*[28] showed significant improvement in the FIB-4 index after achieving SVR by DAA in HCV genotype 4 infected patients. However, they also showed that achieving SVR did not guarantee improvement in cirrhosis (61% of cirrhotic patients showed liver stiffness > 12.5 kPa), and cirrhotic patients still had a risk for HCC development despite achieving SVR by DAA. Thus, from the clinical point of view, several liver fibrosis markers may be helpful for the early detection of HCC occurrence and recurrence.

CHANGE IN CYTOKINES AND CHEMOKINES IN HCC OCCURRENCE AND RECURRENCE AFTER SVR BY DAA TREATMENT

Previous reports suggested that DAA changes the cytokine/chemokine levels compared to the pretreatment levels, and it may be related to hepatocarcinogenesis. Sung *et al*[29] investigated the level of type I interferon, interferon-β in HCV genotype 1b infected patients. Type I interferons bind to a common cell surface receptor, resulting in the activation of the Jak-STAT signal transduction system[30]. Interferon-β may be important not only to prevent patients with acute hepatitis C from developing chronic infection[31] but also to reduce the risk of HCC[32]. After DAA treatment, the expression levels of interferon-β, interferon-induced protein 44 (IFI44) and C-X-C motif chemokine ligand 10 (CXCL10) significantly decreased and rapidly normalized at EOT in the peripheral blood mononuclear cells (PBMCs)[29]. IFI44 and CXCL10 correlated with the pretreatment expression level of interferon-β.

Carlton-Smith *et al*[33] exhibited similar interferon-stimulated gene results in PBMC

at treatment week four and EOT and showed a reduction in CXCL10, CXCL11 and macrophage inflammatory protein (MIP)-1β levels. Hengst *et al*[34] suggested that the expression level of 22 cytokines/chemokines including type I interferon and CXCL10 decreased significantly from baseline to 12 wk after treatment by DAA treatment in patients with HCV genotypes 1, 2, 3 and 4. CXCL10 level was increased in interferon-based therapies by the responsiveness to interferon. In contrast, the interferon system, including CXCL10, is not increased during DAA treatment. A similar effects were also observed in patients infected with HCV genotype 2 or 3 who were treated with DAA[35]. The interferon-stimulated intrahepatic and peripheral gene expression declines with HCV eradication[36].

DAA treatment increased the serum vascular endothelial growth factor (VEGF) level[37,38], and it remained stably elevated at the 3-mo follow-up[38]. These were observed in patients with HCV genotypes 1a, 1b, 2, 3 and 4. VEGF was significantly related to the serum angiopoietin-2 level, and angiopoietin-2 expression in HCC or in cirrhotic tissue before DAAs was related to the risk of HCC recurrence or occurrence. They[38] showed that the extremely high expression of angiopoietin-2 in recurrence of HCC and de novo HCC had its counterpart in the increased levels of circulating VEGF during DAA therapy. Therefore, they suggested that the interaction between the local overexpression of angiopoietin-2 by the slow blood flow of portal hypertension arising through the progression of chronic liver damage and circulating VEGF is a risk factor for developing HCC that is linked with the advanced stage of cirrhosis in patients treated with DAAs[38].

Tumor necrosis factor (TNF)-α, known as an important inflammatory mediator that induces immune responses, was originally found to induce tumor lysis. TNF induces the cellular apoptosis of hepatoma cell lines and HCV core and NS5A proteins block TNF-induced cellular apoptosis[39,40]. TNF-α related apoptosis-inducing ligand (TRAIL) also induces the apoptosis of human hepatic stellate cells and HCV blocks TRAIL-induced cellular apoptosis[41]. Spaan *et al*[42] reported that there is down-regulation in TRAIL-mediated killing by NK cells during DAA therapy in patients infected with HCV genotype 1b. Further studies will be needed. Thus, rapid changes in several cytokines and chemokines are observed during and after DAA treatment and may have several effects on HCC occurrence and recurrence.

IMMUNOLOGICAL MECHANISMS OF HCC OCCURRENCE AND RECURRENCE AFTER SVR BY DAA TREATMENT

Several reports showed that a rapid decreased or normalized immuno-surveillance causes early HCC recurrence or occurrence after DAA therapy. The activating receptor natural killer group 2, member D (NKG2D) and its ligands play a crucial role in the immune response to HCC. Reduced NKG2D ligand expression in HCC correlates with early recurrence[43]. NKG2D predicts the early emergence of HCC after interferon-free DAAs[44].

Major histocompatibility complex class I-related chain A (MICA), which is one of the human ligands of NKG2D, has been known to be a key molecule in viral HCC immune surveillance, as the interaction with NKG2D triggers NK cell-mediated cytotoxicity toward the stressed cells[45,46]. Moreover, HCC sheds membrane-bound MICA as soluble MICA and down-regulates the expression of NKG2D on the NK cell surface because escape immune surveillance[47]. Chu *et al*[44] reported that 12% of DAA-treated HCV genotype 1 infected patients developed HCC recurrence or occurrence within 24 wk after EOT. They suggested that a rapid decrease in NKG2D levels at EOT correlated with early HCC emergence in DAA-treated patients. Of interest, this phenomenon was not found in patients treated with the interferon-based regimen. These data may suggest a risk of early HCC after interferon-free DAA treatment, different from interferon-based therapy. Golden-Mason *et al*[48] reported that the frequency of clusters of differentiation (CD) of 56^{bright} immature NKs decreased 2 wk after DAA therapy started and was maintained at SVR12 in HCV genotype 1 infected patients. Moreover, the downregulation of receptors of cytotoxic signaling including TRAIL, NKp30 and NKp46, was observed 12 wk after DAA therapy started and was maintained at SVR12[48]. They suggest that rapid viral clearance induced by DAA therapy normalizes NK cell function and reduces cytotoxic activity.

The other mechanism was explained by the numbers of peripheral $FOXP3^+CD25^+CD4^+$ regulatory T cells[49]. In their report[49], peripheral $CD4^+$ T cells numbers persisted in DAA treatment groups even approximately 51 wk after EOT in HCV genotype 1 infected patients. In HCV genotype 1a/1b patients, DAA therapy reduced the T-cell compartment in the peripheral blood and re-differentiation of the T lymphocyte memory compartment and resulted in a reduction in the expression of the

coinhibitory molecule T cell immunoglobulin and immunoreceptor tyrosine-based inhibition motif domains (TIGITs) in bulk T lymphocytes[50]. They reported that HCV eradication after DAA therapy involves immune reconstitution[50]. These immune reconstitutions may support the successful treatment of oral lichen planus after DAA therapy[51]. Thus, rapid immunological changes, including in NKG2D systems are observed during and after DAA treatment, and they may have several effects on HCC occurrence and recurrence.

HOST GENETIC FACTORS OF HCC OCCURRENCE AND RECURRENCE AFTER SVR BY DAA TREATMENT

In the interferon era, genome-wide association studies (GWAS) identified that several genetic variants in close proximity to interleukin 28B (IL28B; also known as interferon-lambda 3) variants were strongly associated with the response to pegylated-interferon-α plus ribavirin therapy for chronic HCV infected patients[52-54]. Moreover, IL28B variations are independent predictors of the progression of hepatic fibrosis[55,56] and seems to be involved in the hepatocarcinogenesis[57,58]. However, with the induction of interferon-free regimens, the importance of IL28B genetic variants may be diminishing in HCV genotype 1 infected patients[59]. There have been some reports that showed the relation between single nucleotide polymorphisms (SNPs) and hepatocarcinogenesis.

Lange *et al*[60] revealed a SNP in HLA complex P5 (HCP5) rs2244546, which is a upstream of MICA, as a strong predictor of HCV-related HCC. The differentially methylated cytosine-phosphate-guanine (dmCpG) loci were also reported[61]. The dmCpG loci were highly enriched for enhancers, promoters, or CpG islands and the surrounding regions and were hypermethylated in HCC infected with HCV. The dmCpG loci were associated with cellular growth and proliferation although this report has several limitations[61].

Of interest, Matsuura *et al*[62] investigated GWAS data on hepatocarcinogenesis specifically in HCV-infected patients after the eradication of HCV by interferon-based therapy. There was no difference of development of HCC in HCV genotype 1 or 2 infected patients after eradication of HCV. They found a strong association between the SNP rs17047200, located within the intron of the tolloid like 1 gene (TLL1) on chromosome 4, and the development of HCC, and it played a role in hepatic fibrogenesis. It is uncertain whether interferon-free therapy can inhibit TLL1 after the eradication of HCV. Future studies are needed to evaluate this point.

HCV-mediated enhancement of microRNA miR-373 impairs the JAK/STAT signaling pathway[63]. MicroRNAs associated with HCV-related immunopathogenesis which were found to be enriched in exosomes of HCV viremic patients (in particular, miR-122-5p, miR-222-3p, miR-146a, miR-150-5p, miR-30c, miR-378a-3p and miR-20a-5p), were markedly reduced by DAA therapy. Enrichment of immunomodulatory microRNAs in exosomes of HCV patients was correlated with their inhibitory activity on innate immune cell functions[64]. DAAs against HCV may have an impact on extracellular vesicles including microRNAs, leading to immunomodulation. Thus, several host genetic factors and microRNAs are change during and after DAA treatment, which may have several effects on HCC occurrence and recurrence.

CONCLUSION

DAA therapy is more efficacious for HCV eradication with fewer side effects. The use of DAAs does not increase the occurrence or recurrence of HCC according to clinical trials. However, the mechanism that altered the immunological balance because of a rapid decrease of HCV viral load in the short-term after DAA therapy may contribute to early tumor development (Figure 1). Sasaki *et al*[65] demonstrated the changes of complement cascades and neutralizing antibodies after SVR by DAA. Complement-dependent cytotoxic effects[66] and neutralizing antibodies[67] are also important for HCC cells survival. Thus, a longer follow-up period and basic research are required to establish whether there is a risk or advantage of HCC recurrence or occurrence with interferon-free therapy. Moreover, new serum biomarkers that may be altered by DAA therapy should be investigated in the follow-up of HCV-patients with SVR after DAA and interferon-based regimens.

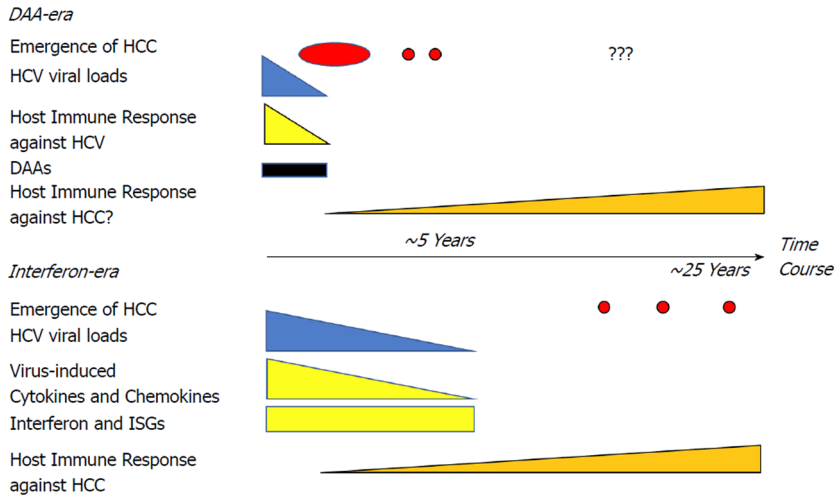

Figure 1 Emergence of hepatocellular carcinoma (HCC) during and after treatment in direct-acting antiviral (DAA) era (upper part) and intreferon era (lower part). It is uncertain whether HCC emerges in patients treated with DAA in more than 5 years after sustained virological response. DAA: direct-acting antiviral; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; ISG: interferon-stimulated gene.

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P- Reviewer: Carneiro BM, Pandey VN, Arriagada GL
S- Editor: Dou Y **L- Editor:** A **E- Editor:** Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 December 27; 10(12): 907-910

DOI: 10.4254/wjh.v10.i12.907

ISSN 1948-5182 (online)

EDITORIAL

Stem cell transplantation for the treatment of end-stage liver disease

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Author contributions: Chen EQ and Tang H designed the structure of this paper. Wu DB searched the literature and wrote the paper; Chen EQ critically revised the paper.

Conflict-of-interest statement: No potential conflicts of interest.

Manuscript source: Invited manuscript

Received: July 6, 2018
Peer-review started: July 6, 2018
First decision: August 8, 2018
Revised: August 14, 2018
Accepted: October 8, 2018
Article in press: October 9, 2018
Published online: December 27, 2018

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Abstract

The past two decades have witnessed an explosion of research and clinical application of stem cells, transforming the field of regenerative medicine. Stem cell transplantation has already been performed to treat patients with cancer, liver diseases, and various types of chronic diseases. Indeed, stem cell-based therapies are effective in many diseases, and provide novel insights into the treatment of end-stage liver disease. Several clinical trials have indicated the efficacy profiles of stem cell transplantation in patients with end-stage liver disease, including liver cirrhosis, liver failure, and liver tumors. Animal models of acute liver failure have also provided important insights into the safety, mechanisms, and efficacy of stem cell therapies. Nevertheless, excitement due to this promising field must be tempered with careful and calculated research. In particular, studies on the quality, safety, and efficacy of stem cell transplantation are needed to ensure that qualified products are tested in well-designed clinical trials and approved by governments. Therefore, further investigations are required to effectively balance the safety with the innovation of stem cell transplantation research toward the effective treatment of end-stage liver disease.

Key words: Stem cell transplantation; End-stage liver disease; Clinical treatment; Efficacy; Safety

Core tip: Stem cells have the capacity for multiple rounds of self-renewal and differentiation, and play important roles in numerous biological functions. Treatment of end-stage liver disease *via* stem cell transplantation has emerged as an effective therapeutic alternative in clinical practice. However, caution should be paid to ensuring the safety and efficacy of stem cell transplantation to avoid the use of products that are not rigorously tested that may put patients at risk.

Wu DB, Chen EQ, Tang H. Stem cell transplantation for the treatment of end-stage

liver disease. *World J Hepatol* 2018; 10(12): 907-910
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/907.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.907

INTRODUCTION

Due to their capacity for multiple rounds of self-renewal and differentiation, stem cells play roles in numerous biological phenomena including immunomodulation, anti-inflammation, anti-apoptosis regulation, angiogenesis, promotion of tissue repair, and production of growth factors[1-3]. The term "stem cells" represents cells of various origins, including mesenchymal stem cells (MSCs), adipose-derived mesenchymal stem cells, embryonic stem cells, induced pluripotent stem cells, hepatic progenitor cells, and hematopoietic stem cells[1,4-8]. However, MSCs are the most common stem cell source for basic and clinical research given the lack of ethical constraints regarding their usage and availability[4,8].

In the past few decades, stem cell transplantation has emerged as a novel and promising therapy for the treatment of patients with cancer, nervous system diseases, eye diseases, orthopedic disorders, diabetes mellitus, and liver diseases. Moreover, advances in stem cell transplantation from basic and translational clinical research have yielded improvements in the survival of patients with benign and malignant hematologic disorders[9] and stem cell transplantation has proven to be an effective therapeutic alternative for central nervous system diseases, including Alzheimer's disease[10]. Moreover, stem cell therapy has been shown to delay or suppress the progression of end-stage liver disease[4,8,11].

TREATMENT OF END-STAGE LIVER DISEASE *VIA* STEM CELL TRANSPLANTATION

To date, there have been numerous clinical studies on stem cell transplantation for the treatment of end-stage liver disease, demonstrating its side effects and efficacy profiles. Furthermore, there were 139 clinical trials registered, including 27 ongoing clinical trials, on the association between stem cell transplantation and liver disease in accordance with the guidelines outlined in ClinicalTrials.gov on July 01, 2018 (http://www.clinicaltrials.gov). Of these, 52 clinical trials were focused on liver cirrhosis (LC), nine on liver failure, and six on liver cancer.

Previous studies indicated that MSC transplantation could constitute an effective treatment for LC. In a multicenter, randomized, open-label, phase 2 trial, autologous bone marrow-derived transplantation of MSCs safely improved liver function and facilitated the quantification of fibrosis following liver biopsy in patients with alcoholic cirrhosis[7]. Another open-label, paired, controlled study from China demonstrated that transplantation of umbilical cord-derived MSCs (UC-MSCs) also improved liver function and reduced ascites in patients with chronic hepatitis B (CHB) and in decompensated LC[1]. MSC transplantation was also shown to improve liver function in LC patients with autoimmune diseases[12]. However, another randomized, controlled phase 2 trial yielded no evidence to support the benefits of granulocyte colony-stimulating factor (G-CSF) administration alone or supplementation of G-CSF with stem-cell transplantation, with no significant dierences in improved liver dysfunction or decreased fibrosis in LC patients after stem cell transplantation[3]. These conflicting results may be associated with differences in LC etiology, an increased frequency of adverse events, and differences in stem cell types used in these studies.

Moreover, studies involving animal models of acute liver failure have shown strong evidence pointing to the success of MSC transplantation in improving liver function, inhibiting hepatocyte apoptosis, and promoting hepatocyte proliferation in animal models of acute liver failure[6], suggesting that MSC transplantation may be used to treat liver failure. In 2012, Shi *et al*[5] performed a case-control study to evaluate the safety and efficacy of UC-MSC transplantation in CHB patients with acute-on-chronic liver failure (ACLF); and found increased survival rates, accompanied by reduced end-stage liver disease scores and enhanced liver function. Another study on MSC transplantation for the treatment of ACLF patients also achieved similar results, in which the treatment increased the 24-wk survival rate, improved liver function, and decreased the incidence of severe infections[11].

Moreover, we recently conducted a systematic review and meta-analysis of MSC transplantation in ACLF patients, which showed that the treatment significantly reduced mortality rates, without increasing the incidence of severe complications[13]. There were also no differences in the incidence of severe complications (*e.g.*, encephalopathy, hepatorenal syndrome, gastrointestinal bleeding) between the standard medical treatment and the MSC treatment group in ACLF patients[5]. Nevertheless, long-term follow-up is needed to confirm the safety of MSC transplantation.

FUTURE PERSPECTIVES

Studies on stem cells and regenerative medicine have received increasing attention in the life sciences in the past 20 years. Stem cells are undifferentiated cells that undergo both self-renewal through symmetric cell division and differentiate into specialized cells, tissues, and organs through asymmetric cell division. Stem cell-based therapies have proven to be effective in many diseases, providing novel insights into the treatment of end-stage liver disease. Indeed, in recent years, numerous studies have reported stem cell-based "cures" for an extraordinary and implausible range of medical conditions.

However, research on the safety and innovation of stem cell transplantation for end-stage liver disease must be well-balanced. Some risky procedures performed without substantial evidence have led to medical accidents, leading to blindness, paralysis, or even death[14].

Moreover, both the administration and the government must be involved in regulations and advisement to ensure the quality, safety, and efficacy of stem cell transplantation. Two finalized tenders regarding guidelines to establish a more stringent policy framework were issued by the Food and Drug Administration (FDA), which included the requirement of sponsors to document a biological license application, request permission from the agency before proceeding to FDA-supervised clinical trials, and obtain agency approval before marketing[15]. To promote rapid yet responsible advancements in the fundamental knowledge and clinical application of stem cells and regenerative medicine, the International Society for Stem Cell Research (ISSCR) has issued three guidelines[2]. The 2016 guidelines revise and extend two prior sets of guidelines (ISSCR, 2006; ISSCR, 2008) and address an integrated set of principles and best practices for ensuring progress in basic, translational, and clinical trials[2]. Overall, safe and effective stem cell transplantation for treating end-stage liver disease will only be achieved from well-designed clinical trials and qualified products approved by the FDA or the government, while avoiding the high risks of unproven cell therapy products.

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P- Reviewer: Gencdal G, Matteucci C

S- Editor: Dou Y **L- Editor:** Webster JR **E- Editor:** Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 December 27; 10(12): 911-923

DOI: 10.4254/wjh.v10.i12.911

ISSN 1948-5182 (online)

MINIREVIEWS

Autoimmune hepatitis: Appraisal of current treatment guidelines

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Author contributions: Lowe D performed the literature review and wrote the manuscript; John S co-authored, edited and reviewed the manuscript.

Conflict-of-interest statement: Lowe D and John S do not have any affiliations with or involvement in any organization or entity with any financial or non-financial interest in the subject matter pertaining to this manuscript.

Manuscript source: Invited manuscript

Received: August 28, 2018
Peer-review started: August 28, 2018
First decision: October 5, 2018
Revised: October 16, 2018
Accepted: October 23, 2018
Article in press: October 23, 2018
Published online: December 27, 2018

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Abstract

Autoimmune hepatitis affects patients of all ages and gender, across all geographic regions. Although still rare, its incidence and prevalence are increasing. Genetic predisposition conveyed by human leucocyte antigen is a strong risk factor for the disease and may be responsible in part for the wide variation in presentation in different geographic regions. Our understanding of the underlying pathogenic mechanisms is evolving and may lead to development of more targeted immunotherapies. Diagnosis is based on elevated levels of serum aminotransferases, gamma globulins, autoantibodies and characteristic findings on histology. Exclusion of other causes of chronic hepatitis is important. Although undiagnosed disease is associated with poor outcomes, it is readily treatable with timely immunosuppressive therapy in the majority of patients. International guidelines are available to guide management but there exists a disparity in the standard treatment regimens. This minireview aims to review the available guidelines and summarize the key recommendations involved in management of this complex autoimmune disease.

Key words: Autoimmune hepatitis; Treatment; Hypergammaglobulinemia; Autoantibodies; Azathioprine

Core tip: Autoimmune hepatitis, is a rare inflammatory condition of the liver that can affect all ages and gender, across all geographic regions. It has a wide variability in clinical presentation and thus, diagnosis can be challenging. While undiagnosed disease leads to significant morbidity and mortality, timely initiation of treatment leads to favorable outcomes in the majority of cases. Guidelines are available by international societies but there exists a disparity in the standard treatment indications and regimens. In this minireview, we summarize key points from the available literature and guidelines, focusing on appropriate indications and different treatment regimens available.

