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REVIEW

Hepatitis E in immunocompromised individuals

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Abstract

Hepatitis E virus (HEV) originally identified as a cause of acute icteric hepatitis in developing countries has grown to be a cause of zoonotic viral hepatitis in developed countries such as the United States. While there are eight identified genotypes to date, genotype 1 (HEV1), HEV2, HEV3, HEV4 are the most common to infect humans. HEV1 and HEV2 are most common in developing countries including Latina America, Africa and Asia, and are commonly transmitted through contaminated water supplies leading to regional outbreaks. In contrast HEV3 and HEV4 circulate freely in many mammalian animals and can lead to occasional transmission to humans through fecal contamination or consumption of undercooked meat. The incidence and prevalence of HEV in the United States is undetermined given the absence of FDA approved serological assays and the lack of commercially available testing. In majority of cases, HEV infection is a self-limiting hepatitis requiring only symptomatic treatment. However, this is not the case in immunocompromised individuals, including those that have undergone solid organ or stem cell transplantation. In this subset of patients, chronic infection can be life threatening as hepatic insult can lead to inflammation and fibrosis with subsequent cirrhosis and death. The need for re-transplantation as a result of post-transplant hepatitis is of great concern. In addition, there have been many reported incidents of extrahepatic manifestations, for which the exact mechanisms remain to be elucidated. The cornerstone of treatment in immunocompromised solid organ transplant recipients is reduction of immunosuppressive therapies, while attempting to minimize the risk of organ rejection. Subsequent treatment options include ribavirin, and pegylated interferon alpha in those who have demonstrated ribavirin resistance. Further investigation assessing safety and efficacy of anti-viral therapy is imperative given the rising global health burden. Given this concern, vaccination has been approved in China with other investigations underway throughout the world. In this review we introduce the epidemiology, diagnosis, clinical manifestations, and treatment of HEV, with emphasis on immunocompromised individuals in the United States.

Key Words: Hepatitis E; Hepatitis E virus; Chronic hepatitis; Acute hepatitis; Immunocompromised; Liver transplant

Core Tip: Hepatitis E Virus is a leading cause of acute icteric hepatitis in developing countries. Despite being self- limiting in most cases, immunocompromised individuals are at a risk of chronic hepatitis, which can be life threatening. Hallmark of treatment includes reduction of immunosuppressive therapies followed by possible need of anti-viral therapy, which has shown to be ineffective.

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INTRODUCTION

Hepatitis E Virus (HEV) was first reported as a non-A, non-B hepatitis in 1980 (another distinct type from post transfusion non-A, non B hepatitis), causing epidemic water-borne acute hepatitis[1]. Although significant improvements have occurred regarding virology, epidemiology, diagnosis, prevention, and treatment of hepatitis E, it is still the leading cause of acute icteric hepatitis in developing countries[2]. Previously, HEV was known as epidemic viral hepatitis in endemic areas. Currently, it is also identified as a zoonotic viral hepatitis in developed countries[3]. Blood transfusions and tissue transplantation are recognized as new routes for virus transmission worldwide[4].

HEV can cause a wide range of clinical manifestations, including acute hepatitis that can be self-resolving, chronic hepatitis (mostly in immunocompromised patients), and extrahepatic manifestations including renal and neurologic symptoms and complications[3]. Despite the development of several serological tests, screening and diagnosis of HEV is still challenging. In most cases, HEV infection is a self-limiting disease despite any treatment. Treatment of acute HEV at times can be imperative, especially in immunocompromised patients, as it can decrease the risk of chronic hepatitis, cirrhosis, and subsequently death[5]. A potentially effective vaccination strategy has been developed for HEV prevention, altering the incidence in Asian countries[6]. The World Health Organization (WHO) recommends consideration of vaccination for high-risk patients such as pregnant women[7]. In this review we summarize the epidemiology of Hepatitis E in the United States, review the clinical manifestations and treatment options with emphasis on their implications in immunocompromised individuals.

EPIDEMIOLOGY

HEV is one of the leading causes of viral-induced acute liver failure worldwide[8]. HEV1, HEV2, HEV3, and HEV4 are the main genotypes which have clinical implications on humans. Genotypes 1 and 2 cause infection in hyperendemic areas such as Asia, Africa, Mexico, and the Middle East (Table 1). Humans are the main reservoir for these genotypes and contamination of drinking water supplies with human feces is the main route of transmission. Consequently, endemics can emerge after heavy rainfall and flooding[9,10]. Transmission through blood transfusion[11] and vertical transmission[12] are well documented for sporadic infection worldwide.

HEV3 and HEV4 are the most prevalent genotypes in industrialized countries[13]. In contrast to HEV1 and HEV2, genotypes 3 and 4 can infect both humans and animals. Pigs, wild boars, and deer are identified as the reservoirs for these genotypes[14]. Transmission by consuming raw or undercooked meat, or close contact with the infected animal is responsible for autochthonous infection[15,16]. There is only one case report of HEV7 infection in humans who regularly consumed camel meat and milk in the United Arab Emirates[17].

Epidemiology of HEV in the United States

The precise incidence and prevalence of HEV infection in the United States is undetermined. HEV is not amongst the nationally notifiable diseases leaving systematic collection, analysis, and evaluation of HEV data a challenge. The absence of sensitive and specific FDA-approved serology assays poses another obstacle in assessing the incidence of HEV in the United States[18]. The lack of commercially available tests also leads to misdiagnosed HEV infection at alarming rates. Reviewing several national drug-

Table 1 Hepatitis E epidemiology according to genotypes

	HEV1, HEV2	HEV3, HEV4
Geography	Developing countries (Asia, Africa, and South America)	Developed countries (Europe, United states, Japan, and Hong Kong)
Disease pattern	Endemic	Sporadic
Seasonal pattern	Yes	No
Reservoir	Only human	Animals (Pigs, wild boars, deer)
Transmission	Fecal-oral	Food-born, blood products, transplantation
Age	More common among young adult	More common among older adults
Risk factor	Chronic liver disease, pregnancy	Chronic liver disease, immunocompromised
Safety measure	Clean water, sanitation, and hygiene	Avoid contact with high-risk animals, cook meat adequately
Chronic infection	Not reported	In immunocompromised patients.

HEV1: Hepatitis E virus genotype 1; HEV2: Hepatitis E virus genotype 2; HEV3: Hepatitis E virus genotype 3; HEV4: Hepatitis E virus genotype 4.

induced liver injuries (DILI) registries revealed HEV infection as the true cause of liver injury in patients initially diagnosed with DILI[19].

National Inpatient Sample (NIS) data from Healthcare Cost and Utilization Project showed the rate of hospitalization due to hepatitis E increased from 3.7 per 10 million in 2010 to 6.4 per 10 million in 2015. Although hospitalization is still low in the United States, the increasing rate is worrisome[20]. The National Health and Nutrition Examination Survey (NHANES) data also demonstrated an increase in HEV seropositivity (IgG/IgM) from 5% in 2013-2014 to 7.7% in 2015-2016. Simultaneously, the rate of IgM seropositivity (recent infection) almost doubled in US-born individuals[21]. The multivariate logistic regression model identified a strong association of HEV seropositivity with aging, female gender, and non-Hispanic Asian ethnicities[21]. Testing serum samples from 681 adult Americans with acute liver failure (ALF) revealed a low rate of acute HEV infection (0.04%) in this population. However, the rate of positive anti-HEV IgG (signifying prior exposure) was significantly higher in the ALF patients than in the general US population[22].

HEV TRANSMISSION

All autochthonous HEV infections detected in the United States are caused by HEV genotype 3. Caitlin reported the risk of anti-HEV seropositivity in people who consumed undercooked meat was 12.9 times higher than the general population. This observation confirmed undercooked meat as a route of zoonotic HEV infection in the US[23]. In one study, serum samples from pigs at 25 slaughterhouses in 10 states were tested for HEV infection. HEV RNA and anti-HEV seropositivity was 6.3% and 40%, respectively. Blood of HEV RNA-positive pigs potentially can contaminate slaughterhouses' supply chains, making it a key source of infection control[24]. A recent study suggested consuming self-grown food as another possible source for zoonotic HEV infection[25].

Ticehurst *et al*[26] reported the possible HEV transmission through blood transfusion for the first time in the United States. A random sample from 5040 blood donations showed 11.4% and 1.8% positive anti-HEV IgG and anti-HEV IgM, respectively[27]. Stramer *et al*[28] reported two positive HEV RNA among 18829 samples of blood donated from six geographic regions. Despite low contamination rates, they suggested providing HEV-negative blood for patients at risk of developing hepatitis, such as severely immunosuppressed patients. Among 128,020 samples of plasma from 27 states, the prevalence of HEV RNA positivity was reported at 0.002%. Therefore, routine screening for HEV contamination in plasma donation was not suggested[29]. Several countries are considering HEV screening in blood donors. Delage *et al*[30]evaluated cost-benefit and the quantitative risk of blood donation screening for HEV infection in the United States. Due to the lower rate of HEV in North America, HEV blood donation screening will be more expensive than in other countries, and have minimal clinical benefits.