Lowe D, John S. Autoimmune hepatitis: Appraisal of current treatment guidelines. *World J Hepatol* 2018; 10(12): 911-923
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/911.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.911

INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory disease of the liver of unknown cause that can affect children and adults of all ages. The course of the disease can occasionally be fluctuating, but is generally progressive. It is marked by interface hepatitis and lymphoplasmacytic infiltration on histology, serum hypergammaglobulinemia and characteristic circulating autoantibodies. Since it was first described by Waldenström in 1950 as a disease affecting young women characterized by jaundice, high serum gammaglobulins, and amenorrhea causing liver cirrhosis, it has been known by many different labels including "lupoid" hepatitis, but AIH has been accepted as the most appropriate term[1].

Many variant, overlapping forms of AIH exist, particularly with coexisting cholestatic features, primary biliary cholangitis (PBC) or primary sclerosing cholangitis (PSC). Diagnosis requires exclusion of other causes of chronic hepatitis such as drug induced liver injury (DILI), viral hepatitis, alcoholic hepatitis or non-alcoholic steatohepatitis (NASH) as some of these cases respond to immunosuppressive therapy. A therapeutic response to corticosteroids in AIH was observed in the early 1950s, as well as an early relapse after withdrawal of corticosteroids[2]. By the late 1950s, combined approach with immunomodulators was described and it remains the cornerstone of therapy. Wide heterogeneity of clinical presentation and relatively rare incidence of the disease has limited the advancement in clinical trials. Thus, more than 50 years after its original description, AIH remains a diagnostic and therapeutic challenge.

Guidelines by the American Association for the Study of Liver Diseases (AASLD) (2010) and European Association for the Study of the Liver (EASL) (2015) provide practice guidance on the management of this complex disease[3,4]. The aim of this minireview is to provide an overview of the current treatment guidelines, with an emphasis on appropriate immunosuppressive therapy and difficult to manage cases.

EPIDEMIOLOGY

AIH is a disease that affects all age groups, occurs in all ethnicities and geographic regions but affects the female gender disproportionately. In the United States, women are affected 3.5 times more than men, and 76% of patients in a Swedish study were women[5,6].

Previously considered to be a disease of the young, a recent large Danish nationwide population-based study demonstrated the peak age of incidence at more than 60 years for both men and women. It also showed that both the incidence and prevalence of AIH is rising[7]. Although it is still considered a rare disease, as its prevalence ranges from 16 to 18 cases per 100000 persons in Europe. In Europe and the United States, it accounts for 2% to 3% of the pediatric and 4% to 6% of the adult liver transplantations[3].

The occurrence and clinical course appear to vary according to ethnicity. The disease appears to be more common and more severe in the North American aboriginals compared to the Caucasian population; African-Americans are more likely to present with cirrhosis; patients with Asian or other non-European Caucasoid back ground have poor outcomes. These diverse clinical outcomes between different ethnic groups, within and between countries may reflect differences in genetic predisposition, environmental stimuli as well as complex socioeconomic reasons such as delivery of healthcare[8].

PATHOGENESIS

The model for the pathogenesis of AIH follows the general hypothesis underlying many autoimmune diseases. The disease is thought to arise in a genetically predisposed individual when a potential environmental antigenic trigger sets of a T-

cell mediated immune response directed at liver antigens, leading to a progressive inflammatory process and scarring[9].

Although a definite antigenic trigger has not been found, some of the proposed triggering factors include drugs, toxins and infectious agents. Genetic predisposition to AIH is primarily conveyed by human leucocyte antigen (HLA) haplotype which determines the autoantigen presentation and $CD4^+$ helper T-cell recognition. HLA-DR3 was shown to be strongly associated with the onset of AIH in the Caucasian population. Subsequently characterized as HLA DRB1*0301, it is associated with a younger age of onset and a more severe phenotype. This HLA class II locus determines the shape of the peptide binding groove of the Major histocompatibility complex (MHC) class II complex which presents peptide antigens to the $CD4^+$ T cells. Strong association of DRB1*0301 haplotype with AIH suggests that a specific peptide bound to this complex is recognized by T cells within the liver which then become autoreactive[10].

Various other haplotypes have been found to be associated with AIH in different geographic populations such as DRB1*0401 in Europeans and DRB1*0405 in Japanese[10]. When negative for DRB1*0301, these patients demonstrate a milder form of the disease with older age of onset. Association with varying haplotypes suggests diversity among the peptide antigens triggering the disease but provide a stronger evidence for a T-cell mediated immune reaction driving inflammation and fibrosis.

HLA haplotypes convey the strongest genetic predisposition to AIH. In addition, many other genetic risk factors have been identified, predominantly affecting the immune regulatory function. Particular variant of cytotoxic lymphocyte antigen-4 (CTLA-4)[11], important co-stimulator of T-cells has been associated with AIH. Mutations in the autoimmune regulator (*AIRE*) gene, important for inducing central immune tolerance, leads to a complex autoimmune phenotype, with majority developing AIH.

Knowledge of the underlying genetic predisposition may lead to identification of potential environmental triggers, better understanding of the disease phenotype and development of therapeutic targets in the future, but this as of now appears to be clinically dispensable.

CLINICAL FEATURES

AIH is a heterogenous disease, characterized by a fluctuating course of activity. Therefore, the clinical manifestations are variable. The spectrum of presentation ranges from asymptomatic disease to acute severe hepatitis or debilitating smoldering cirrhosis. Thus, the diagnosis of AIH should be entertained in any patient presenting with signs or symptoms liver disease, whether acute or chronic.

Presentation

Up to a third of adult patients are found to have acute icteric hepatitis[12]. Presentation is similar to acute viral hepatitis and patients may develop non-specific symptoms such as malaise, fatigue, anorexia, nausea, abdominal pain, and arthralgias. Physical exam may be normal or reveal jaundice, hepatomegaly and splenomegaly. Occasionally, patients may have a severe or fulminant presentation with elevated prothrombin time and serum aminotransferase levels in thousands leading to acute liver failure and need for liver transplantation. This presentation is more common in children and relatively rare after 30 years of age.

Many patients with an acute presentation can undergo spontaneous recovery and the initial episode misdiagnosed as a transient illness. Subclinical disease can progress and lead to cirrhosis. Approximately, one third of all adult patients and almost 50% of children already have cirrhosis at the time of diagnosis[13,14]. AIH, can therefore, present for the first time with signs and symptoms of decompensated cirrhosis including ascites, variceal bleeding or hepatic encephalopathy.

Due to the improvement in diagnostic modalities, more than half of all patients diagnosed with AIH have no specific symptoms. They are usually diagnosed upon work up of abnormal liver enzymes detected on routine blood work for other indications. AIH may rarely be diagnosed during pregnancy, or manifest for the first time during post-partum period. Patients with AIH can undergo spontaneous remissions during pregnancy and typically experience flare up in the immediate post-partum period, likely due to immune reconstitution[15].

It is important to keep in mind the concomitant occurrence of other autoimmune diseases with AIH, particularly autoimmune thyroiditis, rheumatoid arthritis, ulcerative colitis, type 1 Diabetes mellitus and celiac disease[16].

Laboratory features

Abnormalities of the liver biochemistry predominantly reflect a hepatocellular pattern with elevated aminotransferases and variable, but usually mild elevation of serum alkaline phosphatase. Any magnitude of serum aminotransferase elevation is possible, and higher elevations are associated with a more severe course and poor outcomes[17].

Generalized elevation of serum gammaglobulins, particularly the IgG fraction is a characteristic feature of AIH. It can be seen in up to 90% of patients with AIH and a diagnosis of AIH should be questioned in patients without hypergammaglobulinemia[18,19].

Autoantibodies

Presence of serum auto-antibodies is a characteristic hallmark of AIH and serological testing is an important part of the diagnostic work up of the disease[1,18]. Antibodies important for the diagnosis of AIH are described in Table 1. Diagnostic value of serological testing also depends on the technique used. Performance parameters for indirect immunofluorescence assays are well defined for diagnosis of AIH and this is the recommended technique for antibody detection[19].

This is performed using rodent tissue or Hep2 cell lines and results are given in titers. It provides the best sensitivity and specificity profile for antinuclear antibody (ANA) and anti-smooth muscle antibody (ASMA). However, it is labor and time intensive, subject to intra-observer variation and requires experienced lab technicians. Therefore, solid phase enzyme immunoassays have gained popularity and are replacing indirect immunofluorescence. These tests are very antigen specific, easy to perform and give rapid results. However, diagnostic parameters for ANA, ASMA detected by enzyme-linked immuno sorbent assay (ELISA) are not well defined and as the recombinant antigens may differ from those detected by indirect immunofluorescence, the results of the two assays should not be equated[20].

Several other antibodies have been evaluated such as antibodies to asialoglycoprotein receptor which are closely associated with histologic activity and may prove useful in defining the treatment endpoint. Antibodies to liver cytosol type 1 can coexist with anti LKM1 in type 2 AIH and are associated with early age of disease onset and severe phenotype. Newer antibodies continue to be characterized to improve the diagnostic accuracy and prognostic value[21].

Histology

Histological confirmation is a prerequisite for diagnosis of AIH[1,18]. It is also useful in guiding therapeutic decisions. Certain characteristic features have been described but none are pathognomonic. Interface hepatitis characterized by inflammation at the parenchymal portal junction is the hallmark feature. Hepatocyte rosette formation, dense plasma cell rich infiltrate and emperiopolesis (active penetration of lymphocytes into hepatocytes) are other common findings. Multi acinar and bridging necrosis is associated with severe disease[22].

DIAGNOSIS AND DIAGNOSTIC CRITERIA

In patients presenting with signs and symptoms of acute or chronic hepatitis, diagnosis of AIH is made on the basis of aforementioned biochemical and serological lab results, and confirmed by liver histology. Difficulties may arise due to the wide variability of presentation, fluctuating disease course, variant forms and presence of co-existing liver diseases. Therefore prior to confirming AIH, it is crucial to exclude other causes of inflammatory hepatitis such as alcoholic or NASH, viral hepatitis and DILI. Other causes of chronic liver disease such Wilson's disease, hemochromatosis and alpha 1 antitrypsin deficiency should be ruled out as well.

Different scoring systems have been proposed to assist in the diagnosis of AIH. In 1999, the International Autoimmune Hepatitis Group (IAIHG) published a comprehensive scoring system which grades every clinical, laboratory and histological feature of AIH, including response to corticosteroid treatment. Initially designed as a research tool for clinical trials, it was useful in clinical practice for patients with few or atypical features of disease. Its complexity and failure to distinguish AIH from cholestatic syndromes limited the clinical utility[1]. In 2008, a simplified scoring system was proposed by the same group for every day clinical practice (Table 2) [18]. It considers the key diagnostic criteria (autoantibodies, degree of hypergammaglobulinemia, liver histology and exclusion of viral hepatitis). It performs well with good sensitivity and specificity (both more than 90%) in diverse populations, and with chronic disease. Its relative ease of use makes it friendly for clinical practice. However, this has not been validated in prospective clinical trials and its utility in acute or fulminant presentations is limited.

Table 1 Serologic markers of autoimmune hepatitis

ANA	Variably expressed with ASMA in type 1 AIH
	Heterogenous antigen profile
	No single staining pattern is pathognomonic for diagnosis of AIH
	Most useful when found with ASMA (diagnostic accuracy 74%)[20]
ASMA	Marker of type 1 AIH along with ANA
	Reacts to several cytoskeletal elements, especially F-actin.
	ELISA against F-actin as the substrate can be used instead of indirect immunofluorescence but may miss the diagnosis in 15% to 20% of cases[20]
Anti-SLA/LP	Only disease specific antibody with specificity of 99% for AIH
	Present in only 15% patients with AIH in the United States
	Known to have a defined antigen, SEPSECS. ELISA is the preferred methodology of testing
	Closely associated with HLA DRB1*03 and Anti-Ro/SSA
	Have prognostic value as it is associated with severe disease, higher risk of relapse and need for lifelong treatment
Anti-LKM1	Serologic marker for type 2 AIH.
	CYP2D6 is the target antigen. Shares homology with hepatitis C virus antigen
	Present mainly in children, worldwide. Rare in adults in the United States (< 4%)
	Associated with HLA DRB*07
Atypical pANCA	Common in type 1 AIH, and absent in type 2 AIH
	Associated with PSC, UC

ANA: Antinuclear antibodies; ASMA: Anti-smooth muscle antibodies; AIH: Autoimmune hepatitis; ELISA: Enzyme linked immunosorbent assay; Anti-SLA/LP: Anti-soluble liver antigen/liver pancreas antibody; SEPSECSA: Sep (phosphoserine) tRNA: Sec (selenocysteine) tRNA synthase; Ro/SSA: Ribonucleoprotein/Sjögren's syndrome A protein; Anti-LKM1: Antibodies to liver kidney microsome type 1; pANCA: Perinuclear antineutrophil cytoplasmic antibodies; PSC: Primary sclerosing cholangitis; UC: Ulcerative colitis.

Scoring systems are not particularly helpful in making the distinction from DILI during acute or hyperacute presentation. Some drugs, such as minocycline and nitrofurantoin, can induce a drug induced autoimmune-like hepatitis and appropriate diagnosis can only be made with passage of time[23]. In severe presentations, steroids should be started and the most likely offending drug should be withdrawn. After normalization of biochemical parameters, steroids should be tapered. *De novo* AIH will typically recur after treatment withdrawal whereas DILI often resolves with the removal of offending agent and does not recur.

TREATMENT

All patients with AIH must be considered candidates for treatment and the timing of therapy rather than the need for therapy is the most important variable to consider. Early studies in 1970s and 1980s showed that untreated patients with moderate to severe AIH, had very poor outcomes, and the 6-month mortality reached as high as 40%. It was also shown that patients treated with immunosuppressive therapy did very well with improvement in biochemical parameters, clinical symptoms and overall mortality[17,24,25].

Liver biopsy should be performed in all patients to make a diagnosis of AIH and before starting treatment. Transjugular liver biopsy may be performed if there is severe coagulopathy. There is general consensus that patients with active AIH [these include patients with aspartate transaminase (AST) > 10 times the upper limit of normal (ULN), AST > 5 × ULN and total IgG > 2 × ULN, or with hepatic activity index > 4/18 on histology] need timely initiation of immunosuppressive therapy. As per the AASLD guidelines, absolute indications for treatment are (1) AST > 10 × ULN; (2) AST > 5 × ULN along serum IgG > 2 × ULN; (3) bridging necrosis or multiacinar necrosis on histology; and (4) incapacitating symptoms such as fatigue and arthralgia[3]. The EASL clinical practice guidelines consensus group recommends treatment for all patients with active AIH[4]. Our recommendation based on review of the literature and clinical guidelines is that all patients with clinical, laboratory or histological features of active liver inflammation should be considered as candidates for treatment as long as they do not have contraindications or risks for significant

Table 2 Simplified diagnostic criteria of the International Autoimmune Hepatitis Group

Parameter	Discriminator	Score
ANA or ASMA	≥ 1:40	+ 1[1]
	≥ 1:80	+ 2[1]
Anti-LKM	≥ 1:40	+ 2[1]
Anti-SLA/LP	Any titer	+ 2[1]
Total IgG	> ULN	+ 1
	> 1.1 × ULN	+ 2
Liver histology	Compatible with AIH	+ 1
	Typical of AIH	+ 2
Absence of viral hepatitis	No	0
	Yes	+ 2

[1]Addition of all points for autoantibodies must be done, maximum 2 points allowed. Definite autoimmune hepatitis (AIH) ≥ 7; Probably ≥ 6. Typical histology for AIH: Each of the following should be present, interface hepatitis, plasma cell infiltrates, emperiopolesis, and hepatic rosette formation. Compatible liver histology: Chronic hepatitis with lymphocytic infiltrations without typical features as above. AIH: Autoimmune hepatitis; ANA: Antinuclear antibody; ASMA: Anti-smooth muscle antibody; Anti-LKM: Anti-liver kidney microsomal antibody; Anti-SLA/LP: Anti-soluble liver antigen/liver pancreas antibody[18].

adverse effects from corticosteroid or azathioprine therapy. Patients with advanced fibrosis and even cirrhosis with active ongoing inflammation on histology should receive therapy as regression of scarring with successful treatment has been reported[17,24,25]. Acute presentation with jaundice as well as subclinical development of fibrosis is more common in childhood, such that more than 50% of the children diagnosed with AIH already have cirrhosis[26]. Therefore, most children with AIH need to be started on treatment. Risks of therapy outweigh any benefits when cirrhosis is already decompensated or there is minimal or no disease activity on histology. Therapy should not be started in such patients.

Benefits of treatment in asymptomatic older individuals without cirrhosis or advanced fibrosis and mild disease activity are unclear. Treatment in such cases must be individualized. The risks of immunosuppression must be weighed against the risk of progression of subclinical disease. Ten-year survival in patients with mild disease without treatment has been reported to range from 67% to 90%[27]. Therefore, the urgency of initiation of treatment is much less in such a patient population. However, AIH can have a fluctuating course with spontaneous remissions and relapses. A significant proportion of asymptomatic patients become symptomatic over time and risk for progression to cirrhosis and HCC is possible. Therefore, the guidelines agree that treatment should be offered to patients with mild disease especially if they do not have contraindications to immunosuppressive therapy. If decision is made to withhold treatment then these patients should be closely monitored with measurement of ALT and total IgG every 3 mo[3,4]. An algorithm for decision making regarding initiation of immunosuppressive therapy is shown in Figure 1.

Induction of remission

The goal of treatment is to obtain complete biochemical and histological resolution of disease. Two treatment regimens are equally effective and are recommended by the AASLD and British Society of Gastroenterology (BSG). First is prednisone monotherapy starting at 60 mg daily, tapered down over 4 wk to 20 mg daily which is then continued until treatment end point. Dose can subsequently be tapered by 5 or 2.5 mg per week to achieve the lowest effective dose of steroids. The BSG, on the other hand, recommends treatment in all cases where the serum aminotransferases are greater than 5 times the ULN, irrespective of the other criteria for treatment[28]. The other is the combination regimen of prednisone starting at 30 mg daily tapered over 4 wk to 10 mg daily and azathioprine 50 mg daily (United States) or 1-2 mg/kg per day (Europe)[3,28].

The evidence for mortality benefit of immunosuppressive therapy with steroids and/or combination with azathioprine was established in a number of controlled trials in the 1960s and 1970s. A sentinel study performed in Mayo clinic in 1972 compared prednisone monotherapy (starting with 60 mg/d, tapered down to 20 mg over four weeks), azathioprine monotherapy (100 mg/d), combination therapy (prednisone starting at 30 mg/d, decreased to 10 mg/d maintenance after 4 wk combined with azathioprine at 50 mg/d) and placebo. There was a significant but similar mortality benefit with prednisone monotherapy and combination therapy

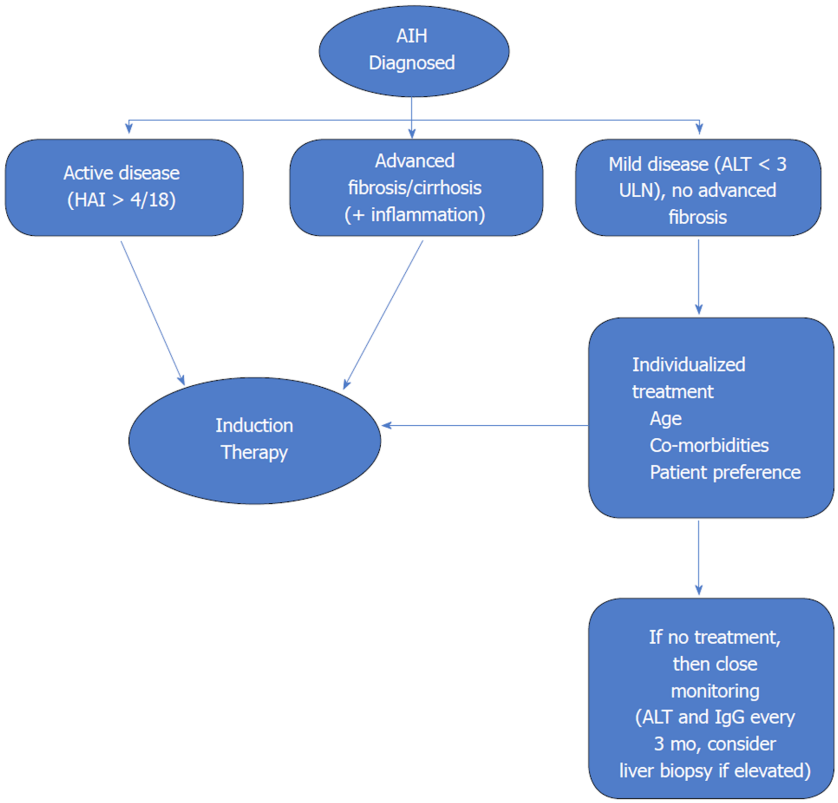

Figure 1 Algorithm for decision making regarding initiation of induction immunosuppressive therapy. Patients with active disease and advanced fibrosis/cirrhosis need initiation of therapy. Patients with mild or asymptomatic disease need an individualized approach. Patients with cirrhosis who have decompensated disease or no inflammatory activity on histology do no benefit from treatment. AIH: Autoimmune hepatitis; ALT: Alanine aminotransferase; ULN: Upper limit of normal; HAI: Hepatic activity index[4].

when compared to placebo (6 % *vs* 7% *vs* 41 %). The combination regimen, however, was associated with fewer side effects (10% *vs* 44%). Seventy-five percent of the patients achieved histological remission, several months after clinical and biochemical remission[17]. This, along with other trials at the time, demonstrated high mortality with azathioprine monotherapy when used for remission induction, likely due to slow onset of action and this was discarded as a valid option.

Thus, the combination regimen was shown to have the best therapeutic profile, and is universally recommended as the first line option[3,4,28].

Upfront combination therapy is especially useful in patients with uncontrolled hypertension, brittle diabetes mellitus, osteoporosis, emotional liability or morbid obesity who are unlikely to tolerate higher doses of steroids well. Similarly, addition of azathioprine may not be appropriate for patients with severe cytopenias, underlying malignancy, pregnancy or established deficiency of thiopurine methyltransferase enzyme (TPMT). Therefore, it is important to individualize regimens based on patient factors and co-morbidities.

Prednisolone is the preferred steroid used in Europe, in contrast to prednisone in the United States, as it does not require intrahepatic conversion to the active form. It also achieves quicker peak plasma concentrations and has greater systemic bioavailability compared to prednisone. Although there does not appear to be a difference in outcomes, it makes sense to use prednisolone in the acute fulminant variant of AIH.

In addition to the classical regimen recommended by the AASLD and BSG, several modifications have been proposed and are being used in clinical practice. A recent questionnaire study by IAIHG evaluated the real-world management of AIH and it suggested wide variations in the initial doses of standard induction therapy and steroid tapering protocols among expert centers[29]. As a general principle, higher the initial steroid dose, faster is the biochemical response with a slightly increased but transient risk of steroid related side effects. Faster induction overall reduces the time to tapering of steroids and thus limiting overall duration of steroid related side effects. In a German cohort, dose of predniso(lo)ne (up to 1 mg/kg per day) in combination with azathioprine lead to more rapid normalization of the

transaminases[30]. A retrospective analysis from Turkey showed a faster biochemical response, less relapses and better survival over 12 mo with starting prednisolone dose of 40 mg and slow taper over 9 weeks in combination with azathioprine in comparison to the standard combination regimen recommended by AASLD[31]. As absence of an early biochemical response is a negative prognostic indicator, strategy to use the most effective dose of steroids for the patient and guide further management based on response is most prudent.

Rate of therapeutic response and dose limiting side effects typically influence the tapering schedule of steroids. Initiation of azathioprine maintenance therapy as soon as possible can help achieve reduction of steroid dose faster. However, in contrast to the AASLD recommendations, and as suggested in the EASL guidelines, it seems reasonable to delay the introduction of azathioprine until early biochemical response is seen from steroid use (usually 2 wk) as that can help clarify diagnostic uncertainties, and differentiate between primary non-response and azathioprine induced hepatotoxicity (although rare, its frequency is increased in advanced liver disease).

It is important to counsel patients about steroid and azathioprine related side-effects. Appropriate adjunctive therapies such as vitamin D and calcium supplementation to prevent bone loss should be given. Vaccination against hepatitis A and B should be completed.

Budesonide, an oral steroid, with a very high first pass metabolism, has been shown in a large, double blind randomized clinical trial to be an effective alternative therapy for AIH[32]. Dose is started at 9 mg daily (3 pills of 3 mg each) in combination with standard azathioprine regimen until remission is induced. A high first pass metabolism leads to less dose limiting side effects when compared to prednisone (28% *vs* 53%). However, this is negated in patients with cirrhosis who may have unpredictable systemic levels due to porto-systemic shunting. Also, the rate of remission induced by budesonide in the study was lower compared to appropriately dosed prednisone[30]. Data regarding the long-term use of budesonide is currently lacking. Therefore, there is a role of budesonide in management of non-cirrhotic patients with AIH who are unable to tolerate systemic side effects of steroids but it is not appropriate for the majority of the patients as a first line agent.

Maintenance therapy

Azathioprine is the drug of choice for maintenance therapy of AIH, as it has been shown to maintain remission effectively in up to 90% patients with fewer side effects compared to low-dose steroid therapy[25,33]. Target dose is usually 1 to 2 mg/kg, but can be titrated up to 2 mg/kg to decrease risk of relapse after steroid withdrawal. To test the tolerance of the drug, it is recommended to start at a lower dose, usually 50 mg daily. Patients should be counseled about the side effects of the drug which include risk of bone marrow suppression, rare risk of malignancy, small risk of drug induced hepatoxicity and pancreatitis. In addition, up to 5% patients demonstrate intolerance to azathioprine manifested by abdominal discomfort, malaise, nausea and fever. Symptoms usually dissipate within 2 to 3 d of stopping the drug. Azathioprine is a category D drug for teratogenic risk, however, all reports from patients treated during pregnancy suggest that it is safe. The risk for maternal and fetal mortality from a disease flare up during pregnancy outweighs any potential harms from the drug, therefore it should be continued at the lowest effective dose to maintain remission during pregnancy.

TPMT is one of the enzymes that is involved in azathioprine metabolism. Patients with genetically determined TPMT deficiency (present in up to 2% of the general population) may be at a higher risk for severe bone marrow suppression. EASL guidelines recommend that when available, serum TPMT testing be performed prior to initiation of azathioprine in patients with AIH. However, not all patients with low levels of TPMT develop bone marrow toxicity and screening for blood TPMT activity has not reduced the frequency of azathioprine related side effects compared with unscreened patients with AIH[34,35]. Therefore, a more pragmatic approach appears to be the initiation of the drug at low dose (50 mg daily) and close monitoring of complete blood count (CBC) (every 2 wk) in the first few months of therapy.

In the first three months of therapy, monitoring of blood counts is done every 2 wk after which it can be spread out. Dose of predniso(lo)ne is tapered in parallel down to 10 mg daily until normalization of transaminases and IgG occurs. Subsequently, steroids can be tapered slowly (in steps of 2.5 to 5 mg daily) every 4 to 12 wk. Transaminases should be closely monitored during this time to detect reactivation of the disease which can be controlled by a transient increase in the steroid dose. Rarely, azathioprine related hepatotoxicity can occur. Usually, IgG levels remain normal in this case and can help differentiate with insufficient treatment response, insufficient dose or lack of compliance. There may be a role of checking thiopurine metabolites (6

thioguanine, 6 methylmercaptopurine) as low levels may indicate lack of compliance. Liver biopsy can help when differentiation between reactivation of AIH or azathioprine related hepatoxicity remains unclear.

Patients who are intolerant to azathioprine, have several alternative modalities for maintenance. 6-mercaptopurine, the active metabolite of azathioprine, can be used in up to 50% of the patients intolerant to azathioprine. However, this should not be tried in patients have had pancreatitis, hepatotoxicity or severe bone marrow suppression secondary to azathioprine.

Mycophenolate mofetil (MMF) has been established as an effective second line agent for AIH[35,36]. It appears to be more useful for patients who suffer from azathioprine intolerance rather than treatment failure with azathioprine. It is tolerated very well and at a target dose of 2 g/d, it can maintain a stable remission rate around 70%[37]. It has been reported to have teratogenic properties and should be prescribed to women of childbearing age with due precaution. Lastly, for patients with mild disease and good tolerance to steroids, chronic low dose predniso(lo)ne at 10 mg daily or less is a viable option to maintain remission.

With good compliance and standard treatment regimens, majority of the patients achieve and maintain sustained remission. 10-year life expectancy for patients with and without cirrhosis and AIH is 89% and 90% respectively, in tertiary referral centers. Overall 10-year survival rate of 93% approaches that of age matched cohorts of the general population[38].

Treatment withdrawal

Most patients with AIH will need lifelong maintenance therapy. This is because only 20% of patients with AIH can maintain a sustained remission after withdrawal of all immunosuppressive therapy[39]. However, this does not preclude a consideration of trial of treatment withdrawal in appropriate candidates. Treatment should be continued for at least 2 years after complete biochemical remission (normal transaminases and normal total IgG) has been achieved. This is because histological resolution lags behind biochemical remission. Patients with persistent mild elevation of transaminases and/or IgG, or intermittent flares during maintenance therapy are likely to experience disease relapse, and treatment withdrawal should not be attempted in them. A recent study showed that patients with ALT levels less than half the ULN and IgG levels in the lower range of normal (< 12 g/L) were much more likely to maintain sustained remission of medications[40]. Liver biopsy can be helpful in excluding a trial of withdrawal as mild ongoing inflammation [hepatic activity index (HAI) > 3] is a strong predictor of relapse. However, a normal liver biopsy is a poor predictor of the probability of relapse. When a decision is made to attempt treatment withdrawal, azathioprine is slowly decreased with careful monitoring of transaminases. Patients who have been successfully weaned off medical therapy should be monitored at regular intervals as up to 50% suffer a relapse within 6 mo, most within 2 years but relapses decades after remission have also been described. Treatment with the initial induction regimen usually helps to get the disease under control. Patients who undergo repeated relapse have higher incidence of cirrhosis, death from liver failure, higher rate of drug induced side effects and overall adverse outcomes[41]. Therefore, lifelong maintenance therapy is needed in patients who suffer a relapse.

Difficult to treat patients: Incomplete and non-responders

Most patients respond well to standard immunosuppressive regimen and at least 10% to 15% appear to be refractory. This can be due to non-compliance, partial response or true non-response.

As biochemical response to immunosuppressive regimen is the norm, non-response to treatment (lack of more than 25% reduction in transaminases after two weeks) should lead to re-evaluation of the diagnosis. Alternative etiologies such as Wilson's disease, DILI, NASH should be definitively ruled out. Occasionally variant forms with overlapping features of PBC, PSC preclude full normalization of enzymes.

Compliance with treatment regimen should be ascertained. Measurement of 6 thioguanine (6TGN) levels can be helpful in this regard. A level > 220 pmol per 8×10^8 red blood cells has been shown to be associated with remission in AIH patients[42]. Lack of detectable serum levels would indicate lack of compliance.

Patients who present with a severe acute hepatitis are more likely to fail standard therapy. Limited data is available on management of such patients. Overall mortality is high (19% to 45%) and liver transplant (LT) evaluation should be initiated. A trial of high dose intravenous corticosteroids (> 1 mg/kg) should be given however a definite futility threshold is not defined. Generally, failure to improve model for end-stage liver disease (MELD)-Na, or serum bilirubin within 7 d of initiation of therapy should lead to alternative treatment strategies including LT.

Some patients with AIH fail to achieve full clinical, laboratory and histologic remission after 3-year standard therapy and are said to have incomplete response. Attempt should be made to optimize dosing of the standard regimen (increasing azathioprine to 2 mg/kg per day with addition of 5 to 10 mg of predniso(lo)ne). If complete response remains elusive, then the goal of therapy is to maintain lowest possible biochemical activity while minimizing side effects. Serum transaminases less than 3 times the ULN are acceptable and azathioprine monotherapy at 2 mg/kg per day is usually reasonable.

Fortunately, true non-responders to standard regimens rare (< 5%) and alternative immunosuppressive agents are needed for these patients. Data for use of these agents in AIH is limited and based on small, mainly retrospective case series as no randomized control trial has been conducted. Largest experience is available for calcineurin inhibitors (CNI) cyclosporine and tacrolimus, primarily as salvage therapy with very effective biochemical response (> 90% for both)[43,44]. These drugs are associated with significant long-term side effects including risk of infections, hypertension, renal dysfunction and diabetes mellitus, and once initiated, they need to be continued permanently.

Recently, role of anti-tumor necrosis factor (TNF) monoclonal antibody, infliximab has been shown to have a positive response in difficult to treat patients with AIH[45]. In addition to inducing stable remission, it was shown to have a beneficial effect on liver histology. Its efficacy is supported pathophysiologically by presence of increased TNF secretion and TNF-positive T cells in the liver of patients with AIH. It should be noted, however, that anti-TNF biologics can themselves induce an AIH-like drug induced syndrome.

LT

AIH and its complications account for up to 5% of all liver transplants, typically for acute fulminant presentations or for advanced decompensated cirrhosis. LT for AIH is an effective intervention, with 10-year patient survival of approximately 75%[3]. Both recurrent and "*de novo*" AIH can occur post liver transplantation, and are treated similarly, using a combination of glucocorticoids and azathioprine. Anti-rejection medications including CNI have not been shown to prevent nor effectively treat recurrent AIH. For refractory cases, switching azathioprine to MMF or changing the calcineurin inhibitor has been recommended[3].

CONCLUSION

Management of AIH, since its initial description, has seen tremendous growth (Figure 2). Most patients can expect near normal life expectancy and a reasonable quality of life. However, there still exists wide disparity in delivery of care and patient outcomes. Our understanding of the underlying pathogenetic mechanisms, although advanced over the years, is still limited and far away from having significant clinical implications. Rarity and heterogeneity of the disease, excellent response to standard treatment regimens besides economic factors guiding the pharmaceutical industry has limited the development of more specific therapies. Further research into the pathogenesis of the disease may lead to development of more definitive serological diagnostic tests as well as targeted immunotherapies addressing the underlying inflammatory mechanisms in the future.

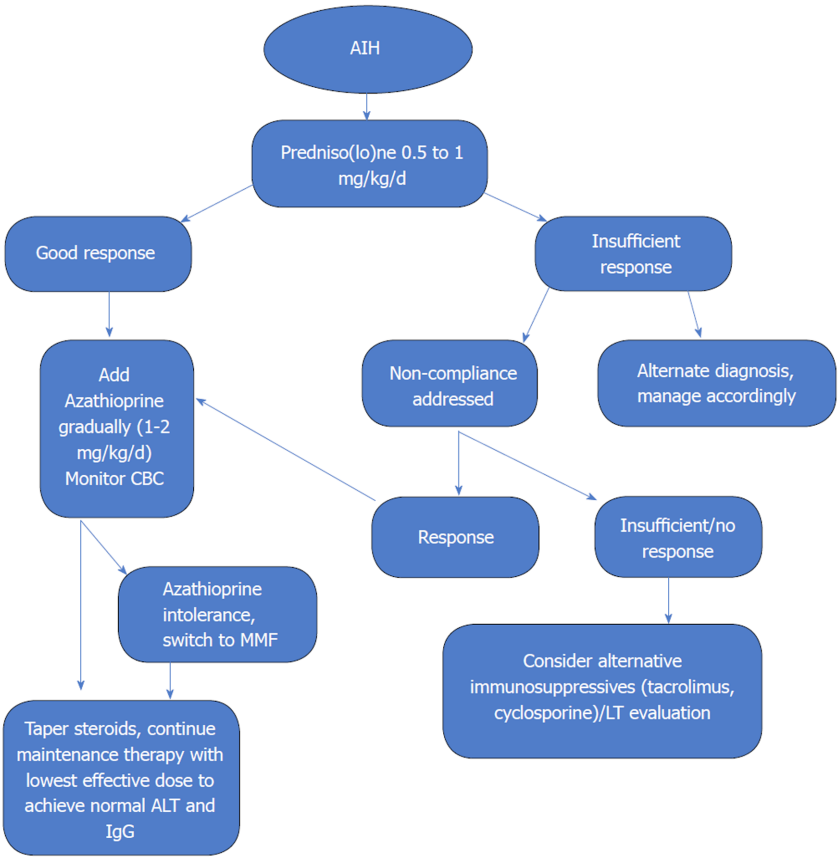

Figure 2 Treatment strategy in autoimmune hepatitis. Treatment includes induction and maintenance therapy to achieve biochemical remission. Induction is achieved by steroids and after a positive response (more than 25% reduction in serum aminotransferases after two weeks) is seen, azathioprine is introduced to achieve long term remission. Timely and appropriate maintenance therapy with azathioprine allows for steroid withdrawal. AIH: Autoimmune hepatitis; ALT: Alanine aminotransferase; CBC: Complete blood count; MMF: Mycophenolate mofetil; LT: Liver transplant[4].

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P- Reviewer: Andreone P, Macedo G
S- Editor: Ma RY **L- Editor:** A **E- Editor:** Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 December 27; 10(12): 924-933

DOI: 10.4254/wjh.v10.i12.924

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Basic Study

Body mass index and its effects on liver fat content in overweight and obese young adults by proton magnetic resonance spectroscopy technique

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Author contributions: Pasanta D performed the majority of experiments and analyzed the data; Chancharunee S, Tungjai M, Sajomsang W and Kothan S designed and coordinated the research.

Supported by Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand, No. 6593(4)1180.

Institutional review board statement: Approved by the Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (AMSEC-61EX-016).

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.

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Abstract

AIM

To assess the association between liver fat content (LFC) and weight status in young adults using proton magnetic resonance spectroscopy (^{1}H MRS) technique.

METHODS

Seventy-eight healthy young adults, between 19-30 years of age participated in this study. This group was then separated into a control of 39 subjects and an overweight/obese group (OW/OB group) consisting of 39 subjects. Blood biochemical quantity and ^{1}H MRS was performed for LFC assessment.

RESULTS

LFC was found to be almost three times higher in OW/OB group when compared to the control group. A 48.7% incidence of non-alcoholic fatty liver disease in the OW/OB group was found. Blood biochemical measurements showed statistically higher low-density lipoproteins and triglyceride, lower high-density lipoproteins, and increased glycosylated hemoglobin and fasting glucose in the OW/OB group. Body mass index was a significant independent predictor for LFC after adjusting for age and sex (multiple linear regression; $\beta = 0.459$, $P < 0.001$).

CONCLUSION

Due to the prevalence of high LFC in the OW/OB group, it can be proposed that weight gain and obesity are sensitive indicators of high hepatic fat content.

Manuscript source: Unsolicited manuscript

Received: June 11, 2018
Peer-review started: June 11, 2018
First decision: July 11, 2018
Revised: August 3, 2018
Accepted: August 21, 2018
Article in press: August 21, 2018
Published online: December 27, 2018

Key words: Young adults; Overweight; Obesity; Non-alcoholic fatty liver disease; Body mass index; Proton magnetic resonance spectroscopy; Cholesterol

Core tip: Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases. The prevalence of NAFLD in young adults is a growing public health concern. Interestingly, the liver fat content (LFC) of an overweight/obese group was approximately three times higher than the control group. This result suggests that obesity can increase LFC and is a risk factor for higher NAFLD in overweight and obese young adults. This current study also demonstrated the importance of Body Mass Index as a tool for risk prevention and control of NAFLD and metabolic syndromes.

Pasanta D, Tungjai M, Chancharunee S, Sajomsang W, Kothan S. Body mass index and its effects on liver fat content in overweight and obese young adults by proton magnetic resonance spectroscopy technique. *World J Hepatol* 2018; 10(12): 924-933
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/924.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.924

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases and is increasing at an alarming rate. Previous studies have reported a positive correlation between BMI and lipid accumulation in the liver, which leads to a higher risk of NAFLD, cirrhosis[1,2], and dyslipidemia[3].

Due to modern lifestyles and diet, there has been a persistent increase in the number of NAFLD patients. This increase occurred at the same time that there were also increases in the number of people considered to be obese all over the world[4,5]. NAFLD in young adults is a topic that has received slight recognition, yet this age group is the most likely to gain weight and develop obesity from diet and lifestyle as they are transitioning into adulthood[6]. The prevalence of NAFLD in young adults has increased almost 2.5 times over 30 years with half of morbidly obese young adults having NAFLD[7]. However, despite the growing public health concern about obesity and NAFLD in young adults, necessary information addressing the effects of obesity and NAFLD pathogenesis in this age group is lacking, and there is an urgent need for better consideration of its effects and mechanisms[8,9]. Proton magnetic resonance spectroscopy (^{1}H MRS) is a well-established non-invasive technique for liver metabolite assessment and is known for its high accuracy for determining liver fat quantification when compared to biopsy[10,11]. As far as we know, there's no study to date that has investigated the effects of obesity on liver fat content (LFC) by ^{1}H MRS in healthy young adults.

The aim of this present study is to assess the association between LFC by ^{1}H MRS technique, blood serum biochemical measures of total cholesterol (Cho), low-density lipoproteins (LDL), high-density lipoproteins (HDL), fasting plasma glucose (FG), glycosylated hemoglobin (HbA1c), and being overweight/obese (OW/OB) as a young adult.

MATERIALS AND METHODS

Study population

The subjects of this current study were 78 healthy young adults between 19-30 years of age. Subjects were randomly chosen from a young adult population residing in Chiang Mai, Thailand through recruitment efforts using posters, or were personally invited to join the study. The control group was comprised of 39 subjects who had engaged in moderate physical activity, and who had a body mass index (BMI) in the normal range according to the World Health Organization (15.8-24.9 kg/m^2)[12]. The OW/OB group was comprised of 39 subjects who had a BMI that was in the overweight and obese range (> 25 kg/m^2)[12]. Exclusion criteria for both groups was diagnoses with a chronic disease or liver injury in any form, alcohol consumption of more than 150 g/wk, hyperglycemia (FBS > 140 mg/dL), hypertriglyceridemia (TG >

300 mg/dL), hepatotoxic medication usage, athletes, contraindication for magnetic resonance imaging (MRI), and poor ^{1}H MRS resolution. Subjects were given a questionnaire about health and lifestyle in order to include or exclude subjects for the study. Eating and exercise habits, occupation, and personal and family medical history were also provided. The Ethics Committee of the Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand (AMSEC-61EX-016) approved all procedures.

LFC assessment by 1H MRS

Liver metabolite spectra were obtained by ^{1}H MRS technique on MRI 1.5 T (Achieva, Philips Medical Systems, Best, The Netherlands) using sense cardiac coil. T2-weighted turbo spin echo (TSE) transverse (TR/TE = 871/80 ms) and coronal T2-weighted (TR/TE = 829/80 ms) images were applied for localization. PRESS sequence with TR = 2000 ms, TE = 43 ms, number of signal averages = 96. Voxel size of 10 × 10 × 10 mm^3 was carefully placed in right lobe of the liver (Couinaud lobe segment V-VIII), carefully avoiding any large vessels and bile duct. The liver metabolite signals without water suppression were obtained and analyzed for metabolized quantification by AMARES algorithm available on jMRUI software[13-15]. Spectrum fitting and quantification was done for water peak (4.72 ppm), and major lipid spectrum peaks (CH_3= 0.9 ppm, CH_2= 1.3 ppm, 2.1 ppm) with prior knowledge and Gaussian line shape was then applied[16]. Signal intensity correction was done for T2 relaxation using linear least-square equation with previous determination for T2 of water and fat. LFC was calculated by a validated method described elsewhere[17,18]. NAFLD was determined as LFC > 5.56%[18].

Blood examination

Blood collection of subjects was done by The Associated Medical Science Clinical Service Center, Chiang Mai University. Ten milliliters of intravenous blood was drawn from antecubital veins and was biochemically analyzed using a fully automated analyzer (Architect ci8200, Abbott Diagnostic). The test focused on Cho, HDL, VLDL, TG, FG, and HbA1c. Subjects were told to fast for 10-12 h prior to blood examination. Later, LDL concentration was calculated from novel adjustable LDL estimation equations[19,20].

Dyslipidemia was described as an abnormality of Cho levels in plasma including increased Tri and LDL, and decreased HDL. The National Cholesterol Education Project (NCEP) Adult Treatment Panel (ATP) III has defined dyslipidemia as Cho ≥ 200 mg/dL, Tri ≥ 150 mg/dL, LDL ≥ 130 mg/dL, and HDL ≤ 40 mg/dL[21]. Normal FG ranges should be between 70-100 mg/dL, and FG between 100-125 mg/dL is considered prediabetes. Normal HbA1c levels should be less than 6%[22].