HEV IN IMMUNOCOMPROMISED

For the first time in the US, Kuniholm *et al*[31] reported a chronic HEV infection in an HIV-positive patient. They also confirmed that chronic infection could persevere even with a CD4+ count > 200 cells/mm^3[31]. Assessing 311 patients who received allografts revealed 4% posttransplant HEV

infection. Although no chronic infection was reported, developing posttransplant infection was associated with graft rejection[32]. A recent study on 145 post-liver transplant patients with a history of hepatitis C virus (HCV) infection showed 6 (4.1%) patients developing anti-HEV IgM antibodies in 5 years. All samples were negative for HEV RNA. Treatment of HCV with Interferon and Ribavirin may contribute in clearance of HEV infection[33].

CLINICAL MANIFESTATIONS

Acute icteric hepatitis

The majority of acute HEV infections are asymptomatic or can cause minor nonspecific systemic illness, that is often self-limiting. It has been estimated that approximately 5-30% of patients acutely infected go on to develop acute icteric hepatitis[34]. Acute icteric hepatitis is characterized by malaise, fever, body aches, anorexia, nausea and vomiting, which occurs for about a one-week period of time classified as the prodromal phase. Following the prodromal phase, patients enter the icteric phase characterized by jaundice and dark urine, which can be coupled with a marked increase in aminotransferases (greater than 8-10 time the upper limit of normal) and a variable degree of hyperbilirubinemia[35]. These symptoms collectively resolve over the course of a few days to weeks, marking the convalescent phase.

In a small percentage of patients, the acute icteric phase can progress to acute liver failure (ALF) or acute on chronic liver failure (ACLF) in those with underlying chronic liver disease[36]. Pregnant women are of particular risk to developing ALF during their second and third trimester, with mortality rate of nearly 25% as a result of hepatic failure or obstetric complications[37]. ACLF is defined by the European Association for the Study of Liver Diseases (EASL) as acute deterioration of pre-existing chronic liver disease usually related to a precipitating event and is associated with increased 28-day mortality due to multi-system organ failure[38]. Typical manifestations include acute worsening of liver function with complications such as worsening ascites, hepatic encephalopathy or coagulopathy[36]. The impact of acute HEV infection in patients with chronic liver disease in the United States has been reported. In a study conducted by Kyvernitakis *et al*[39], 11% of 115 patients with chronic HCV infection diagnosed with cancer were positive for HEV IgG. Seropositivity was significantly associated with older age, place of birth outside the United States, cirrhosis, and history of reused needles/syringes during vaccination[39]. In another study, HEV related ALF was assessed in 681 adults with ALF by testing for anti HEV IgM, IgG and HEV-RNA. A total of three men demonstrated repeatedly detectable anti HEV IgM, but negative HEV RNA, signifying rarity of acute HEV infection in ALF patients (0.4%). 43.4% of ALF patients tested positive for anti HEV IgG, with prevalence being highest from the Midwest and in those of older age[22]. There has also been documentation of a fatal hepatic decompensation caused by HEV4 in an orthotopic liver transplant recipient following a prolonged visit to Hong Kong[40]. In another prospective study in the United States, HEV infection was noted to contribute to a small but important percentage of cases of acute liver injury that was initially suspected to be caused by drug induced liver injury[41].

Chronic HEV in immunocompromised individuals

Chronic HEV infection in solid organ transplant (SOT) recipients can be defined as HEV replication (viremia) present for more than 3 mo after the onset of infection[42]. Chronic infection was initially reported by Kamar *et al*[43] in 2008, when patients who received kidney or liver transplants developed a persistent increase in aminotransferase levels, evidence of histological activity, and liver fibrosis during follow-up after acute HEV. It has been suggested that up to 66% of SOT recipients exposed to HEV go on to develop chronic infection, which is mostly asymptomatic but can most commonly include fatigue and or mild to moderate aminotransferase rise, diarrhea and arthralgias[44]. Chronic infection has been most commonly reported with HEV3 infection[45] however, there have been reports of persistent hepatitis when infected with HEV4[46].

Prevalence of post liver transplant HEV infection in non-endemic regions has been estimated to be between 1% and 2%[47]. Chronic HEV infection has been shown to cause structural injury to the liver including formation of nodules, fibrotic changes and subsequent cirrhosis[48], with reports that approximately 10% of those who develop chronic infection progress to cirrhosis within 2-5 years[49]. Injury caused by viral infection including inflammation has been shown to regress following the clearance of HEV[50]. In persons with prior liver transplantation, chronic infection can result in post-transplant hepatitis, rapid progression to cirrhosis and liver failure, and even the need for re-transplantation which can lead to recurrence of HEV infection in the newly transplanted liver[51].

The effects of chronic HEV can be seen beyond those with SOT, affecting various immunocompromised individuals. Chronic infection has been reported in an individual with non-Hodgkin's lymphoma undergoing treatment[52], and in stem cell transplant recipients on immunosuppression [53]. International studies have demonstrated significantly greater seroprevalence of IgG and IgM antibodies in cancer patients[54], and reported self-resolving acute infection, and even the need for ribavirin treatment in patients with gynecological malignancies treated with chemotherapy[55]. Such findings should spark further investigation when treating cancer patients with elevated transaminases.

Chronic infection has also been seen in patients with human immunodeficiency virus with low CD4+ cell count of less than 200[56,57]. Rheumatological patients receiving mild immunosuppressive treatments are also at increased risk of chronic infection[58].

Extrahepatic manifestations

Infection with HEV can lead to a variety of extrahepatic manifestations including neurological, hematological, renal, and other immune-mediated manifestations. The exact mechanism remains to be elucidated, and suggestions include cross reactions between viral epitopes and self-antigens in tissues, and possible viral replication in other non-hepatic tissues[59].

Neurological manifestations are the most commonly encountered, and include Guillain- Barré syndrome (GBS), neuralgic amyotrophy (NA), encephalitis, myelitis, myositis, vestibular neuritis, peripheral neuritis, and Bell's palsy[60]. In a European study, 16.5% of HEV infected patients reported neurological manifestation, which were more common in immunocompetent patients compared to immunosuppressed individuals (22.6% *vs* 3.2%, $P < 0.001$)[61]. GBS can occur both after acute or chronic infection with various HEV genotypes and is the most frequently described extrahepatic manifestation [62]. In a case- control study from the Netherlands comparing GBS patients to healthy controls, the prevalence acute HEV was higher in GBS patients compared to controls (5% *vs* 0.5%)[63]. Similar manifestations were seen in a study from the United Kingdom and France, in which more than 5% of those infected with HEV3 developed neurological complications during follow-up[64]. NA is an acute and painful neuropathy in the upper extremity characterized by rapid multifocal motor weakness and sensory loss, followed by atrophy[65]. A cohort study from the United Kingdom and the Netherlands demonstrated that 10% of patients with NA had acute hepatitis E[66]. Central nervous system infections including encephalitis and meningitis have been described, with HEV RNA being present in the serum and cerebrospinal fluid in immunosuppressed individuals after SOT[62]. It remains unknown if these neurological manifestations are a result of immune mediated molecular mimicry or direct cytopathic effects of the virus[36]. Based on findings from a variety of studies, it is recommended that clinicians consider infection with HEV as a culprit when encountering patients with neurological disorders and concomitant elevations in liver enzymes[67].

Renal manifestations of HEV include kidney injury, membranoproliferative glomerulonephritis and cryoglobulinemia. In a retrospective study assessing kidney function and histology in SOT recipients with HEV3 infection, there was a statistically significant decrease in glomerular filtration rate during infection (-5L/min, $P = 0.04$). Histological examination of those with high proteinuria and decreased GFR during both the acute and chronic phase of infection demonstrated relapse of IgA nephropathy, membranoproliferative glomerulonephritis, and the majority of patients having cryoglobulinemia that resolved after clearance of HEV[68]. The relationship between cryoglobulinemia and HEV infection remains unclear. In a study assessing SOT recipients infected with HEV, the prevalence of cryoglobulinemia was increased during chronic infection (52.9%) compared to the acute phase of infection (36.4%) and HEV negative SOT recipients (23.6%, $P < 0.01$); also identifying HEV as a predictive factor for cryoglobulinemia (odds ratio 2.3)[69]. Although the exact mechanism is unknown, it is possible that immune complex deposits may play a critical role, as seen in Hepatitis C infection where HCV antigen, anti-HCV IgG antibodies and rheumatoid factor deposit in glomeruli[70]

Over the years many hematological manifestations from HEV infection have been reported. One such manifestation is hemolytic anemia secondary to glucose-6-phosphate dehydrogenase deficiency, leading to oxidative stress in red blood cells during viral hepatitis infection. Several cases have been documented, and there have also been reports of renal failure secondary to renal tubule obstruction by hemo -globin and bilirubin during hemolysis incited by acute HEV[71]. As in other hepatotropic viruses such as cytomegalovirus, hepatitis A virus and hepatitis B virus; there have been reports of autoimmune hemolytic anemia secondary to infection with hepatitis E[72]. Hepatitis associated aplastic anemia, is a life-threatening variant of aplastic anemia in which pancytopenia occurs two to three months after hepatitis[73]. Cases of HEV related aplastic anemia have been reported, leading to recovery following treatment, and even death[74,75]. A variety of different mechanisms leading to thrombocytopenia secondary to hepatotropic viruses have been postulated including bone marrow suppression and development of anti-platelet antibodies and platelet associated immune complexes[76]. There have been several reports of HEV causing thrombocytopenia which were either self-limited or required transfusion or intravenous globulin and corticosteroid administration[77,78]. Several extrahepatic manifestations have been documented for which the pathophysiology remains unclear. A complete list of these manifestations across various organs can be seen in (Table 2).

DIAGNOSIS

The incubation period of HEV is approximately 2 to 6 wk and precedes the IgM response detected during the same time that liver enzyme abnormalities arise. Diagnosis of HEV can be accomplished either directly by detecting the HEV RNA or capsid antigen in the blood and other body fluids or indirectly by detecting anti-HEV antibodies in infected individuals' serum[79]. The detection of anti-

Table 2 Extrahepatic manifestations of hepatitis E viral infection

Organ/System	Manifestation
Neurological	Guillain- Barré syndrome, Bell's palsy, myelitis, peripheral neuropathy, neuralgic amyotrophy, encephalitis, meningitis vestibular neuritis, mononeuritis multiplex, seizure, pseudotumor cerebri, oculomotor palsy, polyradiculoneuropathy
Hematological	Thrombocytopenia, hemolytic anemia, aplastic anemia, hemophagocytic syndrome, thrombotic thrombocytopenic purpura, Cutaneous T cell lymphoproliferative disorder, monoclonal gammopathy of uncertain significance
Cardiovascular	Myocarditis, Henoch-Schönlein purpura
Renal	Reduction in glomerular filtration rate, IgA nephropathy, cryoglobulinemia, membranoproliferative glomerulonephritis, membranous glomerulonephritis
Musculoskeletal	Myositis, polyarthritis
Thyroid	Autoimmune thyroiditis, subacute thyroiditis
Pancreas	Acute pancreatitis

HEV IgM antibody is an important marker of acute viral infection, and has a short positivity mostly ranging from 3-4 mo, but can be present for up to one year[36]. When testing for anti-HEV IgM with conventional assays and commercially available immunohistochemistry assays, sensitivity has been reported to be > 97% in immunocompetent patients and 80-85% for immunocompromised patients with > 99.5% specificity[80,81]. It is important to consider additional testing for RNA presence in immunocompromised individuals due to the poor antibody response exhibited by this population[13]. IgG antibody response is delayed and long lasting with persistence of several years, although the exact duration remains uncertain. In order to detect these antibodies, enzyme immunoassays are utilized with recombinant ORF2 and/or ORF3 proteins from HEV1 strains, which also cross react with other genotypes, however assay detection varies considerably[82]. Use of commercially available assays have limited detection which vary between 0.25 and 2.5 WHO units per ml, and the determination of anti-HEV IgG concentration can be used to estimate reinfection after natural infection or immunization[79]. It has been suggested that immunocompromised patients with anti-HEV IgG concentration < 7 WHO units per ml can become reinfected with increased risk of developing chronic hepatitis[83]. In addition, it has been suggested that anti-HEV IgG titers > 2.5 units per ml are protective following vaccination [84].

The detection and quantification of HEV RNA in blood and other bodily secretions is the gold standard of detecting both acute and chronic active HEV infection, adding benefit to diagnosis of infection in immunocompromised individuals with inherent poor immunologic response[36] (Figure 1). Other situations in which RNA detection is of great utility includes donor screening, diagnosis of chronic HEV infection, and assessing response to antiviral therapy[85]. HEV RNA becomes detectable during the incubation period and can be present in the blood for about 4 wk and 6 wk in feces[81]. Given the narrow window of detectable RNA, an undetectable HEV RNA does not exclude recent infection, particularly when patients present late in their illness[86]. Persistence of RNA for at least 3 mo defines chronic infection[42]. Available types of nucleic acid amplification tests (NAATs) include reverse transcription polymerase chain reaction (RT-PCR), real time RT-PCR, and reverse transcription loop-mediated isothermal amplification, with varying sensitivity in HEV RNA detection[36]. In response to varying sensitivities the World Health Organization (WHO) has developed the international standard and international reference panel for HEV1, HEV2, HEV3, and HEV4, allowing comparison of results obtained from different NAATs with reports using a common unit, the international unit (IU). NAATs detect HEV RNA targets, particularly conserved domains (ORF2 and ORF3 overlap region), of HEV genotypes 1-4[87].

Viral antigens are present in the blood and liver during the early phase of acute hepatitis persisting longer in chronic infection and can be diagnosed using sandwich enzyme immunoassays detecting HEV capsid antigen derived from ORF2[88]. HEV antigen assays have excellent specificity, however sensitivity is a major concern ranging from 40% to 91%[89]. It has been shown that HEV antigen may remain present for months following clearance of chronic HEV infection, suggesting the presence of antigen does not necessarily indicate presence of virions[90]. Given the simplicity, lesser cost and faster results when compared to HEV RNA detection, HEV capsid testing may become an alternative in diagnosis, however the role of HEV antigen diagnosis is yet to be determined[36,79].

TREATMENT

Unlike in most immunocompetent individuals who require no specific treatment for acute HEV infection, chronic infection in immunocompromised hosts (*i.e.*, solid organ transplant recipients) requires treatment to avoid rapid progression to cirrhosis or even death[5]. In SOT recipients, reduction

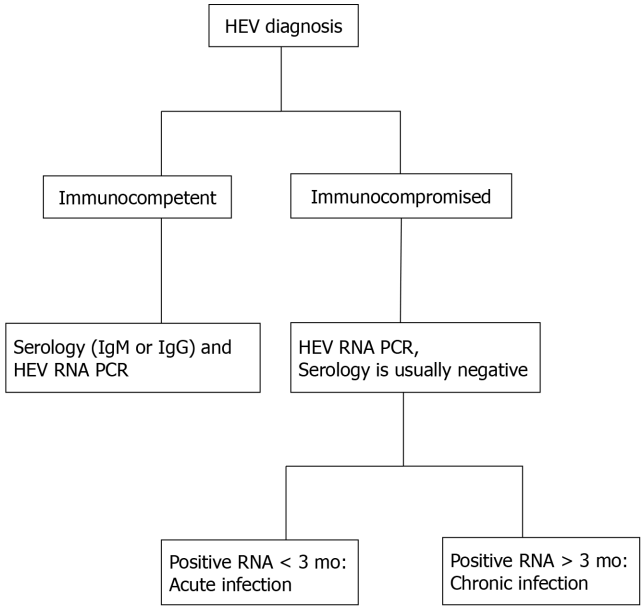

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Figure 1 Diagnosis of hepatitis E virus in immunocompetent *vs* immunocompromised patients. HEV: Hepatitis E virus; PCR: Polymerase chain reaction.

of immunosuppressive therapies is considered the first line therapeutic option, with approximately one third of patients achieving viral clearance after dose reduction[44,50]. However, it is important to remember that reducing immunosuppression can lead to increased risk of organ rejection.

In a large retrospective multicenter case series, Kamar *et al*[42] assessed the efficacy of ribavirin in SOT recipients diagnosed with chronic hepatitis E and HEV viremia. A total of 59 (54 confirmed HEV genotype 3) patients were included of which 37 had received kidney transplants, 10 had liver transplants, 5 heart transplants, 5 combined kidney and pancreas transplants, and 2 patients had undergone lung transplantation. Median dosing of ribavirin was 600 mg/day for a median duration of 3 mo. Following treatment 95% of patients exhibited clearance of HEV and 78% exhibited sustained virological response (SVR). Although 60% of patients unfortunately developed recurrence, 40% of these individuals were able reach SVR following a prolonged treatment course of an additional 6 mo. Adverse events included anemia, requiring dose reduction in 29% of patients, and the use of erythropoietin and blood transfusion[91]. A more recent study conducted by Kamar *et al*[42], retrospectively investigated 30 European centers to assess outcomes of ribavirin therapy in 255 SOT recipients with chronic HEV3. 81% of patients achieved SVR with initial ribavirin treatment (median 600 mg/day for 3 mo), while 90% were able to achieve SVR following an additional course of treatment after initially failing to meet SVR. Interestingly it was also noted that an increased lymphocyte count at the initiation of treatment was a positive predictive factor of SVR, while poor hematological tolerance requiring dose reduction was associated with relapse after completion of therapy[92].