Anthropometry

The same examiner measured every subject. Subjects wore only an examination cloth. Height and bodyweight were measured to the nearest 0.5 cm and 0.1 kg respectively. Waist circumference (WC) and hip circumference (HC) was acquired while instructed to breathe out mildly. Both measurements were done using non-elastic tape. WC was measured at the midpoint of the lower margin of the rib and the top of the iliac crest. HC was measured at the widest section of the buttocks. Waist-to-hip ratio (W/H ratio) was calculated from WC divided by HC.

Statistical analysis

Statistical analysis was performed using SPSS statistical software version 17.0. Normal distribution results are expressed as mean ± SD. The Kolmogorov-Smirnov test and the Shapiro-Wilk test were performed to determine data normality. Comparison of LFC and blood biochemical examination between groups was then further compared with an unpaired samples *t*-test. Relationship between groups was done with Pearson correlation. Multiple stepwise linear regression analysis was used to verify the relationships between LFC and independent of significant corelate variables. Results with P value < 0.05 were considered statistically significant.

RESULTS

A total of 78 healthy subjects in the young adult age group (19-30 years old) participated in this study. The control group of 39 subjects and the OW/OB group of 39 subjects had an average BMI of 20.9 ± 0.3 and 31.3 ± 0.5 kg/m^2, respectively. The characteristics of LFC, anthropometric, and biochemical data of all subjects are shown in Table 1.

Seventy-eight spectra were obtained and were analyzed for LFC. The corrected

Table 1 Characteristic and biochemical analysis of 78 subjects in the control and overweight/obese groups

	Control group	OW/OB group	*P*-value	Correlation with LFC	
				r	*P* value
n	39	39	-	-	-
Gender (male/female)	12/27	24/15	-	-	-
LFC (%)	2.7 ± 0.2	8.1 ± 1.0	< 0.001[b]	-	-
Age	22.3 ± 1.6	22.1 ± 0.3	0.662	-0.058	0.611
BMI (kg/m^2)	20.9 ± 0.3	31.3 ± 0.5	< 0.001[b]	0.531	< 0.001[b]
WC (cm)	74.6 ± 1.4	112.6 ± 7.4	< 0.001[b]	0.259	0.022[a]
HC (cm)	90.7 ± 1.3	122.5 ± 7.5	< 0.001[b]	0.212	0.062
W/H ratio	0.8 ± 0.0	0.9 ± 0.0	< 0.001[b]	0.388	< 0.001[b]
FG (mg/dL)	83.1 ± 1.1	89.9 ± 1.1	< 0.001[b]	0.144	0.21
Cho (mg/dL)	187.3 ± 6.8	200.7 ± 6.1	0.147	0.093	0.419
Tri (mg/dL)	77.8 ± 5.2	117.1 ± 8.8	< 0.001[b]	0.223	0.05
HDL (mg/dL)	59.3 ± 2.5	47.7 ± 1.4	< 0.001[b]	-0.185	0.105
LDL (mg/dL)	111.1 ± 5.6	130.1 ± 5.1	0.014[a]	0.133	0.246
HbA1c (%)	5.1 ± 0.1	5.5 ± 0.1	< 0.001[b]	0.345	0.002[a]

Data expressed as mean ± SD.
[a]$P < 0.05$;
[b]$P < 0.001$. OW/OB: Overweight/obese; LFC: Liver fat content; BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; W/H ratio: Waist-to-hip ratio; FG: Fasting plasma glucose; Cho: Cholesterol; Tri: Triglyceride; HDL: High-density lipoproteins; LDL: Low-density lipoproteins; HbA1c: Glycosylated hemoglobin.

value of liver fat by weight was calculated by a method validated by Longo *et al*[17] and Szczepaniak *et al*[18]. Representative spectrum from the right lobe of the liver was shown in Figure 1.

As expected, LFC, anthropometric, and biochemical results were significantly different between the two groups with the exception of age and Cho. The OW/OB group reported statistically higher BMI, LFC, WC, HC, FG, Tri, LDL, HbA1c, and statistically lower HDL. Cho also was found to be increased in the OW/OB group, but this tendency was not statistically significant. The prevalence of dyslipidemia in the OW/OB group (69.2%) was higher than in the control group (48.7%). There were no subjects in the control group who exceeded the normal FG and HbA1c ranges.

Interestingly, the LFC of the OW/OB group was approximately three times higher than the control group. Additionally, 19 subjects (48.7%) in the OW/OB group had LFC > 5.56%, which is considered to be a cut off point for NAFLD according to a previous large cohort ^{1}H MRS LFC study[18]. Furthermore, dyslipidemia was present in 47.4% of participants in the OW/OB group, and abnormal HbA1c was found in 10.5% of OW/OB subjects.

The data in this study was normally distributed. Pearson correlation analysis was conducted as preliminary analysis for possible predictor variable for LFC and is presented in Table 1. Various statistically significant correlations of LFC and variables were found, with moderate correlation occurring with BMI and mild correlation with W/H ratio, HbA1c, and waist circumference. Among the blood biochemical results, HbA1c showed the highest correlation with LFC followed by Tri. The Pearson correlations and data distribution by sex in both groups is shown in Figure 2. This indicates that the overall data between male and woman in each group is distributed in the same way.

The correlation was then compared between HbA1c and FG to determine the indicator for diabetes. Even if a low positive correlation was found in FG, it is not statistically significant, while the HbA1c showed a statistically significant positive correlation with LFC. The correlation of diabetes (HbA1c and FG) markers is compared in Figure 3.

A multiple linear regression was used to predict the LFC from significantly correlated blood biochemical marker (HbA1c, Tri) and anthropography marker (BMI, W/H ratio). Standardized coefficient and correlations are presented in Table 2. BMI and HbA1c were found to be significant positive independent predictor for LFC after adjusting for age and sex. However, only BMI remained statistically significant as an

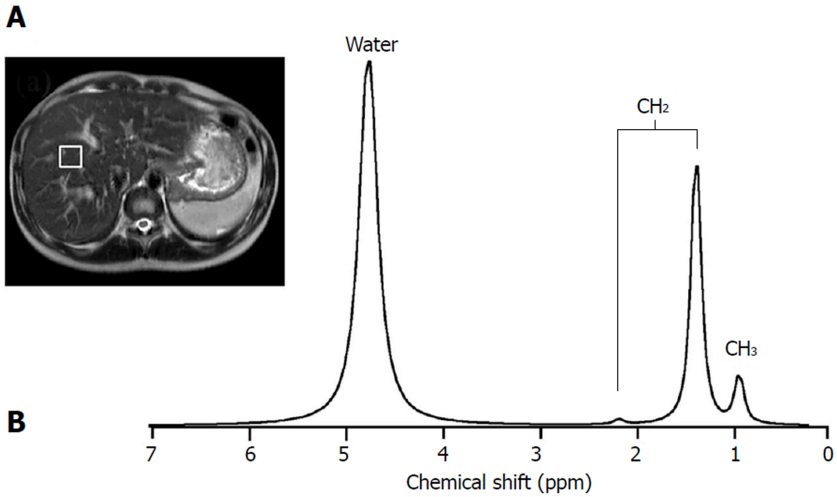

Figure 1 Proton magnetic resonance spectroscopy technique was used for liver fat assessment. Water peak was shown occurring at 4.72 ppm, peaks in fat for CH_3 occurred at 0.9 ppm, and CH_2 peaked at 1.3 ppm and 2.1 ppm. A: Magnetic resonance imaging axial image of abdomen show voxel localization in right lobe liver for liver fat content quantification. B: Representative fitted proton magnetic resonance spectroscopy spectrum of right lobe liver.

independent predictor for LFC after HbA1C adjusting for age, sex, and BMI.

DISCUSSION

In recent years, the prevalence of NAFLD in young adults has been increasing at an alarming rate that parallels with the global epidemic of weight gain and obesity. The prevalence of obesity in young adult is double that of younger ages[8]. Various studies have stated the close relationships between obesity, dyslipidemia, insulin resistance, and NAFLD[23,24]. MRI has proven to be a powerful imaging tool for liver cirrhosis diagnosis[25], and is known for its ability to non-invasively and accurately quantify liver fat using the ^{1}H MRS technique, which is suitable for longitudinal follow-ups when compared to liver biopsy. Liver biopsies are the gold standard, but are an invasive method.

The results of this study confirmed once again the association between BMI and LFC, the higher risk of dyslipidemia, the probability of insulin resistance, and the prospect of metabolic disease in young adults.

The highlight of this study is that LFC in the OW/OB group is higher when compared to the control group, even if both groups were revealed to be healthy. This prevalent rate is consistent with earlier findings where 57.4% of NAFLD subjects in young adults also had high BMI[7,26]. This tendency should also be considered with a higher prevalence of dyslipidemia, prediabetes, and hyperglycemia among subjects with LFC > 5.56%. In accordance with the present results, previous studies have demonstrated that the risk for dyslipidemia starts to increase progressively with a BMI over 21 kg/m^2 and LDL and Tri levels are used to evaluate the risk for coronary artery disease[27].

A new important finding is that the biochemical and anthropographic markers associated with LFC are significantly different between the OW/OB group and the control group. Among the blood lipid markers, Tri and LDL were found to be statistically higher, and HDL was found to be statistically lower when compared to the control group. However, no significant differences were found for Cho, even though the Cho in the OW/OB subjects had increased slightly with almost half of the control group having dyslipidemia. This could be explained by the fact that the two characteristics of the subjects in this age group were that they were exposed to high caloric, low fiber "ready-to-eat" foods, consumed sugary beverages, and had low physical activity. It can be expected that these effects can change the Cho levels in blood[6,28].

The Pearson correlation analysis showed moderate correlation of BMI and LFC and mild correlation with W/H ratio and WC. The association of BMI and LFC was additionally confirmed by multilinearity regression analysis as a significant independent variable after being adjusted for age, sex, and other anthropometric variables. This outcome is dissimilar with previous studies that proposed that W/H ratio can be used as a tool to predict the risks of liver cirrhosis and NAFLD in place of

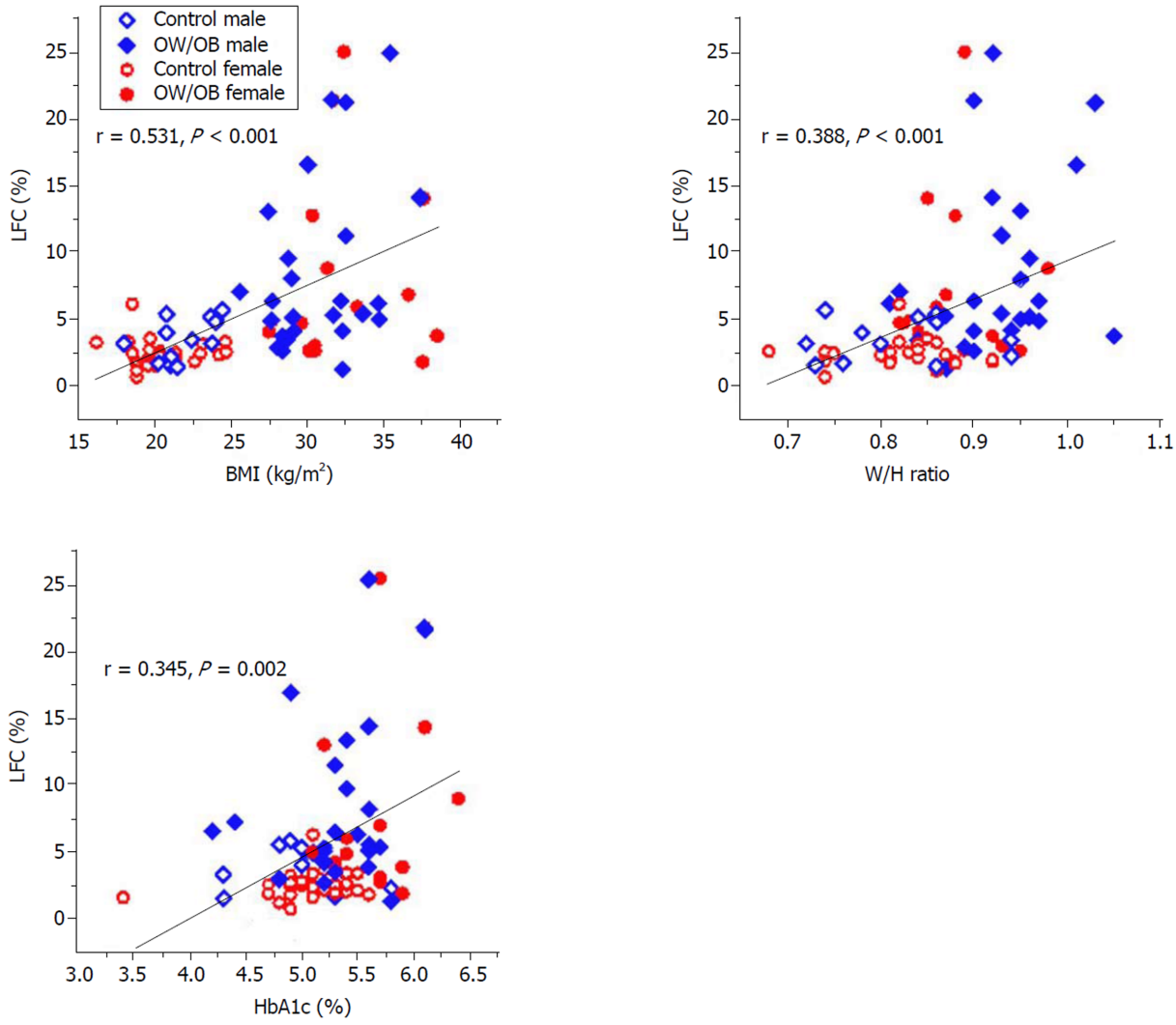

Figure 2 Pearson correlation coefficient (r) and data distribution by sex in each group between body mass index, waist-to-hip ratio, glycosylated hemoglobin, and liver fat content as measured by proton magnetic resonance spectroscopy. BMI: Body mass index; HbA1c: Glycosylated hemoglobin; LFC: Liver fat content; W/H ratio: Waist-to-hip ratio.

BMI[29,30]. A possible explanation is the difference in fat accumulation mechanisms and that weight gain is the main pathogenic mechanism of liver fat accumulation in this age group as was previously proposed by Van Wagner *et al*[31].

HbA1c and FG are also found to be statistically different between the two groups with a slightly positive correlation with LFC. However, only HbA1c is a statistically significant independent variable for LFC after adjusting for age and sex. This result may suggest that HbA1c is a better tool for reflecting the NAFLD effects on insulin resistance than the FG. This assumption is reflected in other research done on the association between HbA1c and NALFD in non-diabetic subjects[32] and on the association of prediabetes characteristics independent of total body fat in obese adolescents with high liver fat assessment by MRI[23]. Elevated HbA1c further confirms the high risk of cardiovascular disease and insulin resistance in overweight and obese young adults.

This study has a few limitations such as the high prevalence of dyslipidemia in the control group that may be caused by the sample characteristics. This group being mostly comprised of young adults engaged in academic studies and whose exercise levels were determined by a questionnaire. There may have been a potential for over reporting by the subjects. A second limitation is that LDL was calculated by an adjustable ratio equation and was not measured directly by biochemical assessment.

To our knowledge, this is the first study on the topic of non-invasive assessment of LFC by ^{1}H MRS technique in healthy young adults without any complications or earlier diagnoses of chronic disease. The high prevalence of NAFLD (LFC > 5.56%) contributed to the impact of silent chronic disease in young adults that had become obese. Although the current study is based on a small sample of subjects, the findings have drawn together various interesting subjects on the effects of BMI, how it

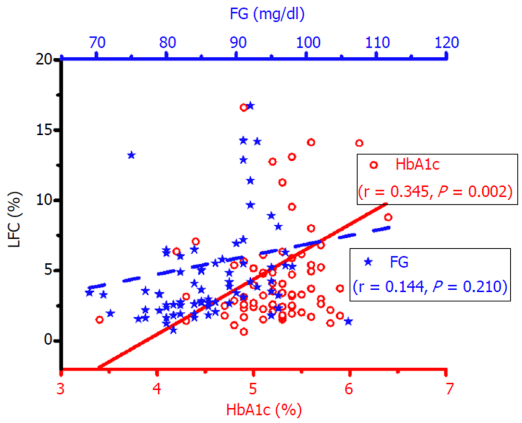

Figure 3 Pearson correlation between glycosylated hemoglobin (circle in red, lower x axis), fasting plasma glucose (star in blue, upper X axis), and liver fat content as measured by proton magnetic resonance spectroscopy. BMI: Body mass index; FG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; LFC: Liver fat content.

contributes to LFC, and how it is a high risk factor of metabolic syndrome in young adults. Previous studies on young adults after a 39 year follow-up has shown that an obese young adult who remained obese throughout their adult life increased their risk of developing severe liver disease[26]. This study suggested a role of BMI in increasing LFC and as a factor in higher NAFLD risk for overweight and obese young adults. The importance of weight control as the primary risk prevention and control of NAFLD and many metabolic syndromes has been proposed. However, this study may reveal the importance in raising awareness for early prevention before NAFLD transitions into chronic liver disease later in adulthood. Future studies on this topic are therefore recommended as young adults are at a high risk for developing severe liver disease. Further, implications of these findings may be forthcoming in future research using longitudinal studies with larger groups of subjects.

In conclusion, it is proposed that the prevalence of high LFC in the OW/OB group can be the result of weight gain and obesity, and may be a leading pathogenic mechanism of liver fat accumulation in young adults. This current study demonstrated the importance of BMI as a tool for the prevention and control of NAFLD and metabolic syndrome in young adults.

Table 2 Multiple linear regression analysis showing relationship of blood biochemical marker and anthropometry marker with LFC as the dependent variable

	Model 1			Model 2			Model 3		
	R^2	β(SE)	*P*	R^2	β(SE)	*P*	R^2	β(SE)	*P*
HbA1c	0.135	0.306	0.002	0.174	0.339	0.004	0.298	0.120	0.327
		(1.273)			(1.283)			(1.379)	
Tri		0.131	0.247		0.065	0.590		-0.029	0.590
		(0.012)			(0.013)			(0.012)	
BMI	0.295	0.463	< 0.001[a]	0.299	0.459	< 0.001[a]			
		(0.109)			(0.111)				
WC		-0.026	0.824		-0.034	0.774			
		(0.016)			(0.017)				
W/H ratio		0.145	0.247		0.136	0.288			
		(8.768)			(9.018)				

Model 1 is unadjusted model. Model 2 is Model 1 adjusted for sex and age. Model 3 is Model 2 adjust for body mass index. Statistical significance: [a]$P < 0.001$. β: Standardized coefficient; SE: Estimated error; R^2: Correction coefficient; LFC: Liver fat content; HbA1c: Glycosylated hemoglobin; Tri: Triglyceride; BMI: Body mass index; WC: Waist circumference; W/H ratio: Waist-to-hip ratio.

ARTICLE HIGHLIGHTS

Research background

In recent years, the prevalence of non-alcoholic fatty liver disease (NAFLD) in young adults has been increasing at an alarming rate that parallels the global epidemic of weight gain and obesity. NAFLD in young adults is a topic that has received little recognition, yet this age group is the most likely to gain weight and develop obesity from their diet and lifestyle as they are transitioning into adulthood. However, despite the growing public health concern about obesity and NAFLD in young adults, necessary information addressing the effects of obesity and NAFLD pathogenesis in this age group is lacking.

Research motivation

NAFLD is a chronic liver disease that is one of the most common health problems among young adults. We aim to identify the effects of obesity on liver fat content (LFC) and health in this age group. This information is crucial for primary prevention and a better understanding of NAFLD pathogenesis in young adults.

Research objectives

The aim of this present study is to assess the association between LFC by proton magnetic resonance spectroscopy (^{1}H MRS) technique. Using biochemical tests, the total cholesterol (Cho), low-density lipoproteins (LDL), high-density lipoproteins (HDL), fasting plasma glucose (FG), glycosylated hemoglobin (HbA1c), and being overweight/obese (OW/OB) will be determined.

Research methods

A total of 78 healthy subjects in the young adult age group (19-30 years old) participated in this study. A control group was made up of 39 healthy subjects, and the experimental group was made up of 39 overweight or obese (OW/OB) subjects. We performed the liver fat assessment by ^{1}H MRS technique on MRI 1.5 T that was calculated into LFC. Intravenous blood was drawn for biochemical analysis. The test focused on Cho, HDL, VLDL, TG, FG, and HbA1c. The waist circumference (WC) and hip circumference (HC) of each subject was measured, and the waist-to-hip ratio (W/H ratio) was calculated.

Research results

LFC from the OW/OB group (8.1% ± 1.0%) was found to be statistically higher when compared to the control group (2.7% ± 0.2%) ($P < 0.001$). Additionally, 48.7% of subjects in the OW/OB group had LFC > 5.56%, which is considered to be a cut off point for NAFLD. The OW/OB group reported statistically higher BMI, LFC, WC, HC, FG, Tri, LDL, HbA1c, and statistically lower HDL. Cho was increased in the OW/OB group compared to the control group, but was not statistically significant. The association of BMI and LFC was additionally confirmed by multilinearity regression analysis as a significant independent variable after being adjusted for age and sex ($P < 0.001$). These findings indicated that BMI is a sensitive marker for LFC in young adults.

Research conclusions

It is proposed that the prevalence of high LFC in the OW/OB group can be the result of weight gain and obesity, and may be a leading pathogenic mechanism of liver fat accumulation in

young adults. Moreover, high BMI is a risk factor for metabolic syndrome in young adults. This current study demonstrated the importance of weight control as a tool for the prevention and control of NAFLD and metabolic syndrome in young adults.

Research perspectives

Further study on this topic may require larger groups of subjects, and should also investigate the alteration of LFC and BMI throughout the adult years as a longitudinal study.

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P- Reviewer: Metin U, Pallav K
S- Editor: Cui LJ **L- Editor:** Filipodia **E- Editor:** Tan WW

World Journal of Hepatology

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World J Hepatol 2018 December 27; 10(12): 934-943

DOI: 10.4254/wjh.v10.i12.934

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

Non-invasive prediction of non-alcoholic steatohepatitis in Japanese patients with morbid obesity by artificial intelligence using rule extraction technology

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Author contributions: Uehara D, Hayashi Y, Seki Y and Kakizaki S designed research; Uehara D, Horiguchi N, Yamazaki Y, Sato K and Yasuda D contributed to data acquisition; Hayashi Y analyzed data using artificial intelligence; Uehara D and Tojima H performed statistical analyses; and Yamada M, Uraoka T and Kasama K supervised the study.

Institutional review board statement: This study was approved by institutional review boards of Yotsuya Medical Cube and Gunma University.

Informed consent statement: For this study using artificial intelligence using rule extraction technology, opt-out was obtained.

Conflict-of-interest statement: There are no conflicts of interest.

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Abstract

AIM

To construct a non-invasive prediction algorithm for predicting non-alcoholic steatohepatitis (NASH), we investigated Japanese morbidly obese patients using artificial intelligence with rule extraction technology.

METHODS

Consecutive patients who required bariatric surgery underwent a liver biopsy during the operation. Standard clinical, anthropometric, biochemical measurements were used as parameters to predict NASH and were analyzed using rule extraction technology. One hundred and two patients, including 79 NASH and 23 non-NASH patients were analyzed in order to create the prediction

Data sharing statement: No additional data is available

Manuscript source: Invited manuscript

Received: June 26, 2018
Peer-review started: July 2, 2018
First decision: August 1, 2018
Revised: August 20, 2018
Accepted: October 17, 2018
Article in press: October 17, 2018
Published online: December 27, 2018

model, another cohort with 77 patients including 65 NASH and 12 non-NASH patients were analyzed to validate the algorithm.

RESULTS
Alanine aminotransferase, C-reactive protein, homeostasis model assessment insulin resistance, albumin were extracted as predictors of NASH using a recursive-rule extraction algorithm. When we adopted the extracted rules for the validation cohort using a highly accurate rule extraction algorithm, the predictive accuracy was 79.2%. The positive predictive value, negative predictive value, sensitivity and specificity were 88.9%, 35.7%, 86.2% and 41.7%, respectively.

CONCLUSION
We successfully generated a useful model for predicting NASH in Japanese morbidly obese patients based on their biochemical profile using a rule extraction algorithm.

Key words: Non-alcoholic steatohepatitis; Artificial intelligence; Rule extraction; Morbid obesity; Liver biopsy; Non-invasive prediction

Core tip: The prevalence of non-alcoholic steatohepatitis (NASH) in Japanese morbidly obese patients is extremely high, and early intervention should be undertaken. However, it is difficult to perform a percutaneous liver biopsy or elastography in routine medical care, especially in morbidly obese patients. We therefore attempted to construct a non-invasive prediction algorithm in order to predict NASH in Japanese morbidly obese patients using artificial intelligence with rule extraction technology. Although further studies with larger numbers of patients are needed to confirm the results, this algorithm may be useful for non-invasively predicting NASH in morbidly obese Japanese patients in the clinical setting.

Uehara D, Hayashi Y, Seki Y, Kakizaki S, Horiguchi N, Tojima H, Yamazaki Y, Sato K, Yasuda K, Yamada M, Uraoka T, Kasama K. Non-invasive prediction of non-alcoholic steatohepatitis in Japanese patients with morbid obesity by artificial intelligence using rule extraction technology. *World J Hepatol* 2018; 10(12): 934-943
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/934.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.934

INTRODUCTIO

Non-alcoholic fatty liver disease (NAFLD) includes non-alcoholic steatohepatitis (NASH) and non-alcoholic fatty liver (NAFL); a pathological diagnosis is the gold standard for the diagnosis of NASH[1,2]. The prevalence of NAFLD is reported to be 29.7% and is increasing in Japan[3]. The number of patients with morbid obesity is also increasing worldwide, and this increase is a major social problem[4]. Although the prevalence of morbid obesity is low in Japan (0.25%-0.3%) compared with to the United States[5,6], it is also increasing in Japan.

Morbid obesity is frequently complicated by metabolic diseases such as type 2 diabetes mellitus, hypertension, hyperlipidemia, and ischemic heart disease. The prevalence of NASH in patients with morbid obesity is also high. We previously reported that the prevalence of NASH in Japanese morbidly obese patients was extremely high (77.5%)[7]. Among non-morbidly-obese patients, the survival rate of NASH patients is significantly lower than in the general population[8]. As a result, an early diagnosis and early intervention are important for NASH patients[7].

A percutaneous liver biopsy is usually performed to diagnose liver diseases, including NASH[9]. Since a percutaneous liver biopsy requires the insertion of a long needle through the subcutaneous tissues to reach the liver, this method is difficult to perform in obese patients with a thick layer of subcutaneous fat. In addition, a percutaneous liver biopsy carries a risk of complications and sampling error in patients with a thick layer of subcutaneous fat. Elastography is non-invasive and sometimes used to estimate the liver stiffness or the extent of fibrosis in patients with

liver disease, including NASH[9]. Since elastography is also performed percutaneously, a thick layer of subcutaneous fat is again an obstacle for the accurate measurement and sometimes leads to a misdiagnosis. Magnetic resonance (MR) elastography is useful for predicting fibrosis and NASH[10]. However, it is also difficult to perform in morbidly obese patients due to limitations in the size or the ability of MR equipment to accommodate the patient. Given the above limitations, a safer, non-invasive, convenient diagnostic method for predicting NASH in morbidly obese patients is needed.

Predictive calculation formulas, such as the NAFIC score, fibrosis-4 index, and NAFLD fibrosis score are usually used to diagnose fibrosis and NASH[11-16]. These formulas are usually constructed using the data from patients whose body weight is within the general range and are not specialized for patients with morbid obesity. The HAIR [hypertension, alanine aminotransferase (ALT), insulin resistance] score[16] and BARD score[17] [includes body mass index (BMI), aspartate aminotransferase (AST)/ALT ratio and presence of diabetes mellitus] were constructed using data of morbidly obese patients from Australia[16] and the United States[17], respectively. However, there are racial differences between Asian people (including Japanese) and Caucasians[7,18,19]. When the BMI is similar, the degree of liver dysfunction and prevalence of NASH is worse in Asian patients than in Caucasians[7,18,19].

Recently, there has been remarkable progress in artificial intelligence, and we are now able to generate highly accurate and interpretable predictive models[20,21]. Rule extraction is a technique that attempts to find a compromise between requirements by building a simple rule set that mimics how well-performing complex predictive models (black-box, *i.e.*, not understandable) make their decisions for physicians and clinicians. We proposed continuous recursive-rule extraction (Re-RX) with J48graft as a promising algorithm for rule extraction[22]; this was based on the Re-RX algorithm[23] to simultaneously enhance the accuracy and interpretability of the extracted classification rules.

In the present study, we extracted new classification rules to predict NASH based on the biochemical profile of Japanese morbidly obese patients using Continuous Re-RX with J48graft. This predictive algorithm, which was named the Japanese algorithm of Morbid Obesity for NASH Prediction (JOMO algorithm), may be useful for predicting NASH in Japanese or Asian morbidly obese patients in the clinical setting.

MATERIALS AND METHODS

Study design

To create the prediction algorithm, we used the data of the cohort of Japanese morbidly obese patients who participated in our previous study[7]. The patient characteristics are shown in Supplemental Table 1 and Table 2 (modified from reference 7). One hundred and two patients, including 79 NASH and 23 non-NASH patients were analyzed using rule extraction technology. The main purpose of this study was to generate a highly accurate and interpretable predictive model for NASH in Japanese morbidly obese patients using an accuracy-priority rule extraction algorithm.

To validate the algorithm, another cohort of 77 patients was collected from October 2012 to September 2014. This cohort included 65 NASH and 12 non-NASH patients. Consecutive obese patients undergoing bariatric surgery for the management of morbid obesity at a single center (Weight Loss and Metabolic Surgery Center, Yotsuya Medical Cube, Tokyo, Japan) underwent a liver biopsy during the operation[7]. The precise methods for liver biopsies and sampling have been described in previous study[7]. NASH was histologically defined as steatosis with at least two of the following[24]: (1) Lobular necro-inflammatory foci; (2) Ballooning degeneration of hepatocytes with or without Mallory bodies; and (3) Perisinusoidal fibrosis. The indications for bariatric surgery followed the criteria established in 2005 by the Asia–Pacific Bariatric Surgery Group consensus meeting[18] as follows: Asian patients with a BMI of > 37 kg/m^2 or > 32 kg/m^2 (with diabetes or 2 other obesity-related comorbidities) and failure to show any improvement with nonsurgical treatment.

The standard clinical, anthropometric (weight, height, waist and hip circumferences) and biochemical measurements, including AST, ALT, γ-glutamyl transpeptidase (γ-GTP), cholinesterase (ChE), uric acid (UA), albumin (Alb), C-reactive protein (CRP), Fe (iron), fasting blood insulin, fasting plasma glucose (FPG), hemoglobin (Hb) A1c, triglyceride, and low density lipoprotein-cholesterol (LDL-cho) levels, were obtained before bariatric surgery. All blood samples were collected after overnight fasting. The homeostasis model assessment insulin resistance (HOMA-IR) index was used as an index of insulin resistance and was calculated by the following

Table 1 The positive predictive value, negative predictive value, sensitivity, specificity and predictive accuracy rate of each category

	Positive predictive value	Negative predictive value	Sensitivity	Specificity	Predictive accuracy rate
HAIR score	92.6%	20.0%	38.5%	83.3%	45.5%
BARD score	78.0%	8.3%	49.2%	25.0%	45.5%
JOMO algorithm	88.9%	35.7%	86.2%	41.7%	79.2%

formula: glucose (mg/dL) × insulin (μU/mL) /405. Computed tomography was performed, and the areas of subcutaneous and visceral fat were calculated. The diagnosis of type 2 diabetes mellitus was based on the Japanese Diabetes Society criteria[25]. Hypertension was diagnosed if the patient had a history of hypertension and was on antihypertensive medication or if the patient had a resting recumbent blood pressure of ≥ 140/90 mmHg on two repeated occasions.

Written informed consent was obtained from every patient, and the study was approved by the institutional review boards (IRB) of Yotsuya Medical Cube (YMC-2009-3). The study was performed in accordance with the principles of the Declaration of Helsinki. For this study using artificial intelligence using rule extraction technology, new IRB statements (Gunma University-2017-004) and opt-out were obtained.

Continuous Re-RX with J48graft

We recently proposed a new accuracy-priority rule extraction algorithm using Re-RX with J48graft[26] and continuous attributes (Continuous Re-RX with J48graft[23,27]). In order to extract more accurate and concise classification rules, we proposed replacing the conventional Re-RX algorithm, which uses C4.5 as a decision tree[28], with Re-RX with J48graft[23,29]. The conventional pruning used in J48 both complements and contrasts with that used in J48graft. The performance of the Re-RX algorithm is thought to be greatly affected by the decision tree.

In contrast, Continuous Re-RX[29] is a rule extraction algorithm that aims to achieve very high accuracy rather than concision. We found that the combination of Continuous Re-RX with J48graft and Continuous Re-RX simultaneously enhanced the accuracy and interpretability[27] and was well suited to generating a considerably better predictive model in this cohort. The age, Sex, BMI, waist-hip ratio, visceral fat area, AST, ALT, γ-GTP, ChE, UA, Alb, CRP, Fe, platelets, LDL-cho, TG, FPG, HbA1c, HOMA-IR, type 2 diabetes mellitus, and hypertension were used as parameters.

Statistical analysis

All data are shown as the mean ± SD. Differences between the groups were analyzed by Fisher's exact probability test and Mann-Whitney *U* tests when a significant difference was obtained by the Kruskal-Wallis test. A value of $P < 0.05$ was considered to be significant.

RESULTS

Patient characteristics

We classified the data of the pathological findings and the results of a blood test obtained from a routine medical examination in the NASH and Non-NASH groups and analyzed the data using Continuous Re-RX with J48graft. The algorithm demonstrated the following rules for classification: R1: ALT ≤ 19 then non-NASH; R2: ALT > 19; CRP ≤ 0.15, HOMA-IR ≤ 6.37, Alb ≤ 4.15 then NASH; R3: ALT > 19, CRP ≤ 0.15, HOMA-IR ≤ 6.37, Alb > 4.15 then Non-NASH; R4: ALT > 19, CRP ≤ 0.15, HOMA-IR > 6.37 then NASH; R5: ALT > 19, CRP > 0.15 then NASH (Figure 1). The patients classified in R1 and R3 were predicted to be non-NASH, while NASH was predicted in the patients who were classified into R2, R4 and R5. This algorithm was based on 10 runs of 5 cross validation (CV)[30].

We validated the algorithm in the other cohort of 77 patients. To validate the performance of the extracted rules in classifying NASH patients, we confirmed the rate of agreement of the results. The 77 cases were classified using the prediction formula as follows: R1, *n* = 6; R2, *n* = 3; R3, *n* = 8; R4, *n* = 1; and R5, *n* = 59 (Figure 2). Fourteen cases were classified as non-NASH (R1 + R3) by the algorithm, and 5 cases were classified as non-NASH according to the results of the histopathological examinations. Sixty-three cases were classified into the NASH group (R2 + R4 +R5), while NASH was histopathologically diagnosed in 56 cases (Figure 2). Figure 3A shows the predictive value using our algorithm. The positive predictive value,

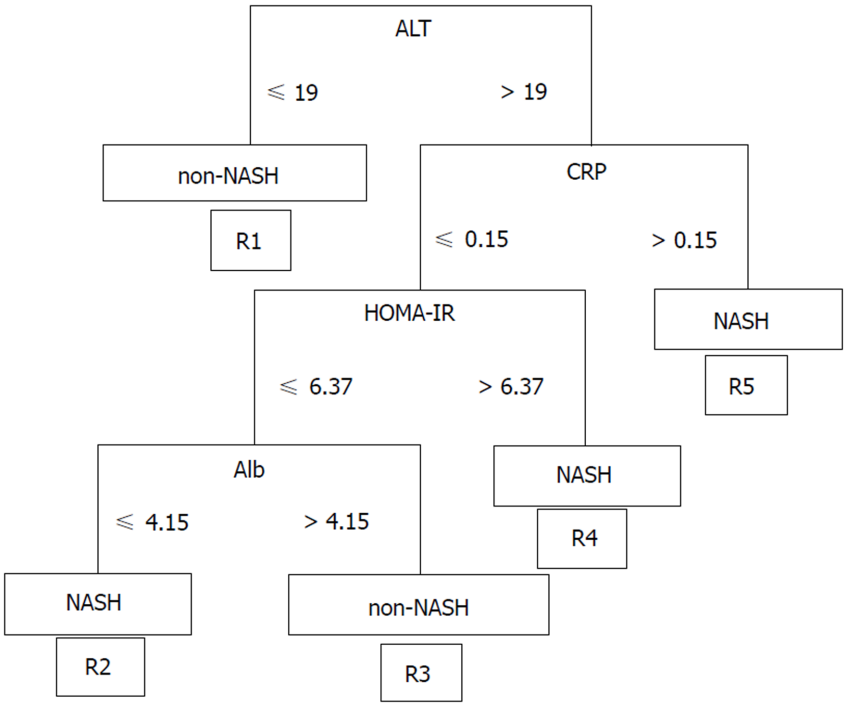

Figure 1 Prediction formula created using a highly accurate rule extraction algorithm. NASH: Non-alcoholic steatohepatitis; ALT: Alanine aminotransferase; CRP: C-reactive protein; Alb: Albumin; HOMA-IR: homeostasis model assessment insulin resistance.

negative predictive value, sensitivity and specificity were 88.9%, 35.7%, 86.2% and 41.7%, respectively (Table 1). The predictive accuracy rate was 79.2%.

Figure 3B shows the predictive value of the conventional predictive method using the HAIR score[16]. The HAIR score was constructed based on data from Australian patients with morbid obesity. The positive predictive value, negative predictive value, sensitivity and specificity were 92.6%, 20.0%, 38.5%, and 83.3%, respectively (Table 1). The predictive accuracy rate was 45.5%.

Figure 3C shows the prediction values using BARD score[17]. The BARD score was constructed based on a large cohort of patients from the United States with a median BMI of 33 kg/m^2. The positive predictive value, negative predictive value, sensitivity and specificity were 78.0%, 8.3%, 49.2%, and 25.0%, respectively. The predictive accuracy rate was 45.5% (Table 1).

The Receiver Operating Characteristic (ROC) curve of our algorithm for all patients is shown in Figure 4. The area of ROC was 0.772.

Our newly developed predictive algorithm showed high predictive accuracy in Japanese obese patients. We named our new predictive algorithm the JOMO algorithm.

DISCUSSION

It is difficult to perform a liver biopsy in morbidly obese patients during the course of a typical consultation. We therefore generated a new predictive model, the JOMO algorithm, to predict NASH in Japanese morbidly obese patients using Continuous Re-RX with J48graft. This algorithm showed substantially better results than conventional predictive formulae. We used 2 cohorts of 102 and 77 Japanese morbidly obese patients to generate and to validate the algorithm, respectively. Although a larger number of cases would enable us to improve the accuracy of the classification and interpretability, it is difficult to collect liver biopsy data from Japanese morbidly obese patients. These cohorts of 102 and 77 patients may be the largest cohorts of Japanese morbidly obese patients to have undergone a liver biopsy. For this reason, we adopted an artificial intelligence approach using Continuous Re-RX with J48graft. Continuous Re-RX with J48graft provided a more accurate predictive accuracy without the need for a validation cohort. Since we generated a predictive model by artificial intelligence using rule extraction technology, we were able to extract more accurate and interpretable classification rules from a limited cohort.

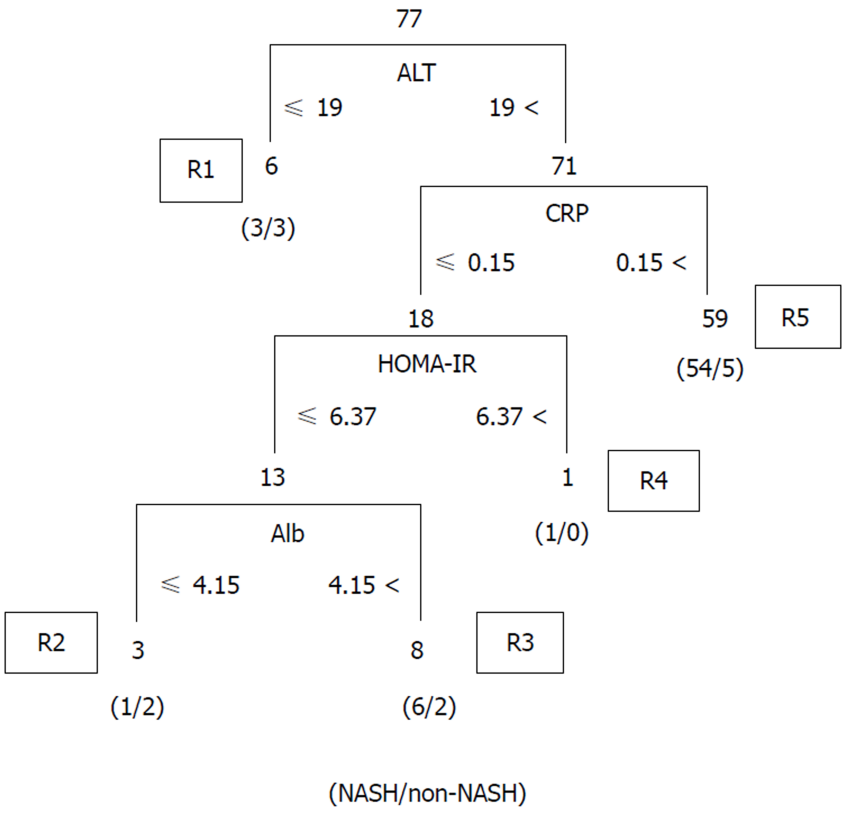

Figure 2 Predictive accuracy of the new developed JOMO algorithm in Japanese morbidly obese patients. NASH: Non-alcoholic steatohepatitis; ALT: Alanine aminotransferase; CRP: C-reactive protein; Alb: Albumin; HOMA-IR: Homeostasis model assessment insulin resistance.

In this study, we performed *k*-fold cross-validation when crafting this algorithm[30,31]. Regarding the experimental settings and data splitting technique, *k*-fold CV[30] was applied, *i.e.*, the original database was partitioned into *k*-subsets/folds of equal sizes where each subset had to be trained and tested. Accordingly, the final output was predicted by taking the averages of all partitions/folds that had been tested. Therefore, we applied 10 runs of 5-fold CV which was averaged to obtain the robust classification accuracy. Although the application of rule extraction technology is new in the medical field, strategies using Continuous Re-RX with J48graft, *i.e.*, rule-based classifiers, have already been established in the field of artificial intelligence[23,27]. Our algorithm using Continuous Re-RX with J48graft can therefore be adapted to different cohorts without a loss of generality.

In our previous study[7], a multivariate analysis for predicting NASH indicated that the waist-hip ratio, ALT level, fasting plasma glucose level, and HOMA-IR were independent factors associated with NASH. However, the JOMO algorithm using Continuous Re-RX with J48graft selected the ALT level, HOMA-IR, CRP level, and Alb level as predictive values. The ALT level and HOMA-IR were both selected in our previous multivariate analysis as well as in this JOMO algorithm. However, the waist-hip ratio and fasting plasma glucose level were not chosen by the JOMO algorithm. On using the predictive factors determined in the previous multivariate analysis, the predictive accuracy rates of the waist-hip ratio, fasting plasma glucose level, ALT level and HOMA-IR were found to be 54.1%, 35.1%, 77.9%, and 79.2%, respectively. The predictive values of the JOMO algorithm were thus considerably better than those of the multivariate analysis. Furthermore, since the attributes selected by artificial intelligence in this study are more concise, they are easily checked by blood sampling. This is an advantage with the JOMO algorithm and fulfills the purpose of this study.

CRP was selected as a predictive parameter in this JOMO algorithm. The predictive value of CRP in the diagnosis of NASH remains controversial at present. Anty *et al*[32] reported the CRP levels were not predictive of the diagnosis of NASH in severely obese patients because the liver as well as the adipose tissue can produce CRP. However, Yoneda *et al*[33] demonstrated that high-sensitivity CRP was useful for making a prediction in cases of NASH compared with in cases of simple nonprogressive steatosis. They also suggested that high-sensitivity CRP might be a

A JOMO algorithm

Number of cases		Pathological diagnosis	
		NASH	non-NASH
Prediction model	NASH	56	7
	non-NASH	9	5

B HAIR score

Number of cases		Pathological diagnosis	
		NASH	non-NASH
Prediction model	NASH	25	2
	non-NASH	40	10

C BARD score

Number of cases		Pathological diagnosis	
		NASH	non-NASH
Prediction model	NASH	32	9
	non-NASH	33	3

Figure 3 A cross table comparing the results of the prediction model and the pathological diagnosis. A: JOMO algorithm; B: HAIR score; C: BARD score. NASH: Non-alcoholic steatohepatitis.

clinical feature that indicates the severity of hepatic fibrosis in cases of NASH[33]. It is of note that there was no statistically significant difference in the CRP levels between the NASH and non-NASH as groups in our cohort (Supplemental Table 2), and thus the introduction of CRP into the predictive model demonstrated the power and usefulness of artificial intelligence.