Treatment of chronic HEV in immunosuppressed individuals who have received SOT poses a challenge following lack of response to ribavirin. A final option includes treatment with pegylated interferon alpha (PEG-IFNa), which has been shown to be effective following liver transplantation. In a study of three post liver transplant patients, a three-month course of PEG-IFNa resulted in an antiviral response with HEV clearance was obtained in two of the study participants[93]. Similar findings were noted by Haagsma *et al*[94] who demonstrated efficacy of PEG-IFNa when reduction of immunosuppressive medications was not adequate. However, it is important to note that PEG-IFNa is contraindicated in lung, heart, renal and pancreas transplant recipients due to the risk of organ rejection[95].

Treatment of HEV in ribavirin resistant infections can be a challenge. Approval of sofosbuvir revolutionized the treatment of chronic hepatitis C and the role of sofosbuvir in the treatment of HEV has also been investigated. Based on *in vitro* studies, sofosbuvir has been considered as a treatment for ribavirin resistant HEV alone or synergistically with ribavirin[96]. Effectiveness of sofosbuvir has been shown to lead to viral clearance in acute HEV when used in combination with ribavirin[97] and for the treatment of refractory HEV in an individual following kidney transplantation[98]. However, other studies have demonstrated inability to reach SVR when treated with combination therapy in a patient with chronic HEV (genotype 3) following multivisceral organ transplantation[99]. A recent case series of 3 SOT recipients treated with combination of sofosbuvir and ribavirin following failed ribavirin monotherapy (inability to achieve SVR) displayed failure of complete elimination of HEV. RNA plasma levels returned to pretreatment levels following cessation of therapy, suggesting antiviral activity of combination therapy[100]. Monotherapy with sofosbuvir has also been shown to be ineffective with

high rates of relapse following only partial response in individuals with chronic HEV[101]. To date none of the mentioned drugs have been approved in the treatment of HEV, and further large-scale studies are indicated to assess safety and efficacy, alone or in combination. Although many clinical trials are actively investigating efficacy of vaccine prevention, there is limited investigation on HEV treatment (clinicaltrials.gov).

VACCINE

Development of a safe and efficacious vaccine has shined light on the prevention of HEV and subsequent worldwide morbidity and mortality. Zhu *et al*[102] published results of a randomized, double blind phase 3 trial of recombinant HEV vaccine (HEV 239: Hecolin®) administered in 3 doses at 0,1 and 6 mo in China. Results demonstrated a near 100% efficacy, with no serious adverse effects at 12 mo follow-up after vaccine administration[102]. Long term efficacy of up to 4.5 years displayed continuous efficacy of 87%, and cross protective efficacy between genotype HEV1 and HEV4 which are prevalent in China[103]. Currently a large, cluster-randomized, blinded trial (NCT02759991) is investigating the effectiveness of Hecolin in pregnant women in Bangladesh[104]. It has been recommended that vaccination against HEV in certain high-risk individuals such as those who are immunocompromised, have chronic liver disease, pregnant women in endemic areas, and those in hyperendemic parts of the world[81]. Further studies are urgently needed to investigate vaccine efficacy toward other prevalent genotypes and to assess safety and efficacy in those with aforementioned underlying chronic medical conditions prior to being garnered approval beyond China. Recently a single investigation was completed in the United States, assessing Hecolin® safety, reactogenicity and immunogenicity in healthy adults (NCT03827395), for which we eagerly await results.

CONCLUSION

Hepatitis E infection is a major global health burden that leads to extensive morbidity and mortality, particularly in developing countries. While most cases of acute HEV infection are self-limiting and only require symptomatic treatment, progression to chronic disease can be fatal. Individuals particularly at risk for chronic infection include solid organ transplant recipients and those with other immunosuppressive conditions such as HIV and rheumatological conditions. Elevation in liver enzymes in the immunosuppressed should prompt urgent serological testing coupled with HEV RNA detection, given inherent poor immunological response. The initial hallmark to treatment is the reduction of immunosuppressive therapies to allow physiological defense and viral clearance. Subsequent treatment options include ribavirin; however, resistance poses a challenge as other treatment options can be harmful to SOT recipients. While vaccine development has proven to be effective, it is imperative that we continue to assure clean drinking water and safe food practices worldwide. Further clinical investigations are essential in order to help develop safe and efficacious viral treatments that can save millions of lives worldwide.

FOOTNOTES

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MINIREVIEWS

Small duct primary sclerosing cholangitis: A discrete variant or a bridge to large duct disease, a practical review

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Abstract

The natural history, associations with inflammatory bowel disease (IBD), and long-term outcomes of large duct primary sclerosing cholangitis (ldPSC) have been well documented. Small duct primary sclerosing cholangitis (sdPSC) is a much less common and relatively more benign variant. The natural history of sdPSC has been difficult to characterize given the limited number of studies in the literature especially with regards to the subset of patients who progress to large duct involvement. It has been unclear whether sdPSC represented a subset of ldPSC, an earlier staging of ldPSC, or a completely separate and distinct entity of its own. Strong associations between sdPSC and IBD have been established with suspicion that concurrent sdPSC-IBD may be a key prognostic factor in determining which patients are at risk of progression to ldPSC. Little is known regarding the discrete circumstances that predisposes some patients with sdPSC to progress to ldPSC. It has been suspected that progression to large biliary duct involvement subjects this subset of patients to potentially developing life-threatening complications. Here the authors conducted a thorough review of the published sdPSC literature using Pubmed searches and cross-referencing to compile all accessible studies regarding cohorts of sdPSC patients in order better characterize the subset of sdPSC patients who progress to ldPSC and the associated outcomes.

Key Words: Small duct primary sclerosing cholangitis; Inflammatory bowel disease; Progression; Primary sclerosing cholangitis; Outcomes

Core Tip: Strong associations between small duct primary sclerosing cholangitis (sdPSC) and inflammatory bowel disease (IBD) have been established with suspicion that concurrent sdPSC-IBD may be a key prognostic factor in determining which patients are at risk of progression to ldPSC.

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INTRODUCTION

Primary sclerosing cholangitis (PSC) is a chronic liver disease with the potential of progression to cirrhosis that is characterized by multi-focal cholestatic inflammation and fibrosis[1-3]. PSC has an incidence of 0.9 to 1.3 cases per 100000 in the United States[2,4]. PSC has a close association with inflammatory bowel disease (IBD) and has a risk of developing various hepatobiliary malignancies including cholangiocarcinoma (CCA)[1-3]. Classic or large-duct primary sclerosing cholangitis (ldPSC) has very distinct clinical, cholangiographic, and histologic features with cholangiography typically establishing a diagnosis[1,3-5]. In 1985, Ludwig *et al*[6] brought into question the possibility of small intra-hepatic biliary duct involvement which led to pathologic studies in 1991 confirming the diagnosis of small duct primary sclerosing cholangitis (sdPSC), also referred to as pericholangitis.

The natural history, associations with IBD, and long-term outcomes of ldPSC have been well documented. Small duct primary sclerosing cholangitis is a much less common and relatively more benign variant[7-9]. In recent years, it has been discovered that this variant can rarely progress to having large biliary duct involvement[7-14]. Several studies have attempted to characterize this unique subset of patients, with the rate of progression to ldPSC ranging from 7.1%-22.9%[7-9,11-14]. Little is known regarding the etiology or discrete circumstances that predisposes some patients with sdPSC to progress to ldPSC. It is known, however, that progression to large biliary duct involvement subjects this subset of patients to potentially developing life-threatening complications[8,9].

The natural history of sdPSC has been difficult to characterize given the limited number of studies in the literature. Describing the subset of patients who have progressed to ldPSC is even more challenging. The authors conducted a thorough evaluation of the published literature to compile all accessible studies regarding cohorts of sdPSC patients using PubMed searches and cross-referencing. Table 1 summarizes the individual studies, the baseline characteristics, and outcomes of each cohort of sdPSC patients.

GENETIC PATHOGENESIS

The etiology of PSC is not well understood however it is believed to be predominantly autoimmune due to its association with elevated levels of antineutrophilic cytoplasmic, antinuclear, and anticardiolipin antibodies in addition to the HLA DR3 and HLA B8 genes[2,4,15]. A strong association between PSC and IBD has also been well established with studies showing a significantly increased risk of developing PSC and UC in first-degree relatives of patients who have PSC with or without UC[3,4,16,17].

The etiology of sdPSC is even less understood, though it carries a more favorable prognosis than its large-duct counterpart[8]. It has been unclear whether sdPSC represented a subset of ldPSC, an earlier staging of ldPSC, or a completely separate and distinct entity of its own[8,10]. A study evaluating the components of sdPSC within the subset of patients with and without concomitant IBD suggested the strongest association existed between HLD-DRB1*13:01 and sdPSC[15]. In contrast to the strong association of HLA-B*08 with ldPSC, HLA-B*08 was found to be more prevalent in sdPSC when compared to healthy controls, but not to the extent found in ldPSC[15]. Additionally, patients that have the DRB1*13:01 haplotype are at an increased risk of developing IBD[15]. A noteworthy hypothesis drawn from this study is the notion that patients with sdPSC and concomitant IBD could represent precursors to classic PSC while those sdPSC patients without IBD may actually represent a different biliary disease process, such as primary biliary cholangitis, or a secondary cause of sclerosing cholangitis, such as those related to the ABCB4 gene[15].

ENVIRONMENTAL PATHOGENESIS

It has been speculated that in addition to genetic factors, environmental factors contribute to the

Table 1 Baseline characteristics and outcomes of small duct primary sclerosing cholangitis cohorts

Ref.	Study design, population	Study location	Total sdPSC	Females	Age at Dx (yr)[1]	F/U (mo)[1]	UC	CD	HCC	CCA	AIH	LdPSC conversion	Conversion time (mo)[1]	Treatment			Liver transplant	Death
														UDCA	Steroids	AZA		
Wee *et al*[31], 1984	Retrospective, Adult	Mayo Clinic	3	1	34	-	3	0	0	3	0	0	-	-	-	-	0	3
Broomé *et al*[11], 2002	Retrospective, Adult	Sweden	32	12	39	63	13	3	1	0	0	4	115	7	7	6	1	0
Björnsson *et al*[7], 2002	Retrospective, Adult	Oslo/Oxford	33	14	38	106	20	7	0	0	0	4	-	7	-	-	2	2
Angulo *et al*[9], 2002	Longitudinal cohort, Adult	Mayo Clinic	18	7	39	126	14	3	0	0	0	3	122	7	0	0	2	1
Nikolaidis *et al*[39], 2005	Retrospective, Adult	Greece	6	-	32	26	2	1	0	0	0	0	-	5	0	5	0	0
Charatcharoenwitthaya *et al*[12], 2007	Longitudinal cohort, Adult	Mayo Clinic	42	14	35	57	13	3	0	0	0	3	-	30	-	5	6	1
Miloh *et al*[32], 2009	Retrospective, Pediatric	New York	16	5	10	78	4	4	0	0	5	0	-	16	-	-	2	0
Olsson *et al*[28], 2009	Retrospective, Adult	Sweden	7	3	24	71	4	0	0	0	7	0	-	-	6	6	0	0
Singal *et al*[10], 2011	Retrospective, Adult	New York/Florida	25	12	37	39	13	0	0	0	0	0	-	15	8	12	1	1
Fevery *et al*[17], 2016	Retrospective, Adult	Belgium	33	-	35	144	3	9	0	0	0	0	-	-	-	-	-	-
Valentino *et al*[13], 2016	Longitudinal cohort, Ped	Boston	24	-	10	44	-	-	-	-	-	6	72	-	-	-	2	2
Liu *et al*[40], 2017	Retrospective, Adult	Australia	10	-	41	96	-	-	0	0	-	-	-	-	-	-	-	-
Deneau *et al*[27], 2017	Retrospective, Pediatric	Multi-institutional	98	34	10.5	-	-	-	-	-	36	-	-	75	-	-	-	-
Weismüller *et al*[30], 2017	Retrospective, Adult	Multi-institutional	254	96	37	-	67	24	0	0	-	-	-	-	-	-	-	-
Umetsu *et al*[41], 2019	Retrospective, Pediatric	Japan	3	-	9	66	-	-	0	0	-	-	-	-	-	-	0	0
Ringe *et al*[14], 2020	Retrospective, Adult	Germany	16	7	29	127	6	4	-	-	1	5	144	16	-	-	-	-

[1]Indicates an average.

AIH: Autoimmune hepatitis; AZA: Azathioprine; CCA: Cholangiocarcinoma; CD: Crohn's Disease; Conversion time (average time from sdPSC diagnosis to ldPSC conversion); F/U: Follow-up (months after initial diagnosis); HCC: Hepatocellular carcinoma; LdPSC: Large duct primary sclerosing cholangitis; SdPSC: Small duct primary sclerosing cholangitis; UC: Ulcerative colitis; UDCA: Ursodeoxycholic acid; -: Unknown.

pathogenesis of PSC in part due to persistent insult to the cholangiocytes[2-4]. More recent studies suggest the involvement of the gastrointestinal microbiome and its metabolites as an important and modifiable component of the pathogenesis of PSC[3,4]. The relationship of PSC with the enteric microbiome, known as the "leaky gut" hypothesis, describes the passive translocation of bacterial products from an inflamed gut to the portal venous system triggering an inflammatory cascade that leads to the characteristic "onion skinning" intrahepatic biliary duct fibrosis that is seen in all variants of PSC[2,4,18,19]. The development of the laminar concentric fibrosis interrupts the arterial and biliary interface causing ischemia to the cells lining the biliary system[4]. Injured cholangiocytes facilitate the pathogenic strictures and fibrosis through the secretion of inflammatory cytokines and chemokines[2,4]. Other theories exist focused on defects in the protective mechanisms against toxicity from bile acids, gut-derived T cell recruitment to the liver, and even disruptions in bile homeostasis as potential key factors in PSC pathogenesis[2,4].

Based on initial investigation of the pathophysiologic association of hepatobiliary disorders and colonic inflammation, the role of bacterial chemotactic peptides in the development of sdPSC has been evaluated[20,21]. Colitis was induced in the specimens using intrarectal infusions of acetic acid and saline, followed by intrarectal infusion of N-formyl L-methionine L-leucine L-tyrosine (fMLT), a bacterial chemotactic peptide produced by *Escherichia coli*. The experimentally induced colitis and rectal fMLT induction resulted in an eight-fold increase in biliary excretion of fMLT. Liver specimens showed evidence of pericholangitis affecting the small biliary ducts suggesting bacterial chemotactic peptides could play a pathogenic role in the development of sdPSC[20].

EPIDEMIOLOGY AND NATURAL HISTORY

Approximately 75% of patients with PSC have both small and large duct involvement, while 15% have only small duct and 10% with only large duct involvement[3,8]. PSC often insidiously progresses to advanced liver disease with an estimated 10-year survival of 65%[2,3]. LdPSC affects men twice as frequently as women and typically presents within the fourth decade of life with the mean age of diagnosis being 41 years[3]. However, a study in Norway suggested that PSC may occur as commonly in females as in males but with a more clinically subtle course[5]. The incidence per year is estimated to be 0.9-1.3 per 100,000 and the prevalence is approximately 0.5-16.2 per 100,000 patients in the United States[4,5]. Studies in Asia and Spain have reported a lower prevalence of up to 10-fold when compared to the US and EU[22-24]. Some studies suggest an increase in the incidence of PSC in recent decades though this trend has also been associated with other autoimmune and idiopathic inflammatory disorders and could be related to increased use of magnetic resonance cholangiography[3]. Approximately 70% of patients with PSC have concurrent IBD with UC accounting for 80% of PSC-IBD patients, while CD and intermediate colitis affects 10% each[2,3,5]. Hepatobiliary malignancies affect up to 10.9%

of PSC patients with a five-fold increased risk of colorectal cancer when compared to IBD patients without PSC[3,25].

SdPSC is more benign when compared to ldPSC with most mortality limited to the small subset that progress to large-duct involvement or who develop liver failure[8]. Studies have shown a median survival of 29.5 years in sdPSC *vs* only 17 years in ldPSC without liver transplantation[8,10,23,26]. SdPSC seems to have a similar predilection for male gender however percentages vary across individual studies. Evaluating the data in Table 1 yielded a 60.9% male predominance of sdPSC across all studies of adult and pediatric populations. The annual incidence of sdPSC is estimated to be 0.15 per 100,000 patients and the median age of diagnosis is 35 and 9.5 years in adults and children respectively[8,10,27]. IBD affects approximately 80% of sdPSC patients with the large majority being diagnosed at initial presentation[8,27]. Of the patients with sdPSC and concomitant IBD, approximately 78% have UC, 21% CD, and 1% an intermediate colitis[8,15]. A study describing differences between sdPSC patients with and without IBD reported a mortality of 9% and 7% respectively and transplantation in 6% and 14% respectively[15]. Hepatobiliary malignancies are extremely rare in sdPSC with very few reported cases. One long-term retrospective, multi-institutional study reported approximately one-fourth of patients with sdPSC may show evidence of progression to ldPSC[8].