The level of CRP can be affected by many factors. However, the cohort used in this study was relatively homogenous: All patients were candidates for bariatric surgery, and blood samples were collected from all patients who were roughly in the same condition before surgery. As such, our cohort may be protected against miscellaneous factors that influence the CRP level. The prevalence of NASH patients was high, and the number of non-NASH patients was low in the cohort used in this study. This may have influenced the accuracy of our predictive algorithm. Therefore, further evaluation of the role of CRP in this algorithm will be needed before it should be applied to other cohorts. It the future, we plan to improve this algorithm further using a larger number of NASH and non-NASH patients.

In this study, we generated a new predictive model that used Continuous Re-RX with J48graft to predict NASH in Japanese morbidly obese patients. This algorithm enables the prediction of NASH using parameters available from blood tests in routine medical examinations. Although further studies with larger numbers of patients are needed to confirm the results, this algorithm may be useful for non-invasively predicting NASH in morbidly obese Japanese patients in the clinical setting.

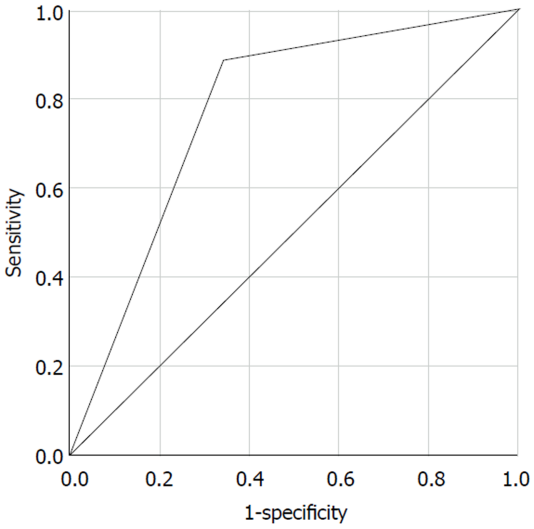

Figure 4 Receiver operating characteristic curve of the JOMO algorithm.

ARTICLE HIGHLIGHTS

Research background

Pathological diagnosis is the gold standard for the diagnosis of non-alcoholic steatohepatitis (NASH). However, it is difficult to perform a percutaneous liver biopsy in routine medical care, especially in morbidly obese patients. Predictive calculation formulas are usually used to diagnose fibrosis and NASH.

Research motivation

Recently, there has been remarkable progress in artificial intelligence. We therefore attempted to construct a non-invasive prediction algorithm in order to predict NASH using artificial intelligence with rule extraction technology.

Research objectives

Morbidly obese Japanese patients who required bariatric surgery underwent a liver biopsy during the operation. Standard clinical, anthropometric, biochemical measurements were used as parameters for making prediction model.

Research methods

One hundred and two patients, including 79 NASH and 23 non-NASH patients were analyzed in order to create the prediction model, another cohort with 77 patients including 65 NASH and 12 non-NASH patients were analyzed to validate the algorithm. We used Continuous recursive-rule extraction with J48graft for rule extraction to predict NASH.

Research results

Alanine aminotransferase, C-reactive protein, homeostasis model assessment insulin resistance, albumin were extracted as predictors of NASH. When we adopted the extracted rules for the validation cohort, the predictive accuracy was 79.2%. The positive predictive value, negative predictive value, sensitivity and specificity were 88.9%, 35.7%, 86.2% and 41.7%, respectively.

Research conclusions

We successfully generated a useful model for predicting NASH in Japanese morbidly obese patients based on their biochemical profile using a rule extraction algorithm.

Research perspectives

Although further studies with larger numbers of patients are needed to confirm the results, this algorithm may be useful for non-invasively predicting NASH in morbidly obese Japanese patients in the clinical setting.

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P- Reviewer: Ozenirler S, Namisaki T, Takahashi T, Soresi M, de Oliveira C, De Silva AP

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World J Hepatol 2018 December 27; 10(12): 944-955

DOI: 10.4254/wjh.v10.i12.944

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Impact of sepsis and non-communicable diseases on prognostic models to predict the outcome of hospitalized chronic liver disease patients

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Author contributions: Abid S contributed to the study idea and design; Qazi Arisar FA, Shaikh PA, Awan S, and Abid S contributed to acquisition, analysis, or interpretation of data; Qazi Arisar FA wrote the initial draft; Abid S and Shaikh PA made critical revision for important intellectual content; all authors approved the final version of the manuscript and are accountable for all aspects of the work.

Institutional review board statement: This study was reviewed and granted exemption by the Ethical Review Committee of the Aga Khan University Hospital, Karachi.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained through a retrospective review of charts.

Conflict-of-interest statement: All authors declare no conflicts-of-interest related to this article.

Data sharing statement: No additional data are available.

Open-Access: This article is an

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Abstract

AIM

To evaluate the impact of sepsis and non-communicable diseases (NCDs) on the outcome of decompensated chronic liver disease (CLD) patients.

METHODS

In this cross-sectional study, medical records of patients with CLD admitted to the Gastroenterology unit at the Aga Khan University Hospital were reviewed. Patients older than 18 years with decompensation of CLD (*i.e.*, jaundice, ascites, encephalopathy, and/or upper gastrointestinal bleed) as the primary reason for admission were included, while those who were admitted for reasons other than decompensation of CLD were excluded. Each patient was followed for 6 wk after index admission to assess mortality, prolonged hospital stay (> 5 d), and early readmission (within 7 d).

RESULTS

A total of 399 patients were enrolled. The mean age was 54.3 ± 11.7 years and 64.6% (n = 258) were male. Six-week mortality was 13% (n = 52). Prolonged hospital stay and readmission were present in 18% (n = 72) and 7% (n = 28) of patients, respectively. NCDs were found in 47.4% (n = 189) of patients. Acute kidney injury, sepsis, and non-ST elevation myocardial infarction were found in 41% (n = 165), 17.5% (n = 70), and 1.75% (n = 7) of patients, respectively. Upon multivariate analysis, acute kidney injury, non-ST elevation myocardial infarction, sepsis, and coagulopathy were found to be statistically significant predictors of mortality. While chronic kidney disease (CKD), low albumin, and high Model for End-Stage Liver Disease (MELD)-Na score were found to be statistically significant predictors of morbidity. Addition of sepsis in conventional

Manuscript source: Invited manuscript

Received: August 4, 2018
Peer-review started: August 6, 2018
First decision: August 24, 2018
Revised: September 7, 2018
Accepted: October 17, 2018
Article in press: October 18, 2018
Published online: December 27, 2018

MELD score predicted mortality even better than MELD-Na (area under receiver operating characteristic: 0.735 *vs* 0.686; $P < 0.001$). Among NCDs, CKD was found to increase morbidity independently.

CONCLUSION
Addition of sepsis improved the predictability of MELD score as a prognostic marker for mortality in patients with CLD. Presence of CKD increases the morbidity of patients with CLD.

Key words: Chronic liver disease; Mortality; Morbidity; Prognostic factors; Non-communicable diseases; Sepsis

Core tip: Chronic liver disease is one of the leading causes of mortality. Child-Pugh and Model for End-Stage Liver Disease scores have been designed to predict the outcome in cirrhotic patients. Infection and renal insufficiency can worsen the outcome in cirrhotic patients. Myocardial infarction, sepsis,, and coagulopathy are associated with poor outcomes in patients with cirrhosis. The addition of sepsis can improve the predictability of the Model for End-Stage Liver Disease score as a prognostic marker for mortality in hospitalized patients with liver cirrhosis. Presence of chronic kidney disease increased the morbidity of cirrhotic patients. There is no direct impact of non-communicable disease over mortality in hospitalized patients with liver cirrhosis.

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URL: https://www.wjgnet.com/1948-5182/full/v10/i12/944.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.944

INTRODUCTION

The massive global burden of chronic liver disease (CLD) has been well documented[1], with more than one million deaths per year worldwide[2] making it the 14th leading cause of death globally[3]. Many prognostic models have been developed over the years to help classify the severity of liver disease and direct the aggressiveness of medical care. The Child-Pugh Turcotte (CTP) Score and the Model for End-Stage Liver Disease (MELD) score are two of the most commonly used scoring systems worldwide[4-6].

Child and Turcotte proposed the CTP score initially using nutritional status, the presence of ascites, hepatic encephalopathy, total bilirubin, and albumin as parameters to determine mortality risk in patients undergoing portosystemic shunt surgery. Later, Pugh *et al*[4] modified it to its current version by replacing nutritional status with prothrombin time or international normalized ratio (INR), making it the most widely used scoring system for estimation of prognosis in CLD patients. However, the subjectivity of variables (ascites, encephalopathy) as well as inter-laboratory variability limited the accuracy of the CTP score, with the waitlist mortality for liver transplantation continuing to rise[7].

MELD score was introduced primarily to determine the survival of patients undergoing transjugular intrahepatic portosystemic shunt placement[5]. MELD score incorporates total bilirubin, creatinine, and INR. It has not only become the mainstay for prioritizing patients for liver transplant, but also for predicting mortality in non-transplant surgical procedures, alcoholic hepatitis, and acute variceal hemorrhage[6,8]. However, there are still several comorbidities such as hepatocellular carcinoma, hepatopulmonary syndrome, and portopulmonary hypertension (HTN) that can affect the prognosis of CLD patients and that are not taken into account by the MELD score[9,10].

Several studies have been done on the MELD score with proposals made for revision of the scoring system to include other factors to improve the predictive accuracy of the score. Some of these include modified CTP, MELD-Na, Reweighted MELD and the Refit MELD, which have been shown to be superior to the current CTP

and MELD scores[11-14]. Such studies have led several leading researchers to investigate other variables and scoring systems to better predict mortality in patients hospitalized for decompensated CLD.

Acute kidney injury (AKI) is a devastating complication that is frequently progressive and independently associated with mortality in a stage-dependent fashion in CLD patients[15]. Bacterial infections are common in liver disease, especially in decompensated patients. Infections increase the mortality four-fold in patients with end-stage liver disease[16]. Inflammation stemming from infections plays a key role in the outcome of cirrhosis. The presence of systemic inflammatory response syndrome with or without infection is a major predictor of prognosis in CLD patients[17].

There is an alarming rise in the prevalence of non-communicable diseases (NCDs) worldwide. Diabetes, HTN, cardiovascular diseases, chronic obstructive pulmonary diseases, and cancers have emerged as leading causes of mortality globally[18]. In a recent cohort of the Asian population, diabetes was found to impact mortality in cirrhotic patients[19]. However, the effect of other NCDs on the outcomes of liver disease has not been elucidated.

Charlson *et al*[20] proposed a comorbidity index to predict all-cause mortality on the basis of a number of comorbid conditions. Seventeen different diseases, each allocated a score from one to six, are incorporated in the Charlson comorbidity index. The total sum gives the total burden of comorbidities in that patient[20]. Since the 1980s, the score has been extensively used to estimate mortality in a different subset of cohorts including liver disease patients[21,22]. However, the severity of liver disease was not incorporated in those studies.

The aim of this study is to evaluate the impact of NCDs on the outcome of patients admitted for decompensated CLD. We also aimed to construct a model over and above the existing scoring system.

MATERIALS AND METHODS

Study design and settings

This study employed a cross-sectional design. Medical records of CLD patients who were admitted to the Gastroenterology unit at the Aga Khan University Hospital in Karachi, Pakistan were reviewed. Patients older than 18 years with decompensation of CLD (*i.e*. jaundice, ascites, encephalopathy, and/or upper gastrointestinal (GI) bleed) as the primary reason for admission were included. Those admitted for reasons other than decompensation of CLD were excluded. Each patient was followed for 6 wk after index admission to assess mortality and morbidity. The study was conducted after approval from the institutional ethical review committee.

Variables analyzed

(1) Demographics: age, gender, weight, duration of documented CLD, smoking, and current alcohol use; (2) Clinical presentation: blood pressure, heart rate, temperature, respiratory rate, Glasgow coma scale; (3) CLD complications/decompensation: presence of jaundice, ascites, encephalopathy, esophageal varices, GI bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis, and hepatopulmonary syndrome; (4) NCDs: Diabetes mellitus, HTN, chronic obstructive lung disease, ischemic heart disease, congestive heart failure, peripheral vascular disease, chronic kidney disease (CKD), cerebrovascular disease (prior strokes), acquired immunodeficiency syndrome, cancer, dementia, connective tissue diseases, and peptic ulcer disease; (5) Laboratory markers: complete blood count, electrolytes, liver function tests, serum albumin, creatinine, prothrombin time, C-reactive protein; (6) Scoring Systems: Child-Pugh Score, MELD score, MELD-Na score, and Charlson comorbidity index. While calculating Charlson comorbidity index, patients with Child A disease were given a score of 1 (mild), while those with Child B and C disease were given a score of 3 (moderate to severe) as per standard score; and (7) Primary outcome: To assess mortality within 6 wk of admission and morbidity defined as either prolonged hospital stay > 5 d (120 h) or readmission within 7 d of the index admission.

Operational definitions

Sepsis: Sepsis was defined as the presence of any source of infection along with at least two of the following[23]: (1) Temperature > 38 °C (100.4 °F) or < 36 °C (96.8 °F); (2) Heart rate > 90 bpm; (3) Respiratory rate > 20 or $PaCO_2$ < 32 mmHg; and (4) Total leukocyte count (TLC) > 12000/mm^3, < 4000/mm^3, or > 10% bands.

AKI: AKI was defined as per kidney disease: Improving Global Outcomes Acute Kidney Injury Work Group, and revised consensus recommendations of the

International Club of Ascites[24], *i.e.* increase in serum creatinine ≥ 0.3 mg/dL within 48 h; or a percentage increase serum creatinine ≥ 50% from the baseline which is known, or presumed, to have occurred within the prior 7 d.

Statistical analysis

The sample size was calculated using Open Epi for proportion, using mortality rate due to infection, and AKI between 36%-38%[16]. Taking into account the 95% confidence interval (CI), 80% power, and an odds ratio (OR) of 1.5, the final sample size was approximately 384 CLD patients.

Data were analyzed using Statistical Package for Social Sciences version 20. The frequency for all variables was calculated. Data were expressed as a mean and standard deviation for normally distributed continuous variables. The significance of association was calculated using the Student *t*-test. Categorical variables were recorded in their absolute value and analyzed using the chi-squared test. Univariate analysis was used to identify parameters associated with mortality and morbidity. Multiple logistic regressions were done in order to identify independent predictors of poor outcome in these patients. These factors were incorporated in the existing MELD score. Receiver operating characteristic curves of MELD, MELD-Na, and the new score were made. Significance tests and CIs were assessed through the nonparametric bootstrap. The area under the curve was compared between the three scores. Fisher exact test was used to determine the association of NCDs with predictors of mortality. All *P* values were two-sided and a *P* value of < 0.05 was considered statistically significant.

RESULTS

Demographics

Records of 399 patients admitted primarily due to decompensation of liver disease were reviewed. Mean age was 54.3 ± 11.7 years and 64.6% (*n* = 258) of patients were male. Hepatitis C was found to be the leading cause of CLD. The length of hospital stay of more than five days was 18%, while readmission rate within 1 wk was 7%. Six-week mortality was observed in 13% (*n* = 52) of patients. Table 1 describes the demographic details of patients.

NCDs were present in 47.4% (*n* = 189) of patients. AKI, sepsis, and non-ST elevation myocardial infarction (NSTEMI) were present in 41% (*n* = 165), 17.5% (*n* = 70), and 1.75% (*n* = 7) of patients, respectively.

Factors predicting mortality

Upon univariate analysis, hypotension, tachycardia, tachypnea, hypoxia, NSTEMI, sepsis, renal insufficiency, encephalopathy, pneumonia, anemia, leukocytosis coagulopathy, high CTP and MELD scores, and frequent admissions in the last 1 to 3 mo were found to be associated with 6 wk mortality.

Upon multivariate analysis, sepsis (OR = 6.50; 95%CI: 3.007-14.06; *P* < 0.001), AKI (OR = 2.69; 95%CI: 1.17-6.20; *P* = 0.02), and INR (OR = 1.75; 95%CI: 1.14-2.69; *P* < 0.001) were significant independent predictors of mortality (Table 2). Figure 1 shows a flow diagram of 6 wk mortality for patients with advanced cirrhosis and concomitant sepsis based on the logistic regression model. Sepsis, AKI, and INR were used in a hierarchical pattern based on the OR.

Factors predicting morbidity

Upon multivariate analysis, CKD, low albumin, and high MELD-Na scores were found to be independent factors in predicting morbidity (Table 3).

Factors predicting both mortality and morbidity

Upon multivariate analysis, CKD, high TLC, and high Child class were found to affect both mortality and morbidity (Table 4).

Addition of sepsis in MELD score

Once the value of sepsis was determined as a significant independent predictor of mortality based on multivariate analysis, we tried to formulate a new score by adding a factor of 6.5 into existing MELD scores. This factor was derived from the odds ratio of sepsis for mortality upon multivariate analysis. The new score labeled as MELD-sep was found to predict mortality better than MELD and MELD-Na as depicted by the area under receiver operating characteristic of 0.735 for MELD-sep in contrast with 0.686 for MELD-Na and 0.671 for MELD score (Figure 2).

Impact of NCDs

Table 1 Baseline demographic details

Variables		*n* (%) or mean ± SD
Age (yr)		54.56 ± 11.74
Gender	Male	258 (64.6)
	Female	136 (34.4)
Etiology (viral)	Hepatitis C	260 (65.1)
	Hepatitis B	31 (7.7)
	Hepatitis B + Hepatitis D	12 (3)
	Hepatitis B + Hepatitis C	6 (1.5)
	Non-B, Non-C	61 (15.2)
	(Unknown etiology)	
	Alcohol	20 (5)
	Autoimmune hepatitis	8 (2)
	Hemochromatosis	1 (0.25)
Duration of chronic liver disease (yr)		4.25 ± 3.71
NCDs	Diabetes	148 (37)
	Hypertension	96 (24)
	Chronic kidney disease	30 (7.5)
	Ischemic heart disease	23 (5.7)
	Chronic obstructive pulmonary disease	13 (3.2)
Infections on admission	Sepsis	70 (17.5)
	Lower respiratory tract infection	24 (6)
	Urinary tract infection	30 (7.5)
Non-ST Elevation myocardial infarction		7 (1.75)
Stroke		3 (0.75)
Decompensation on admission	Ascites	300 (75.1)
	Presence of esophageal/gastric varices	235 (58.8)
	Portosystemic encephalopathy	170 (42.6)
	Acute kidney injury	165 (41.3)
	Upper GI bleed	118 (29.5)
	Hepatocellular carcinoma	98 (24.5)
	Hepatorenal syndrome	86 (21.5)
	Spontaneous bacterial peritonitis	76 (19)
	Hepato-hydrothorax	12 (3)
Investigations	Hemoglobin (g/dL)	9.81 ± 2.17
	Total leukocyte count (× 109/L)	9.87 ± 6.17
	Platelets (× 109/L)	121.29 ± 108.12
	Prothrombin time (s)	17.37 ± 7.87
	International normalizing ratio	1.63 ± 0.65
	Creatinine (mg/dL)	1.65 ± 1.37
	Sodium (mmol/L)	132.3 ± 7.62
	Potassium (mmol/L)	4.17 ± 0.88
	pH	7.37 ± 0.12
	Bicarbonate (mmol/L)	20.13 ± 4.79
	Total bilirubin (mg/dL)	5.47 ± 7.57
	Alanine transaminase (IU/L)	78.7 ± 128.21
	Gama glutamyl transferase (IU/L)	119.26 ± 159.87
	Alkaline phosphatase (IU/L)	178.35 ± 133.8
	Albumin (g/dL)	2.59 ± 0.58
Child class	A	39 (9.8)
	B	142 (35.6)
	C	218 (54.6)
Prognostic scores	MELD score	18.0 ± 8.55

	MELD-Na	21.73 ± 8.31
	Charlson index	4.21 ± 1.63
	Charlson age adjusted score	5.33 ± 2.20
Outcomes	Mortality	52 (13)
	Prolong stay	72 (18)
	Readmission	28 (7)

NCDs: non-communicable diseases; GI: gastrointestinal; MELD: Model for End-Stage Liver Disease.

NCDs were found to be associated with an increased readmission rate (28.6% without NCDs *vs* 71.4% with NCDs; $P = 0.03$). However, there was no effect on length of stay (length of stay > 5 d in 51.4% without NCDs *vs* 48.6% with NCDs; $P = 0.45$). Upon multivariate analysis, among all NCDs, only CKD was directly related with increased morbidity (OR = 3.18; 95%CI: 1.30-7.82; $P = 0.01$). Moreover, the presence of NCDs was not found to be an independent predictor of mortality in our series (mortality rate of 12.4% without NCDs *vs* 13.9% with NCDs; $P = 0.65$). Similarly, the presence of multiple comorbid conditions did not appear to impact the mortality directly (mortality rate of 12.2% without NCDs *vs* 14.1% with 2 or fewer NCDs *vs* 15.6% with 3 or more NCDS; $P = 0.97$). However, the presence of NCDs was directly related with NSTEMI, which was a major predictor of mortality in our study (Table 5). Similarly, Charlson comorbidity index was used to calculate the burden of NCDs in our patients, and this index did not appear to predict the outcome in cirrhotic patients.

DISCUSSION

Decompensated liver disease is a state of organ failure related to multi-organ consequences such as encephalopathy, renal insufficiency, volume overload, GI bleeding, infections, and frailty[25-28]. In this study of hospitalized decompensated cirrhotic patients, we evaluated the impact of sepsis and NCDs on patient outcome.