CLINICAL PRESENTATION

PSC has a highly variable initial presentation with approximately 50% of patients being asymptomatic at presentation and up to 40% of cases being incidentally diagnosed after routine blood work revealing cholestatic elevation of liver enzymes[3,4]. Those who go without early incidental detection can present with the sequelae of advanced liver disease[4]. Patients who develop symptoms at the time of diagnosis typically present with weight loss, jaundice, pruritis, abdominal pain, diarrhea, fever, and fatigue[1]. Patients with sdPSC present with generally similar symptoms though weight loss and jaundice at diagnosis is more significantly seen in ldPSC than in sdPSC[8,10].

DIAGNOSIS

Several factors contribute to the clinical diagnosis of PSC. The first includes a cholestatic elevation in liver biochemical testing, specifically with a significantly higher elevation in serum alkaline phosphatase compared to milder elevations in the serum aminotransferases[1]. Concomitant autoimmune hepatitis may cause more substantial elevations in the serum aminotransferases[1,28]. Second, cholangiographic findings of multifocal intrahepatic, extrahepatic, or a combination of both are typically seen[1]. Lastly, a liver biopsy may be warranted in the appropriate context to exclude other diseases, establish stage of disease, or to diagnose sdPSC[1]. Not all patients will present with a significant elevation in serum alkaline phosphatase so a strong clinical suspicion should warrant further investigation with magnetic resonance cholangiopancreatography (MRCP), endoscopic retrograde cholangiopancreatography (ERCP), or percutaneous transhepatic cholangiography (PTC)[1,5]. Cholangiography is negative in sdPSC due to the involvement of biliary ducts that are less than 100 micro millimeters making liver biopsy necessary to confirm the diagnosis of sdPSC[10]. The subset of sdPSC patients who progress to large-duct involvement will develop the characteristic cholangiographic findings in classic PSC[10,14].

HISTOPATHOLOGIC FEATURES OF SDPSC

Most studies have reported that sdPSC has similar histopathologic features as PSC, albeit with normal imaging findings[10]. As mentioned above, several studies have reported that sdPSC may just be an earlier form of well-developed PSC[8]. Therefore, the histopathologic features may be subtle and easily missed[10]. At our institution, we recently encountered a 35-year-old female that reported intermittent pruritis with previous episodes of jaundice and persistently elevated alkaline phosphatase. An MRCP showed no abnormalities within the biliary tract, sdPSC was suspected, and a liver biopsy was performed. The liver biopsy was evaluated by a board-certified hepatopathologist and showed several portal tracts containing atrophic bile ducts (*i.e.*, evidence of biliary senescence changes). These were subtle by hematoxylin and eosin (H&E) evaluation (Figure 1A); however, additional stains were able to highlight peribiliary sclerosis with focal areas of fibrous bile duct obliteration (Figure 1B). Cytokeratin 7 (Figure 1C) and copper stains (Figure 1D) were helpful to confirm the presence of chronic biliary injury and suboptimal bile flow[29].

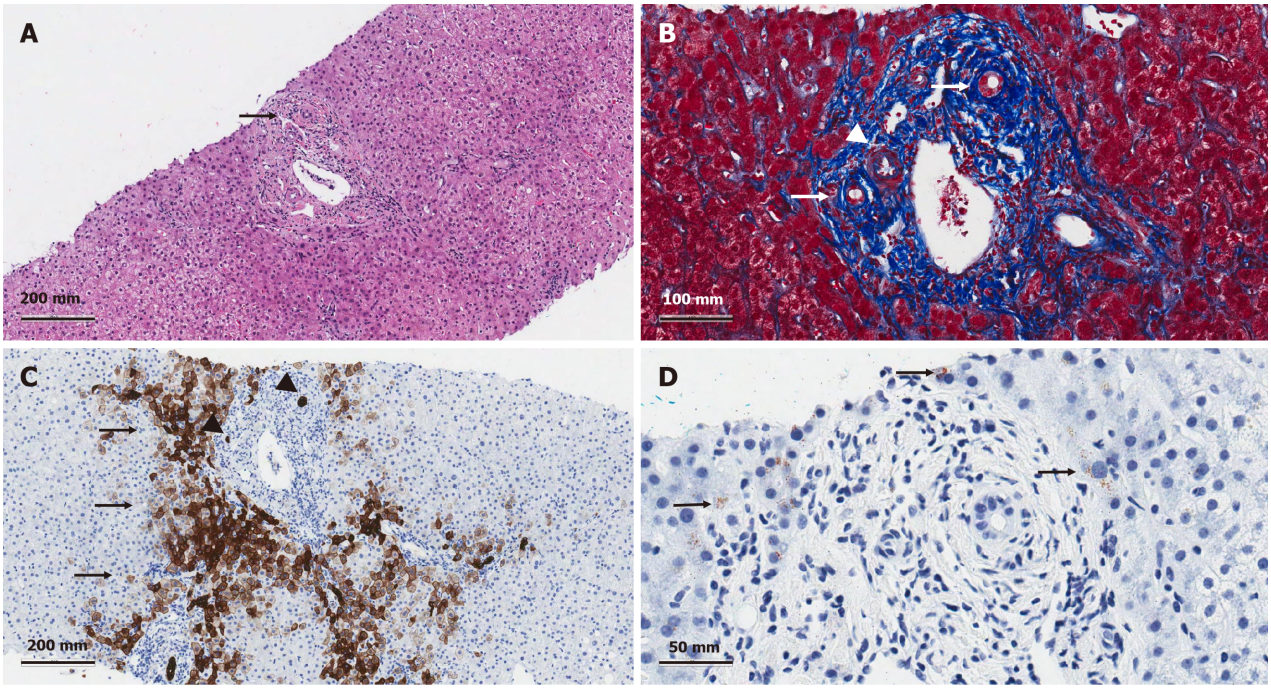

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Figure 1 Histologic features in a liver biopsy collected from a patient with normal magnetic resonance cholangiopancreatography and suspected small duct primary sclerosing cholangitis. A: The H&E shows a portal tract with bile duct senescence (arrow) and mild non-specific, lymphocytic inflammation. B: The Masson's trichrome stain shows a higher power image of this same portal tract, highlighting the smaller size of the intrahepatic bile ducts when compared to the adjacent hepatic artery (arrowhead). This stain also shows peribiliary sclerosis that is causing fibrous obliteration of the bile duct epithelium (arrows). The collagen surrounding the ducts is dense and has a keloid-like appearance. C: A cytokeratin 7 stain was conducted and shows prominent periportal cholangiolar metaplasia of hepatocytes (arrows) with atrophy of the intrahepatic bile ducts (arrowhead). In patients without chronic biliary obstruction or suboptimal bile flow, cholangiolar metaplasia is not present. D: A copper stain was positive for periportal deposition (arrows), further supporting the presence of chronic biliary obstruction.

ASSOCIATED DISORDERS

Similar to ldPSC, sdPSC has a strong association with IBD. The large majority of sdPSC patients present with concurrent UC[8,15]. A key difference from ldPSC is a higher prevalence of Crohn's disease with a study showing a prevalence of 21% in sdPSC *vs* 5-10% in ldPSC populations[8]. Studies have not shown any significant differences in outcomes when comparing sdPSC-UC and sdPSC-CD populations[8,10,15,30]. Hepatobiliary cancers in sdPSC are quite rare with only 1 documented case of hepatocellular carcinoma in all of the evaluated studies[11]. In contrast, cholangiocarcinoma (CCA) is seen in approximately 15% of ldPSC patients while cases seen in sdPSC are exceedingly rare[31]. Additionally, ldPSC patients have five times increased risk of developing colorectal cancer when presenting with concurrent IBD when compared to ldPSC patients without IBD[4,25]. This association with malignancies is the thought behind routine colorectal screening in those with PSC-IBD and may warrant evaluation for the need of routine surveillance in the sdPSC-IBD population. An overlap syndrome exists between PSC and autoimmune hepatitis (AIH) which is more commonly seen in the pediatric population though adult PSC patients can develop superimposed AIH years after the initial PSC diagnosis[27,28]. A similar trend is seen in sdPSC as the majority of sdPSC-AIH patients were seen in the pediatric populations[27,28,32]. Other disorders associated with PSC include type I diabetes mellitus, membranoproliferative glomerulonephritis, hypothyroidism, and autoimmune hemolytic anemia though the prevalence of these conditions in sdPSC have not been as well established[8,10].

TREATMENT

No widely accepted method of therapy has been established for patients with ldPSC or sdPSC in part, due to ambiguity regarding the pathogenesis of the disease. Ursodeoxycholic acid (UDCA) at lower doses improved serum liver biochemical tests however there was little symptomatic improvement and no significant improvement in overall outcomes[33,34]. A study using moderate doses of UDCA failed to produce a statistically significant outcome[35]. Most recently a multi-center study examining high doses of UDCA was aborted due to increased morbidity and mortality despite improvement in serum biochemical profiles[1]. The major gastroenterology societies within the United States recommend

against the use of UDCA in patients with PSC[1]. Additionally, the role of immunosuppressive agents and corticosteroids in the treatment of PSC has been explored[1,36,37]. However, no studies demonstrated significant improvement in morbidity and mortality with these agents.

Ultimately, the only definitive therapy for PSC is liver transplantation which has a five-year survival rate of nearly 85%[1,38]. A possibility of recurrence has been seen in 20-25% of cases, 5-10 years post-transplant[38]. Patients with sdPSC have a significantly longer median survival without liver transplantation compared to those with ldPSC[8]. However, studies have shown that among the cohort of patients who progress from small to large-duct involvement, up to half will develop outcomes of death or liver transplantation[8].

CONCLUSION

SdPSC is a rare disorder with the potential of progressing to ldPSC. The definitive etiology and pathogenesis of sdPSC and the circumstances that lead to progression to large-duct involvement are not well understood. Strong associations between sdPSC and IBD have been established with suspicion that concurrent sdPSC-IBD may be a key prognostic factor in determining which patients are at risk of progression to ldPSC. Additionally, this association may warrant future studies regarding the need for routine colorectal cancer screening in sdPSC patients with concomitant IBD. Evaluation using the current available literature is limited due to small cohorts and limited data regarding this specific subset of patients. It is therefore crucial for clinicians to continue reporting readily accessible data in hopes that future studies can further characterize which patients are at most risk of progression as large-duct involvement carries a more grim prognosis and requires more diligent surveillance.

FOOTNOTES

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MINIREVIEWS

New progress in understanding roles of nitric oxide during hepatic ischemia-reperfusion injury

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Abstract

Hepatic ischemia-reperfusion injury (HIRI) is a major clinical cause of morbidity and mortality in liver surgery and transplantation. Many studies have found that nitric oxide (NO) plays an important role in the HIRI and its increase or decrease can affect the progression and outcome of HIRI. However, the role of NO in HIRI is controversial and complicated. NO derived by endothelial NO synthase (eNOS) shows a protective role in HIRI, while excessive NO derived by inducible NO synthase (iNOS) accelerates inflammation and increases oxidative stress, further aggravating HIRI. Nevertheless, the overexpression of eNOS may exacerbate HIRI and iNOS-derived NO in some cases reduces HIRI. Here we review the new progress in the understanding of the roles of NO during HIRI: (1) NO possesses different roles in HIRI by increasing NO bioavailability, down-regulating leukotriene C4 synthase, inhibiting the activation of the nuclear factorκB (NFκB) pathway, enhancing cell autophagy, and reducing inflammatory cytokines and reactive oxygen species (ROS). And NO has both protective and deleterious effects by regulating apoptotic factors; (2) eNOS promotes NO production and suppresses its own overexpression, exerting a hepatoprotective effect reversely. Its activation is regulated by the PI3K/Akt and KLF2/AMPK pathways; and (3) iNOS derived NO mainly has deteriorating effects on HIRI, while it may have a protective function under some conditions. Their expression should reach a balance to reduce the adverse side and make NO protective in the treatment of HIRI. Thus, it can be inferred that NO modulating drugs may be a new direction in the treatment of HIRI or may be used as an adjunct to mitigate HIRI for the

purpose of protecting the liver.

Key Words: Hepatic ischemia-reperfusion injury; Nitric oxide; Endothelial nitric oxide synthase; Inducible nitric oxide synthase

Core Tip: This review focuses on the new progress in the understanding of the role of nitric oxide (NO) in hepatic ischemia-reperfusion injury (HIRI). NO protects HIRI by increasing NO bioavailability and cellular autophagy, down-regulating leukotriene C4 synthase, inhibiting the nuclear factor κB (NF-κB) pathway, and reducing inflammatory cytokines and reactive oxygen species. While by regulating apoptotic factors, it has dual effects. eNOS exerts hepatoprotective effects by promoting NO production through the involvement of the phosphoinositide 3-kinase/Akt pathway and Kruppel-like factor 2/adenosine monophosphate-activated protein kinase pathways. The function of eNOS overexpression remains controversial. iNOS-derived NO mainly deteriorates HIRI, but it may reduce damage under certain conditions. The balance of eNOS and iNOS is important for the HIRI protection.

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INTRODUCTION

Hepatic ischemia-reperfusion injury (HIRI) is a major complication often seen in liver surgery and organ transplantation. It manifests as cellular damage during the ischemic phase and worsens during reperfusion. Depending on the different conditions of ischemia, HIRI can be divided into warm ischemia-reperfusion (WIR) injury and cold ischemia-reperfusion injury (IRI), which have similar pathophysiology but different clinical injury sites[1].

With a high incidence of cases, liver cancer has increased by 114.0% and ended up with 1007800 cases in 2016[2]. Many liver cancer patients are supposed to be treated by liver transplantation or hepatectomy, where HIRI occurs during the operation [3]. Although HIRI is receiving increasing attention to improve the success rate of surgery and improve prognosis, very few of them are known.

The pathophysiological process of hepatic IRI involves the interaction of many different cell types and numerous signaling pathways such as anaerobic metabolism, acidosis, oxidative stress, and intracellular calcium overload. Among the interactions, the imbalance in the ratio of endothelin (ET) to nitric oxide (NO) is one of the mechanisms involved in HIRI. Normally, their function is to regulate blood flow to the hepatic sinusoids. In contrast, in the first few hours after reperfusion, as ET rises, plasma expression of NO decreases, leading to an increase in the ET/NO ratio and the possible appearance of HIRI[4,5].

Many pieces of evidence show that NO plays an important role in ischemia-reperfusion (I/R)[6,7]. However, as a vasodilator, the role of NO has been controversially discussed by scientists[8,9]. In past studies, NO was regarded as a negative factor because of its cytotoxic effect[10]. Nevertheless, a recent study indicated that NO can induce either a positive or negative effect during the early phase of HIRI and have a protective effect during late HIRI[11]. Therefore, it is important to further explore the protective mechanism of NO in HIRI.

NO is a small molecule free radical that can easily penetrate cell membranes. It is also an important effector and messenger molecule of biological information, which has undergone many extensive types of research in the past few years. There are two sources of NO in the human body—enzymatic production and non-enzymatic production. Non-enzymatic production mainly comes from chemical degradation and inorganic nitrogen transformation on the body surface or ingested. For enzymatic production, NO is oxidized from L-arginine by NO synthase (NOS)[12].

There are Ca^{2+}-independent and Ca^{2+}-dependent NOS in the human body. Ca^{2+}-dependent NOS can be subdivided into neuronal (nNOS) and endothelial (eNOS). eNOS is an enzyme continuously expressed in vascular endothelial cells and exerts biological functions through producing NO. In contrast to eNOS, Ca^{2+}-independent inducible NOS (iNOS) is activated by some exterior factors including viruses, bacteria, pro-inflammatory interferon, and cytokines[13]. iNOS produces a large amount of NO in hepatocytes, cholangiocytes, and Kupffer cells (KCs), helping macrophages to mount an immune response[14].

eNOS and iNOS are believed to take actions in HIRI. While depending on different isoforms of NOS, NO has a dual effect on hepatocellular functions during IR. eNOS-derived NO is hepatoprotective of ischemia following IRI by improving hepatic microcirculation and counteracting the deteriorate functions of reactive oxygen species (ROS) [4]. However, an augmented level of iNOS activation upon reperfusion will produce excessive NO, resulting in endothelial dysfunction and aggravating liver damage in HIRI[14]. It has been reported that iNOS-derived NO may have a positive or negative function in HIRI depending on the different conditions[15].

This review aims to find the role of NO during HIRI and look for candidate ways to alleviate liver damage (Table 1).

DIFFERENT ROLES OF NO DURING HIRI

Recently, studies have showed that NO has a significant role during the HIRI, which can be a positive protective function or negative deleterious function. NO was proved to reduce HIRI through various mechanisms such as increasing NO bioavailability, down-regulating leukotrienes (LTs), inhibiting liver cell apoptosis, enhancing autophagic flux, maintaining liver microcirculation blood flow, stabilizing ATP levels, and reducing oxidative stress injury. Whereas, NO can also regulate some apoptotic signal pathways to accelerate the apoptosis of hepatic tissue.

Increase of NO bioavailability involved in its protective effect in HIRI

Hide *et al*[16] found that NO bioavailability was reduced during reperfusion by detecting the levels of cyclic guanosine monophosphate, a second messenger of NO. They concluded that the decreased NO bioavailability can be explained by the reduction of eNOS activity leading to less synthesis of NO and increased NO clearance by reacting with ROS and forming peroxynitrite, which may later react with cell components such as proteins, lipids, and DNA, further damaging the cell. Therefore, increasing NO bioavailability can protect the liver from further damage during HIRI. It is reported that obestatin enhances NO bioavailability by up-regulating eNOS expression[17]. Also, simvastatin maintains NO bioavailability by preventing kruppel-like factor 2 (KLF2) down-regulation[16].