Traditionally, CTP and MELD scores have been used to predict the mortality in cirrhotic patients. However, the group of patients used to create and validate the MELD score was devoid of acute reversible complications such as sepsis[29]. Infections significantly increase the mortality in end-stage liver disease with some studies reporting a 30% death rate in 1 mo[16]. Prevention and treatment of sepsis were shown to reduce mortality in patients with cirrhosis and AKI[17]. AKI is a common and overwhelming complication in patients with end-stage liver disease. Belcher *et al*[15] also showed AKI to be associated with high mortality and complications in hospitalized patients with cirrhosis. Our study found that AKI, myocardial infarction, sepsis, and coagulopathy on admission were associated with high mortality in cirrhotic patients.

Interestingly, the MELD and Charlson comorbidity index scores were not associated with high mortality in our analysis. However, high Child class appeared to affect both mortality and morbidity. Based on our observations, we propose that the addition of sepsis as a factor in the MELD score gives a better prediction of mortality as compared to conventional MELD and MELD-Na scores in CLD patients admitted with acute decompensation. We also found that CKD, low albumin, and high MELD-Na scores were able to predict morbidity.

Chirapongsathorn *et al*[30] and Shu *et al*[31] related longer hospital stays with a high rate of 30-d readmission while Masadeh *et al*[32] found the opposite relationship. In our analysis, prolonged hospital stay was associated with subsequent early readmission upon univariate analysis but did not stand out as an independent factor upon multivariate analysis.

The prognostic value of chronic NCDs in cirrhotic patients has not been extensively studied. Recently, diabetes has been associated with high mortality[19,33,34], higher readmission rate[35], and a major factor in determining liver-related outcomes in cirrhotic patients[36]. We were unable to relate diabetes directly to mortality and morbidity in our series. However, among NCDs, CKD was found to directly predict morbidity in our series. Moreover, the presence of NCDs was associated with NSTEMI, which was one of the major predictors of mortality in our cohort. This observation indicates NCDs as an important factor that could influence the outcome of CLD patients independent of well-known prognostic variables incorporated in Child-Pugh and MELD scores for advanced liver disease patients.

Despite previous studies supporting the use of the Charlson comorbidity index for prediction of poor outcome in CLD patients[21,22], our study did not find a direct

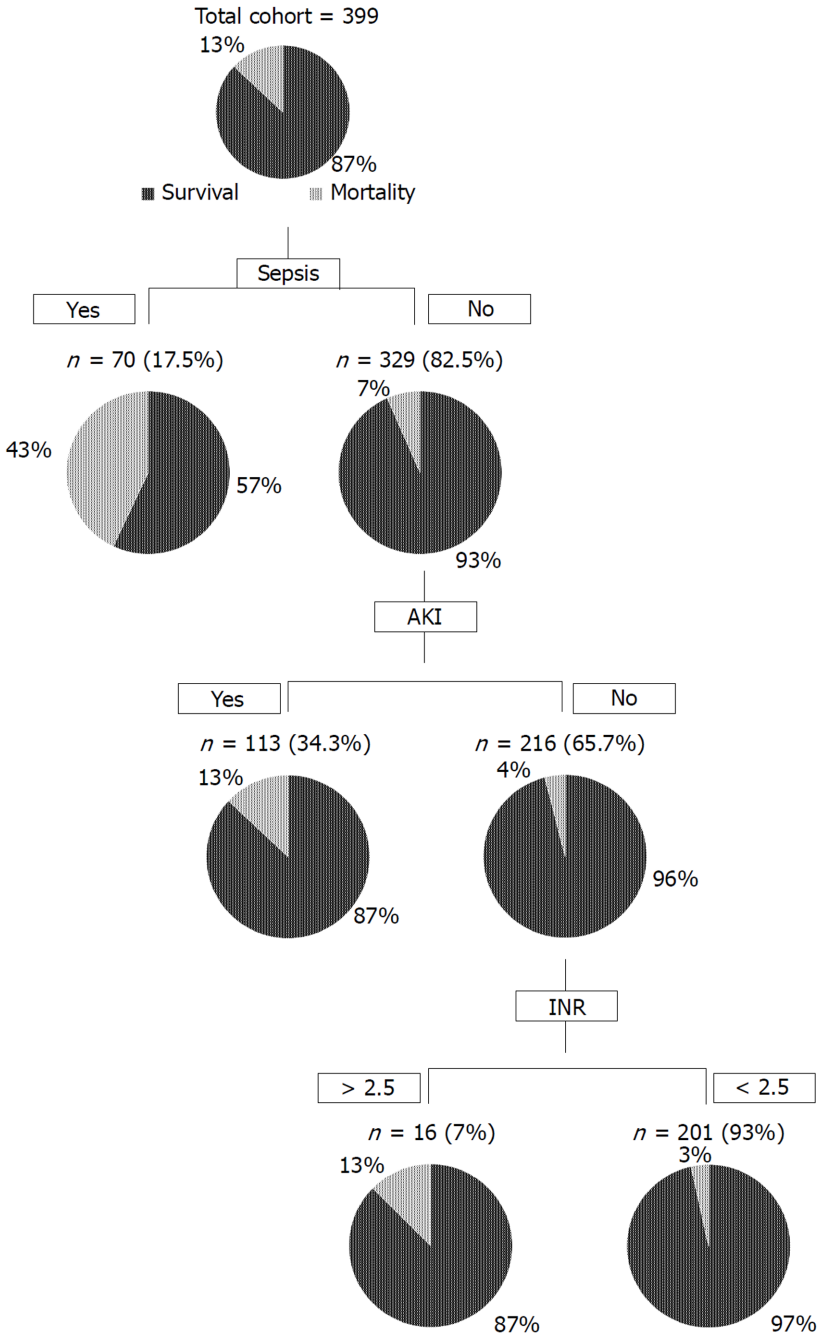

Figure 1 Flow diagram of 6 wk mortality for patients with advanced cirrhosis and concomitant sepsis based on the logistic regression model. AKI: Acute kidney injury; INR: International normalized ratio.

relationship between Charlson comorbidity index and morbidity upon multivariate analysis. This could be due to the difference between patient characteristics. We selectively enrolled patients who were admitted due to decompensated liver disease while previous studies included patients who were labeled as cirrhotic in their database regardless of compensation status[21,22]. Moreover, the follow-up period was longer in the Danish cohort[22].

The present study takes into account readmissions at our center. The possibility of readmissions at other centers was not accounted for. Other limitations include the cross-sectional design, shorter follow-up, and single center focus. Further large-scale multicenter studies with a longer follow-up would be helpful in strengthening the impact of NCDs and sepsis on the determination of outcome in hospitalized cirrhotic patients.

In conclusion, the addition of sepsis improves the predictability of the MELD score as a prognostic marker for mortality in patients with decompensated CLD. Presence

Table 2 Multivariate analysis of factors predicting 6 wk mortality in chronic liver disease patients

		OR (95%CI)	*P* value
NSTEMI	No	1	
	Yes	16.03 (2.01-127.46)	0.009
Sepsis	No	1	
	Yes	6.50 (3.01-14.06)	< 0.001
AKI	No	1	
	Yes	2.69 (1.17-6.20)	0.02
INR		1.75 (1.14-2.69)	< 0.001

NSTEMI: non-ST-elevation myocardial infarction; AKI: acute kidney injury; INR: international normalized ratio; OR: odds ratio; CI: confidence interval.

of CKD increases morbidity of patients with CLD.

Table 3 Multivariate analysis of factors predicting morbidity

		OR (95%CI)	*P* value
CKD	No	1	
	Yes	3.18 (1.30-7.82)	0.01
MELD-Na		1.05 (1.01-1.08)	0.005
Albumin		0.55 (0.32-0.92)	0.02

Prolonged hospital stay > 5 d (120 h) or readmission within 7 d of the index admission; CKD: chronic kidney disease; MELD: Model for End-Stage Liver Disease; OR: odds ratio; CI: confidence interval.

Table 4 Multivariate analysis of factors predicting both mortality and morbidity

		OR (95%CI)	*P* value
CKD	No	1	
	Yes	3.12 (1.21-8.06)	0.01
TLC		1.08 (1.03-1.12)	< 0.001
Child Class		3.57 (2.20-5.79)	< 0.001

CKD: chronic kidney disease; TLC: total leukocyte count; OR: odds ratio; CI: confidence interval.

Table 5 The relationship between predictor of mortality and non-communicable diseases, *n* (%)

	NCDs		*P* value
	Yes	No	
	210 (52.6)	189 (47.3)	
NSTEMI	7 (3.3)	0	0.01
Sepsis	31 (14.8)	39 (20.6)	0.14
AKI	92 (44.7)	76 (40.2)	0.41

NSTEMI: non-ST-elevation myocardial infarction; INR: international normalized ratio; AKI: acute kidney injury; NCDs: non-communicable diseases.

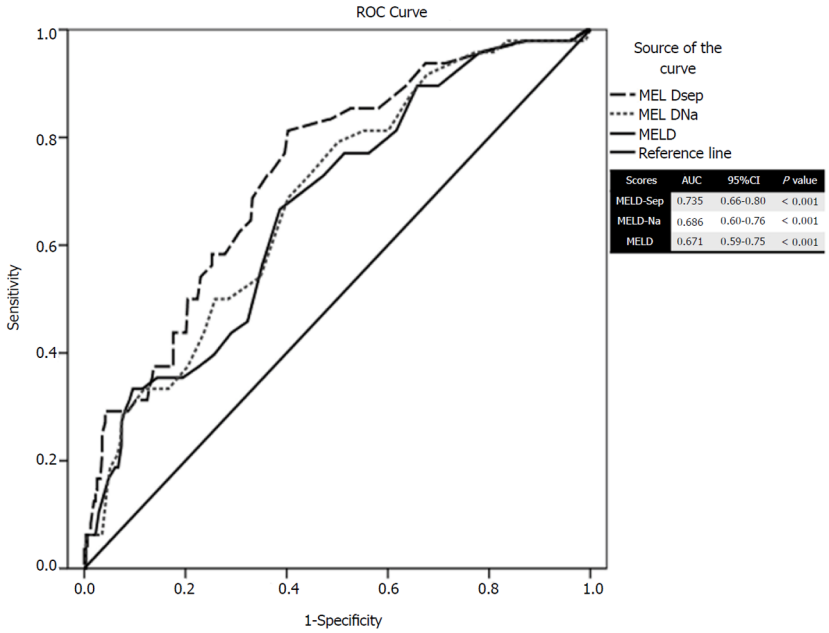

Figure 2 Receiver operating characteristic curve shows 6 wk mortality prediction of MELD-Sep *vs* MELD and MELD-Na. MELD: Model for End-Stage Liver Disease.

ARTICLE HIGHLIGHTS

Research background

Patients with decompensated chronic liver disease (CLD) are at high risk of complications. Various scores have been used to classify the severity of liver disease and to predict mortality. Recently, diabetes was found to impact mortality in cirrhotic patients. However, the impact of other comorbidities on mortality and morbidity has not been studied. Moreover, the impact of sepsis on available predictability scores has not been determined.

Research motivation

Given the limitations with the use of Child-Pugh and Model for End-Stage Liver Disease (MELD) scores, we wanted to come up with a new score to predict mortality and morbidity.

Research objective

The objective for this study included determination of sepsis, non-communicable diseases (NCDs), and acute kidney injury (AKI) in patients admitted with decompensated liver disease, along with their impact of NCDs on mortality and morbidity parameters. We also wanted to evaluate whether the addition of any other variable makes MELD a better tool as a prognostic marker.

Research methods

We performed a retrospective analysis of medical records of patients with CLD admitted at the Aga Khan University Hospital. All adult patients with decompensation of CLD (*i.e.*, jaundice, ascites, encephalopathy, and/or upper gastrointestinal (GI) bleed) as the primary reason for admission were included. Multivariate analysis was performed to assess predictors of 6 wk mortality, prolonged hospital stay (> 5 d), and early readmission (within 7 d).

Research results

Six-week mortality rate was 13%. Prolonged hospital stay and readmission rates were 18% and 7%, respectively. NCDs were present in 47.4% of patients. AKI, sepsis, and NSTEMI were present in 41%, 17.5%, and 1.75% patients, respectively. Factors associated with mortality included AKI, NSTEMI, sepsis, and coagulopathy. The factors found responsible for morbidity included chronic kidney disease (CKD), low albumin, and high MELD-Na score. By adding sepsis to the conventional MELD score, the predictability of mortality increased significantly. CKD was found to impact morbidity independently.

Research conclusion

This study highlighted multiple factors associated with early mortality, readmission, and prolonged hospital stay. This study also determined the significance of the addition of sepsis in the MELD score to improve its predictability as a prognostic marker for mortality in patients with decompensated CLD. Presence of CKD increased morbidity of patients with CLD.

Research perspective

We need to amend factors linked to mortality, readmission, and prolonged stay not only to control mortality and morbidity, but also to minimize the cost burden by patients.

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P- Reviewer: Fedeli U, Reggiani GM
S- Editor: Ma RY **L- Editor:** Filipodia **E- Editor:** Tan WW

World Journal of Hepatology

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

World J Hepatol 2018 December 27; 10(12): 956-965

DOI: 10.4254/wjh.v10.i12.956

ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

One in five hepatocellular carcinoma patients in the United States are Hispanic while less than 40% were eligible for liver transplantation

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Author contributions: Robinson A and Wong RJ contributed to the study concept and design, acquisition of data, and drafting of the manuscript; Wong RJ contributed to statistical analysis and study supervision; all authors contributed to analysis and interpretation of data, and critical revision of the manuscript for important intellectual content.

Institutional review board statement: The study was reviewed and determined to be exempt by the Alameda Health System Institutional Review Board because human subjects were not involved, as per United States Department of Health and Human Services guidelines, and the SEER database is publicly available without individually identifiable private information.

Informed consent statement: Given the observational study design and large registry-based cohort, this study was granted IRB exemption by Alameda Health System Institutional Review Board because human subjects were not involved, as per United States Department of Health and Human Services guidelines, and the SEER database is publicly available without individually identifiable private information. Thus informed consent was not required and not possible.

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Abstract

AIM

To evaluate trends and disparities in hepatocellular carcinoma (HCC) outcomes among Hispanic patients in the United States with a focus on tumor stage at diagnosis.

METHODS

We retrospectively evaluated all Hispanic adults (age > 20) with HCC diagnosed from 2004 to 2014 using United States Surveillance, Epidemiology, and End Results (SEER) cancer registry data. Tumor stage was assessed by SEER-specific staging systems and whether HCC was within Milan criteria at diagnosis. Multivariate logistic regression models evaluated for predictors of HCC within Milan criteria at diagnosis.

RESULTS

Overall, Hispanics accounted for 19.8% of all HCC (73.3% men, 60.9% had Medicare or commercial insurance, 33.5% Medicaid, and 5.6% uninsured). Thirty-eight percent of Hispanic HCC patients were within Milan criteria at diagnosis. With latter time periods, significantly more patients were diagnosed with HCC within Milan criteria, and in 2013-2014, 42.6% had HCC within Milan criteria. On multivariate regression, Hispanic males (OR *vs* females: 0.76, 95%CI: 0.68-0.83, $P < 0.001$), Hispanics > 65 years (OR *vs* age < 50: 0.67, 95%CI: 0.58-0.79, $P < 0.001$), and uninsured patients (OR *vs* Medicare/commercial: 0.49, 95%CI: 0.40-0.59, $P < 0.001$) were significantly less likely to have HCC within Milan criteria at diagnosis.

CONCLUSION

While one in five HCC patients in the United States are of Hispanic ethnicity, only 38% were within Milan criteria at time of diagnosis, and thus over 60% were

Conflict-of-interest statement: Robert J Wong is a consultant, member of the advisory board, research grants, and speaker's bureau for Gilead and member of speaker's bureau for Bayer. The other authors declare no conflicts of interest related to this article.

STROBE statement: Guidelines of the STROBE statement have been adopted.

Manuscript source: Unsolicited manuscript

Received: May 22, 2018
Peer-review started: May 23, 2018
First decision: July 10, 2018
Revised: July 10, 2018
Accepted: August 20, 2018
Article in press: August 21, 2018
Published online: December 27, 2018

ineligible for liver transplantation, one of the primary curative options for HCC patients. Improved efforts at HCC screening and surveillance are needed among this group to improve early detection.

Key words: Liver cancer; Surveillance; Epidemiology; End results; Milan criteria; Hepatocellular carcinoma

Core tip: The Hispanic population represents a major contributor to the hepatocellular carcinoma (HCC) burden. However, our current study demonstrates that over 60% of Hispanic HCC patients were ineligible for liver transplantation given the extent of disease severity. This advanced cancer stage at diagnosis likely reflects suboptimal implementation of early and timely HCC screening and surveillance in high-risk populations. These findings emphasize the need to be more vigilant about HCC screening and surveillance, especially among the Hispanic population.

Robinson A, Ohri A, Liu B, Bhuket T, Wong RJ. One in five hepatocellular carcinoma patients in the United States are Hispanic while less than 40% were eligible for liver transplantation. *World J Hepatol* 2018; 10(12): 956-965
URL: https://www.wjgnet.com/1948-5182/full/v10/i12/956.htm
DOI: https://dx.doi.org/10.4254/wjh.v10.i12.956

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality worldwide[1-3]. The number of deaths per year from HCC is nearly identical to the worldwide incidence, highlighting the incredibly high case fatality rate of this aggressive disease[3]. While the highest prevalence of HCC is among populations in Asia and Africa, the incidence of HCC has been increasing in the United States, due to the growing number of patients with nonalcoholic steatohepatitis (NASH) and chronic hepatitis C virus (HCV) who are at risk for HCC[1,4-8]. However, the rising HCC incidence is not uniform across all groups, and an increasingly disproportionate rise in HCC incidence among minority groups, and in particular Hispanics, have been reported[9-13]. A recent study by White *et al*[7] reported a 4.5% annual increase in overall HCC incidence in the United States from 2000 to 2009. However, race/ethnicity-specific differences were observed with significantly greater increases in HCC incidence among Hispanics, such that beginning in 2012, HCC incidence among Hispanics surpassed that of Asians in the United States. While the exact etiology underlying this epidemiology is unclear, these trends likely reflect the successful impact of HBV vaccination in Asians and the emergence of nonalcoholic fatty liver disease (NAFLD) in the Hispanic population. Because the Hispanic population carries a disproportionate burden of NAFLD, understanding trends in HCC among the Hispanic population in the United States may provide insight on NAFLD-HCC trends[11,14-19]. The aim of the current study is to evaluate overall trends and disparities in HCC outcomes among Hispanic HCC patients in the United States with a focus on HCC tumor stage at presentation.

MATERIALS AND METHODS

The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) population based cancer registry was reviewed to identify all Hispanic adults (age 20 years and older) with HCC from 2004 to 2014 in the United States[20]. The 2004-2014 SEER data includes data from 18 regions in the United States (San Francisco-Oakland, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose-Monterey, Los Angeles, Alaska Natives, Rural Georgia, California excluding San Francisco/San Jose/Los Angeles, Kentucky, Louisiana, New Jersey, and Greater Georgia) and represents approximately 28% of the United States population.

The International Classification of Disease for Oncology, Third Edition was used by the SEER database to define HCC[21]. HCC tumor stage was evaluated using SEER

historic summary staging, which is unique to SEER and is not used particularly for prognosis, but to describe the extent of disease[21]. Tumors confined to only one lobe of the liver with or without vascular invasion were defined as localized. Tumors involving more than one lobe through contiguous growth of a single lesion, extension to adjacent structures (gallbladder, diaphragm, or extrahepatic bile ducts), or spread to regional lymph nodes were defined as regional. Metastatic disease or extension of cancer to distant lymph nodes or nearby organs such as the stomach, pleura, or pancreas was defined as distant. In addition to SEER HCC staging, we also evaluated tumor characteristics including size and number of tumors to determine whether a patient's tumor met Milan criteria (single lesion less than 5 cm or no more than 3 lesions each less than 3 cm) with no extrahepatic or vascular involvement. We specifically chose to evaluate whether or not a patient's tumor met Milan criteria, as this affects eligibility for liver transplantation, which is one of the major curative treatment options for HCC[22].

Insurance status was evaluated using SEER classifications, which included three categories: Medicare or commercial insurance, Medicaid, and uninsured/no insurance. Medicare or commercial insurance includes patients with private insurance (fee-for-service, managed care, HMO, PPO, Tricare) or Medicare (administered through a managed care plan, Medicare with private supplement, or Medicare with supplement, NOS and Military). Medicaid includes patients with Medicaid (including administered through managed care plans, Medicare with Medicaid eligibility, and Indian/Public Health Service). Uninsured is defined as those who were either not insured or self-pay at time of HCC diagnosis. Age-specific comparisons utilized three categories of age at time of HCC diagnosis (age < 50 years, age 50 - 64 years, and age > 65 years).

Statistical analysis

Demographic characteristics of the study cohort were presented as proportions and frequencies. Overall proportion of adult Hispanic patients with localized HCC or HCC within Milan criteria were stratified by sex, age at time of diagnosis, insurance status, and year of diagnosis, and compared across groups using $\chi 2$ test. Predictors of HCC tumor stage at presentation and HCC treatment received were evaluated using multivariate logistic regression models. Forward stepwise regression methods were used with variables demonstrating significant associations in the univariate model (P < 0.10) or those with biologic significance determined a priori (*e.g.*, age and sex) included in the final model. Statistical significance was met with a 2-tailed P-value < 0.05. All statistical analyses were performed with Stata version 14 (Stata Corp, College Station, TX). The study was reviewed and determined to be exempt by the Alameda Health System Institutional Review Board because human subjects were not involved, as per United States Department of Health and Human Services guidelines, and the SEER database is publicly available without individually identifiable private information.

RESULTS

Hispanics accounted for 19.8% of all HCC among United States adults in the 2004-2014 SEER registry, with the majority of subjects with HCC being men (n = 9856, 73.3%). Among both Hispanic men and women with HCC, 60.9% had Medicare or commercial insurance at time of HCC diagnosis, 33.5% had Medicaid, and 5.6% were uninsured (Table 1). The proportion of the HCC cohort represented by Hispanic patients increased from 17.6% in 2004-2006 to 20.1% in 2013-2014.

When evaluating HCC tumor stage at diagnosis, 38.0% of Hispanic HCC patients were within Milan criteria, and 54.0% of Hispanic HCC patients had localized HCC (Table 2). Compared to female HCC patients, male HCC patients had significantly lower rates of localized HCC (52.8% *vs* 57.6%, P < 0.001) and significantly lower rates of HCC within Milan criteria (37.4% *vs* 39.7%, P = 0.01). Compared to HCC patients age < 50 years at time of diagnosis, higher rates of localized HCC and HCC within Milan criteria were observed in those age 50-64, but HCC patients age > 65 years had lower rates of localized HCC or HCC within Milan criteria (Table 2). Compared to Medicare or commercially insured patients, Hispanic HCC patients with Medicaid or no insurance had significantly lower rates of localized HCC or HCC within Milan criteria.

When stratified by year of HCC diagnosis, Hispanic HCC patients in the latter time periods had significantly higher rates of localized HCC at time of diagnosis, and in 2013-2014, 56.9% of Hispanic HCC patients had localized tumor stage. Similarly, the proportion of Hispanic patients with HCC within Milan criteria also increased with

Table 1 Characteristics of the study cohort

	Hispanics		Asians		Non-Hispanic White	
	Percent (%)	Frequency (*n*)	Percent (%)	Frequency (*n*)	Percent (%)	Frequency (*n*)
Sex						
Female	26.7	3584	29.5	3273	24.9	9273
Male	73.3	9856	70.6	7840	75.1	27940
Age						
< 50 yr	10.7	1440	10.7	1191	6.1	2268
50-64 yr	47.1	6325	36.4	4045	45.6	16973
> 65 yr	42.2	5675	52.9	5877	48.3	17972
Insurance						
Medicare or Commercial	60.9	5969	66.3	5177	80.2	21265
Medicaid	33.5	3280	29.5	2299	16.1	4257
Uninsured	5.6	549	4.3	335	3.7	983
Year of diagnosis						
2004-2006	17.6	2722	17.6	2713	51.8	8009
2007-2008	17.4	2115	16.3	1985	52.5	6376
2009-2010	18.9	2550	15.4	2084	51.3	6927
2011-2012	20.1	2870	13.9	2133	51.9	7690
2013-2014	20.1	3183	13.9	2198	51.7	8211
HCC SEER summary stage						
Localized	54	6091	51.8	2250	52.6	8644
Regional	29.5	3324	31	1345	31.2	5129
Distant	16.5	1858	15	747	16.2	2652
HCC within Milan criteria						
No	62	8337	59.1	2832	55.4	10530
Yes	38	5103	40.9	1958	44.6	8465

HCC: Hepatocellular carcinoma; SEER: Surveillance, Epidemiology, and End Results.

time, and in 2013-2014, 42.6% of Hispanic HCC patients were within Milan criteria (Table 3). When stratified by sex, age, and insurance status, similar trends were seen, such that HCC patients in latter time periods had the highest rates of more localized stage of disease.

On multivariate regression, in comparison to Medicare/commercially insured Hispanic HCC patients, uninsured patients were significantly less likely to have HCC within Milan criteria at time of diagnosis (OR 0.49, 95%CI: 0.40-0.59, $P < 0.001$), whereas no significant difference was observed in Medicaid HCC patients (Figure 1). Compared to 2004-2006, Hispanic HCC patients in both the 2011-2012 and 2013-2014 cohorts were significantly more likely to have HCC within Milan criteria at diagnosis (2011-2012: OR 1.17, 95%CI: 1.04-1.32, $P = 0.007$; 2013-2014: OR 1.30, 95%CI: 1.16-1.45, $P < 0.001$). Compared to patients age < 50 years at the time of diagnosis, Hispanic HCC patients age 65 years and older were significantly less likely to have HCC within Milan criteria (OR 0.67, 95%CI: 0.58-0.79, $P < 0.001$), whereas patients age 50-64 years old were significantly more likely to have HCC within Milan criteria (OR 1.33, 95%CI: 1.15-1.54, $P < 0.001$). Males were less likely than females to be within Milan criteria (OR 0.76, 95%CI: 0.68-0.83, $P < 0.001$) (Figure 1).

Similar trends were observed when evaluating predictors of SEER localized stage of HCC at diagnosis. Uninsured Hispanic HCC patients and those with Medicaid were significantly less likely to have localized disease compared to those patients with Medicare or commercial insurance (Uninsured: OR 0.43, 95%CI: 0.33-0.55, $P < 0.001$, Medicaid: OR 0.85, 95%CI: 0.74-0.97, $P = 0.018$). Patients diagnosed with HCC during 2013-2014 were more likely to have localized disease as compared to those diagnosed from 2004-2006 (OR: 1.28, 95%CI: 1.08-1.52, $P = 0.004$), however there was no significant difference when comparing 2011-2012 or 2007-2008 to 2004-2006. Males were less significantly less likely to have localized disease compared to females (OR 0.79, 95%CI: 0.68-0.91, $P = 0.001$) (Figure 2).

Table 2 Proportion of Hispanic hepatocellular carcinoma patients with localized hepatocellular carcinoma or hepatocellular carcinoma within Milan criteria at time of diagnosis

	Localized HCC at Time of diagnosis			HCC within Milan criteria at time of diagnosis		
	Percent (%)	Frequency (*n*)	*P*-value	Percent (%)	Frequency (*n*)	*P*-value
Total	54	6091		38	5103	
Sex						
Female	57.6	1680	< 0.001	39.7	1422	0.01
Male	52.8	4411		37.4	3681	
Age						
< 50 yr	51.7	630	< 0.01	36.8	530	< 0.001
50-64 yr	55.5	3030		44.6	2820	
> 65 yr	52.9	2431		30.9	2753	
Insurance						
Medicare or Commercial	56.2	3004	< 0.001	42.7	2547	< 0.001
Medicaid	53.8	1607		42.9	1407	
Uninsured	39.4	191		29.9	164	
Year of Diagnosis						
2004-2006	52.8	1195	< 0.001	33.4	908	< 0.001
2007-2008	31.7	553		33.8	714	
2009-2010	52.7	1368		37.3	951	
2011-2012	56.3	1368		40.9	1173	
2013-2014	56.9	1525		42.6	1357	

HCC: Hepatocellular carcinoma.

DISCUSSION

Using United States population-based cancer registry data, the current study evaluated disparities in HCC tumor stage at diagnosis with a focus on Hispanics with HCC. Overall, the proportion of HCC in the Hispanic population increased during the study period such that over 20% of HCC patients in the United States during 2013-2014 were of Hispanic ethnicity. Despite this huge disease burden among the Hispanic population, less than half of Hispanic HCC patients were within Milan criteria at the time of diagnosis in the most recent time period studied. Furthermore, significant disparities among this group were observed, with a disproportionately higher risk of advanced tumor stage at presentation among Hispanic men, older Hispanic patients, and uninsured Hispanic patients.

Nearly one in five HCC patients in the United States are of Hispanic ethnicity with Hispanic patients showing the greatest increase in the incidence of HCC from 2004-2006 to 2013-2014[20]. These observations are consistent with previous SEER registry based studies evaluating trends in HCC incidence. For example, El-Serag *et al*[23] reported significantly greater age-adjusted HCC incidence rates in Hispanic patients in comparison to non-Hispanic white and black populations between 1992 and 2002. This increasing HCC incidence among the Hispanic population in the United States has continued to rise such that HCC incidence among Hispanics surpassed HCC incidence among Asians in 2012 to become the ethnic group with the greatest incidence of HCC in the United States and representing over 20% of all HCC among adults in the United States[7]. While our current study is limited by the ability to evaluate the liver disease etiology contributing to HCC, the Hispanic population in the United States carries a disproportionate burden of NAFLD[14-17,24-28]. Furthermore, during this same period where Hispanics have become the ethnic group with the most rapidly rising incidence of HCC, it is also important to note that prevalence of NASH-related HCC has also risen in parallel[8,18,26,29-31]. Two recent studies using the SEER-Medicare database and the United Network for Organ Sharing registry demonstrate the continued rising prevalence of NAFLD-related HCC in the United States[8,18].

While the growing burden of HCC among the Hispanic population is concerning, it is even more alarming that the Hispanic population faces significant disparities in access to treatment options for HCC, which contributes to significantly lower survival[32-35]. A recent study by Ha *et al*[34] using the SEER database found that Hispanic patients with HCC had significantly lower rates of curative HCC treatment

Table 3 Trends in the proportion of Hispanic hepatocellular carcinoma patients with localized hepatocellular carcinoma and hepatocellular carcinoma within Milan criteria over time

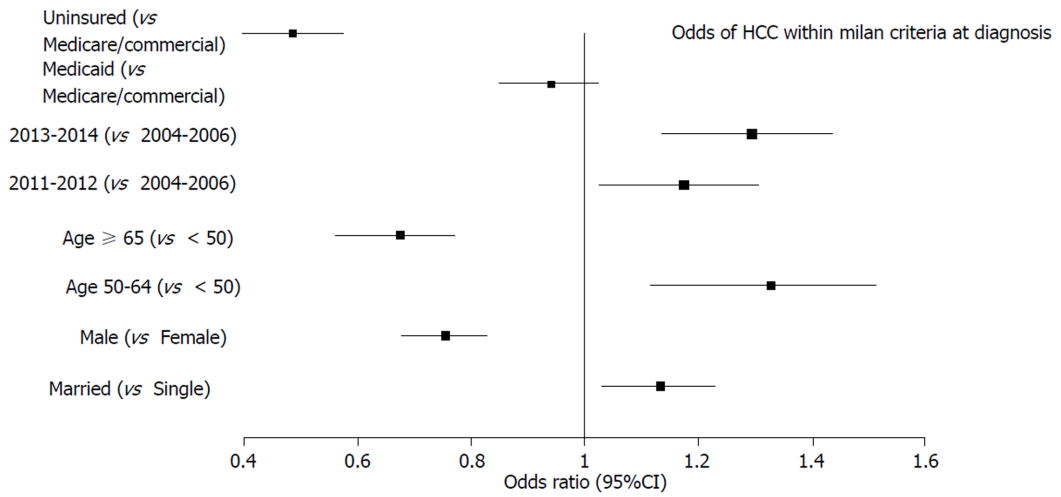
Localized HCC	Overall		Female		Male		Age < 50		Age 50-64		Age 65 and over		Uninsured		Medicaid		Medicare/Commercial	
	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
2004-2006	52.8	1195	58	342	51	853	48.7	165	54.1	544	52.9	486	-	-	-	-	-	-
2007-2008	31.7	553	55.5	236	48.1	635	46	115	50.9	424	50.1	332	33.9	38	49.3	281	52.1	531
2009-2010	52.7	1368	55.3	588	51.8	844	51.5	121	55.4	589	49.6	422	36	40	52.6	397	54.4	675
2011-2012	56.3	1368	58	363	55.7	1005	60.2	121	57.5	706	54.1	541	39.6	53	56.4	456	58	835
2013-2014	56.9	1525	59.7	451	55.7	1074	56	108	57.6	767	56.1	650	46.9	60	55.5	473	58.4	963
P-Value		< 0.001		< 0.03		<0.001		< 0.02		< 0.02		< 0.01		0.17		0.14		< 0.001
HCC Within Milan	**Overall**		**Female**		**Male**		**Age < 50**		**Age 50-64**		**Age 65 and Over**		**Uninsured**		**Medicaid**		**Medicare/Commercial**	
	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*
2004-2006	33.4	908	34.6	255	34.3	653	32.9	141	39.9	462	26.4	305	-	-	-	-	-	-
2007-2008	33.8	714	32.8	179	35.5	535	34.1	105	40.6	397	25.2	212	27.5	36	37.8	237	37.6	428
2009-2010	37.3	951	40.6	260	35.3	691	36.2	97	45.5	559	28.2	295	30.3	37	40.1	337	40.4	558
2011-2012	40.9	1173	41.1	310	40.2	863	40.8	92	47.7	676	33.1	405	30.7	47	47.5	420	42.4	687
2013-2014	42.6	1357	46.1	418	36.8	939	41.2	95	47.1	726	30.9	536	30.8	44	44.2	413	47.8	874
P-Value		< 0.001		< 0.001		< 0.001		0.3		< 0.001		< 0.001		0.92		< 0.001		< 0.001

HCC: Hepatocellular carcinoma.

when compared to non-Hispanic whites. Hispanics were also significantly less likely to receive liver transplantation even after correcting for stage of disease at time of diagnosis. Our current analysis of the most recent SEER database continues to highlight these disparities among the Hispanic HCC population. Despite improvements in earlier tumor stage of diagnosis over time, even in the most recent 2013-2014 period only 42.6% of Hispanic HCC patients were within Milan criteria at the time of diagnosis, which means that nearly 60% of patients were not eligible for liver transplantation, one of the main curative options for HCC patients.

A greater frequency of HCC was observed among male patients within our study, which is consistent with prior data showing HCC rates in males are two to four times as high as rates in females[35,36]. This sex-specific trend in HCC risk is also observed among NAFLD HCC populations. Corey *et al*[36] evaluated at risk factors for HCC in 94 patients with NAFLD with HCC and 150 patients with NAFLD without HCC. The strongest association with risk of HCC was being male, with males having a four-fold higher risk of developing HCC compared to females. The majority of Hispanic HCC patients in our study were men, and they had more advanced HCC at diagnosis compared to females. Similarly Yang *et al*[37] observed significantly better outcomes in females with HCC. In their study of primarily non-Hispanic Caucasian patients, the distribution of tumor burden showed a higher incidence of single lesions among women and a higher incidence of vascular invasion among men.

Our study utilized a large population-based cancer registry, which represents a large proportion of the United States population. The SEER registry allowed for a large sample size and comprehensive analysis of HCC epidemiology and outcomes. Despite this, the current study has several limitations that should be acknowledged. SEER does not include etiology of HCC (*e.g.*, chronic hepatitis B virus, chronic HCV, alcoholic liver disease, or NAFLD), which may have affected rates of disease progression or rates of timely HCC screening and surveillance. Along the same lines, liver disease-specific treatment data (*e.g.*, antiviral therapies) were not available for inclusion in the analysis. While our study observed more advanced tumor stage among older patients, this observation may be confounded by other factors. Older patients may have had more significant co-morbidities (hypertension, diabetes mellitus, or cardiac disease) that may have affected referral for HCC screening and surveillance. However, these additional co-morbidities and risk factors such as alcohol use, obesity, and concurrent diabetes mellitus were not available in the database for inclusion in our analyses. While our study also investigated disparities related to insurance status, we acknowledge that insurance status is only one key factor that is tightly linked to other demographic and socioeconomic factors in a complex manner. Furthermore, in the SEER database, Medicare and commercially

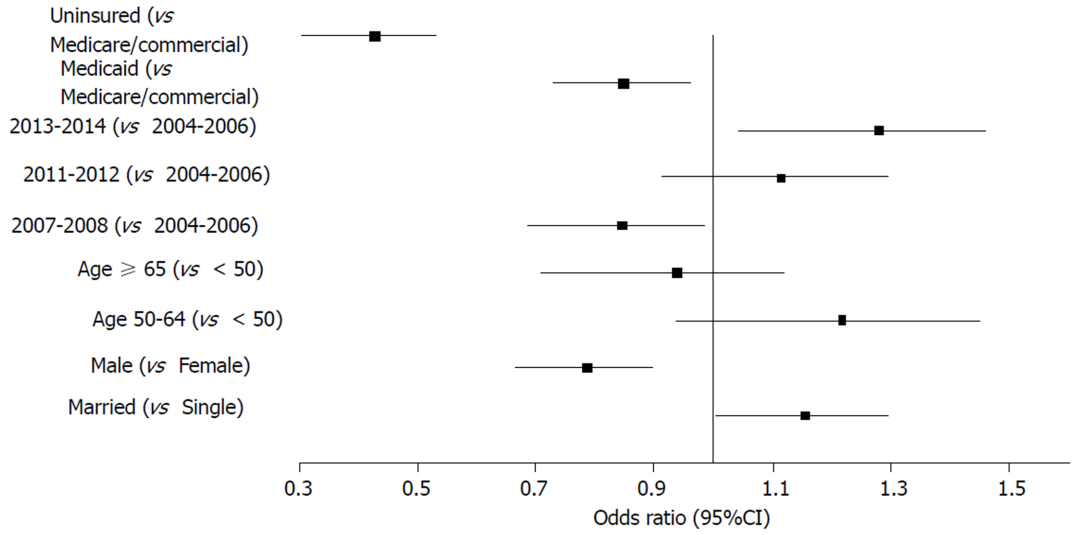


	Odds ratio	95%CI	*P*-Value
Uninsured (*vs* Medicare/commercial)	0.49	0.40-0.59	< 0.001
Medicaid (*vs* Medicare/commercial)	0.94	0.86-1.03	0.203
2013-2014 (*vs* 2004-2006)	1.30	1.16-1.45	< 0.001
2011-2012 (*vs* 2004-2006)	1.17	1.04-1.32	0.007
Age ⩾ 65 (*vs* < 50)	0.67	0.58-0.79	< 0.001
Age 50-64 (*vs* < 50)	1.33	1.15-1.54	< 0.001
Male (*vs* Female)	0.76	0.68-0.83	< 0.001
Marital status (Married *vs* Single)	1.13	1.04-1.24	0.005

Figure 1 Multivariate logistic regression model evaluating predictors of hepatocellular carcinoma within Milan criteria at diagnosis. These data specifically evaluate the independent risk of having hepatocellular carcinoma (HCC) within Milan criteria at diagnosis when evaluated by insurance status at time of diagnosis, the year of HCC diagnosis, the age at time of HCC diagnosis, sex, and marital status. An odds ratio of > 1.00 indicates increased likelihood of having HCC within Milan criteria at time of diagnosis.

insured patients are combined into one category, and demographic and clinical differences between those with Medicare and commercial insurance may affect HCC stage at diagnosis. However, access to care, including HCC screening and surveillance that would affect tumor stage and HCC treatment are expected to be similar between those with Medicare and commercial insurance in the United States. While our study found significant disparities in HCC stage among uninsured patients, these differences may reflect difficulty accessing healthcare due to complex socioeconomic needs. However, these data were not available to assess further in the database. Another factor not readily available for analysis is concurrent substance use and high-risk behavior, which may have affected timely access and adherence to HCC surveillance. Lastly, surveillance data were not available to evaluate how the differences in successful screening and surveillance rates contributed to the observed disparities in HCC stage at diagnosis. Despite these limitations, our study provides valuable information regarding a vulnerable subset of the United States population.

Understanding HCC trends provides valuable data to guide clinicians and policy makers to identify high-risk groups as well as those that suffer disparities towards which resources can be targeted to improve HCC screening and surveillance for early detection and treatment. Our current study focused specifically on Hispanic HCC patients, a group that has the highest incidence of HCC in the United States since 2012 and represents 20% of all adults with HCC. Among this group less than 40% had HCC within Milan criteria at the time of diagnosis, and thus over 60% were ineligible for liver transplantation, one of the major curative options for HCC patients. Part of this problem revolves around effective HCC screening and surveillance, and despite established guidelines, overall HCC screening rates remain low[38-40]. In one United States population-based study, less than 20% of patients with cirrhosis who developed HCC received regular surveillance, and only 29% of patients who had a diagnosis of HCC had undergone annual surveillance in the three years before receiving the diagnosis[41]. Tavakoli *et al*[42] observed that less than half of cirrhotic patients received first time HCC screening among safety-net populations with cirrhosis, with the largest disparity occurring among patients with NASH cirrhosis. Improved efforts at HCC screening and surveillance are needed, particularly among Hispanic patients, a growing population with a high prevalence of NAFLD.



	Odds ratio	95%CI	*P*-Value
Uninsured (*vs* Medicare/commercial)	0.43	0.33-0.55	< 0.001
Medicaid (*vs* Medicare/commercial)	0.85	0.74-0.97	0.018
2013-2014 (*vs* 2004-2006)	1.28	1.08-1.52	0.004
2011-2012 (*vs* 2004-2006)	1.12	0.94-1.32	0.208
2007-2008 (*vs* 2004-2006)	0.85	0.71-1.01	0.072
Age 65+ (*vs* < 50)	0.94	0.76-1.17	0.573
Age 50-64 (*vs* < 50)	1.22	0.99-1.50	0.063
Male (*vs* Female)	0.79	0.68-0.91	0.001
Married (*vs* Single)	1.16	1.02-1.31	0.025

Figure 2 Multivariate logistic regression model evaluating predictors of localized stage hepatocellular carcinoma at diagnosis. These data specifically evaluate the independent risk of having localized cancer stage hepatocellular carcinoma (HCC) at diagnosis compared to advanced cancer stage when evaluated by insurance status at time of diagnosis, the year of HCC diagnosis, the age at time of HCC diagnosis, sex, and marital status. An odds ratio of > 1.00 indicates increased likelihood of having localized cancer stage HCC at time of diagnosis.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is a leading cause of morbidity and mortality worldwide. The Hispanic population represents a major contributor of HCC prevalence, particularly in the United States. Understanding HCC epidemiology and disparities in HCC outcomes among this cohort will help guide interventions to improve HCC care.

Research motivation

Given the significant burden of HCC among the Hispanic population, understanding HCC epidemiology and outcomes among this group is critical. Data gathered from studying HCC epidemiology can help identify potential areas where quality improvement programs can be developed and implemented to improve management of HCC.

Research objectives

The main objective of this study was to evaluate disparities in cancer stage at diagnosis among Hispanic HCC patients.

Research methods

The current study utilized a large United States national-based cancer registry. We utilized a retrospective observational cohort study design to evaluate HCC epidemiology and outcomes among Hispanic adults diagnosed with HCC from 2004 to 2014.

Research results

We identified that over 60% of Hispanic HCC patients were diagnosed with advanced cancer stage that was beyond eligibility for liver transplantation. This highlights an important disparity and may reflect suboptimal utilization of timely HCC screening and surveillance among this population.

Research conclusions

These findings may suggest that the Hispanic population at risk for HCC may experience suboptimal receipt of or delays in timely HCC screening and surveillance. These study findings further add to the existing literature highlighting the poor adherence to HCC screening and surveillance among at-risk populations. Specifically, our study identified a high-risk group in the Hispanic population, which is particularly concerning given the higher risk of nonalcoholic fatty liver disease in this population, a disease that is increasing in prevalence.

Research perspectives

Our study findings emphasize the importance of timely and consistent implementation of HCC screening and surveillance that will translate into improved early HCC detection. This will ultimately improve treatment options for curative intent and thus improve long-term survival outcomes among HCC patients.

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