NO can down-regulate LTC4S by inhibiting the nuclear factorκB pathway

Many studies reveal that cysteinyl leukotrienes are directly associated with hepatic IRI. Leukotriene C4 synthase (LTC4S) is one of the enzymes that are responsible for LTC4 synthesis, showing a strong relationship with the NO[18,19]. In I/R rats, the gene expression level of LTC4S is much higher. However, this is reversed by V-PYRRO/NO, which acts as an NO donor. Hong *et al*[20] also found that another NO donor, sodium nitroprusside, could down-regulate the mRNA expression of *LTC4S* by inhibiting nuclear factor-kappa B (NF-κB) activation in an NF-κB α inhibitor-independent manner by detecting the protein levels of NF-κB p65 and p50 in the nuclear extracts using Western Blot.

NO can regulate some apoptotic signal transduction pathways and factors

NO has a significant role in regulating some apoptotic signal transduction pathways which can be potentially activated to induce or inhibit the hepatic cell apoptosis process caused by HIRI during the hepatic operation or other hepatic diseases. The signal pathways or apoptosis-related genes including caspases[21-25], Bcl-2 gene family[26-30], mitogen-activated protein kinase (MAPK)[31], and NF-kB[32]. Studies have shown that the caspase family is strongly related to hepatic cell apoptosis[33]. Zhao *et al*[21] found that steatosis-induced decline in adenosine monophosphate-activated protein kinase (AMPK)-catalyzed phosphorylation permits caspase-6 activation, leading to hepatocyte death. And Gao *et al*[22] indicated that caspase-3A is involved in cadmium (Cd)-induced cell apoptosis in common carp, which showed 71.8% sequence similarity and 59.3% sequence identity to human caspase-3. Zhang *et al*[23] found that Cd treatment increased the level of iNOS and NO. The overexpression of NO leads to chicken hepatic cell apoptosis by inducing the mitochondrial apoptotic pathway. In two other studies, mouse liver cell apoptosis can be inhibited by reducing NO content, down-regulating Bax protein expression, and increasing Bcl-2 protein expression[24,25]. Besides, an imbalanced Bax/Bcl-2 ratio is caused by decreasing levels of NO and iNOS and increasing Bcl-2 expression through the NF-kB pathway. And this imbalanced ratio may show a protective role in the damaged liver[30]. Jiang *et al*[34] also found that 7-mer peptide can increase the level of Bcl-2 and decrease the level of Bax expression to reduce apoptosis and protect against IRI.

NO protects against I/R-induced liver injury by enhancing autophagic flux

NO has an important role in protecting against I/R-induced liver injury by enhancing autophagic flux. During severe environments such as IRI, the cell will undergo an autophagic process, which is an adaptive response to reduce the injury. Studies have found that the protective mechanism of NO during HIRI is associated with autophagic flux. Shin *et al*[35] demonstrated that NO could enhance light chain-3 lipidation and autophagosome-lysosome fusion during hepatic I/R. Also, eNOS-induced NO enhances autophagy *via* p38 MAPK activation during liver I/R. Simvastatin, which is used to protect the donor

Table 1 Roles of nitric oxide, inducible nitric oxide synthase, and endothelial nitric oxide synthase in various conditions and pharmacological protection against hepatic ischemia-reperfusion injury

Pretreatment	NO/ iNOS/ eNOS levels	Animals	Experimental cells	Mechanism	Liver cell necrosis and liver damage	Ref.
NMP, BMMSCs, and liver CDC	eNOS↑, iNOS ↓	SPF rats	Liver tissue	Macrophage activation, ICAM-1, VCAM-1, vWF↓	↓	[5]
L-NAME and BDL	iNOS mRNA↓	Male Wistar rats	Liver tissue	TGF-β, NOx, HA↓; AMDA↑		[6]
Simvastatin and WIR	NO↑	Male Wistar rats	Primary LSEC	Nitrotyrosines, O^{2-}↓; Nrf2, HO-1↑	↓	[14]
Simvastatin and WIR	NO, eNOS↑	Male Wistar rats	LSEC	KLF2, p-eNOS, cGMP↑; O^{2-}, VCAM-1↓	↓	[16]
Obestatin and HIRI	eNOS↑, iNOS ↓	Adult Wistar albino male rats	Liver tissue samples	Reducing oxidative stress and inflammatory process	↓	[17]
HIRI	NO↑	Male Sprague-Dawley rats	Liver	Decrease synthesis of LTC4S, NF-κB↓	↓	[18,19]
V-PYRRO/NO and HIRI	NO↑	Male Sprague-Dawley rats	Liver	Decrease synthesis of LTC4S, NF-κB↓	↓	[20]
AMPK–caspase-6 axis and nonalcoholic steatohep-atitis	-	LAKO mice	Hepatocyte	Caspases-6 activation	↓	[21]
Cd-Induced Apoptosis	NO↑ iNOS↑	Hy-Line Brown laying hen	Liver	Mitochondrial apoptotic pathway	↓	[23]
Punicalagin and cyclophosphamide	NO, iNOS↓	Sprague-Dawley male rats	Liver	Reduce cell apoptosis	↓	[25]
*Emblica officinalis*Gaertn and NAP	NO↓	Male Wistar rats	Liver	Apoptosis, autophagy, inflammation↓	↓	[28]
Tormentic acid and LPS/ D-GalN	NO, iNOS↓	Male C57BL/6 mice	Liver samples	TNF-α, NF-κB↓; imbalanced Bax/Bcl-2 ratio	↓	[30]
Selenocysteine-containing 7-mer peptide	NO↓	Adult male Wistar rats	Liver tissue	Inhibit oxidant peroxynitrite, Bax↓; Bcl-2↑	↓	[34]
IPC and HIRI	NO↑	Male C57BL/6 mice	Liver tissue	p38 MAPK↑; autophagy↑	↓	[35]
Simvastatin and hepatic transplantation	NO↑	Male rats	Liver	Autophagy↑	↓	[36]
IPC	NO↑	Male Sprague-Dawley rats	Liver	Oxygen species liberation and proinflammatory cytokine↓; microcirculation ↑	↓	[37]
Oxytocin and HIRI	NO↑	Adult male albino (Sprague Dawley strain)	Liver	NO bioavailability↑	↓	[38]
TELL and HIRI	eNOS↑	Male Wistar rats	Liver	Activate PI3K/Akt pathway, suppress TLR4, p-PI3K, p-Akt, Nrf2, p-NF-κB p65, p-MAPK p38, TNF-α, GSH, MyD88, HMGB-1, TBARS↑; NF-κB↓	↓	[42]
Apelin preconditioning and HIRI	eNOS↑	Male albino rats	Liver tissue	Activate PI3K/Akt pathway, suppress AT1R, counteract Ang II/AT1R system	↓	[43]
Simvastatin and WIR	eNOS↑	Male inbred Sprague Dawley rats	Liver tissue	Activate KLF2 pathway, TM, p-eNOS↑, TGF-β, TNF-α, IL- 1β↓	↓	[44]
HMP and liver DCD	NO, eNOS↑	Adult male Sprague-dawley rat	Liver	KLF2↑; NF-κB p65, IL-1β, TNF-α↓	↓	[45]
TMZ and WIR	eNOS↑	Male Wistar rats	Liver tissue	p-MAPK↑; activate MAPK pathway	↓	[46]

IGL-1 and Fatty Liver Graft Cold Storage	eNOS↑	Homozygous (obese) Zücker rats	Liver	Activate MAPK pathway, ATP↑	↓	[48]
SEW2871 and WIR	eNOS↑	Male C57BL/6 mice	SECs	VE-cadherin, p-Akt↑; IFN-γ, TNF-α, IL-6, VCAM-1↓	↓	[49]
EPO and liver transplantation	eNOS↑	Female landrace pigs	Liver tissue	Activate JAK2/PI3/AKT pathway, AMPK↑; βcR2-VEGFR-2 complex	↓	[51]
HIRI	-	Female domestic (Landrace) pigs	Liver tissue	IL-6, STAT-3 and E-selectin mRNA↑	-	[52]
TDF and HIRI	eNOS, iNOS↓	Female Wistar albino rats	Liver tissue	cGMP↑, activate mitochondrial K-ATP channels, mitochondrial Ca^{2+}↓	↓	[53]
PTX and HIRI	eNOS, iNOS↓			cAMP↑ TNF-α, IL-1, IL-6, IL-12, TGF-β, IFN-γ, procollagen-I mRNA↓	↓	
L-NNA and HIRI	iNOS↑, eNOS, NO↓	Male Wistar rats	Liver	TNF-α, NF-κB↑, Bcl-2↓	↑	[54]
L-Arginine/CDN and HIRI	NO, eNOS↑, iNOS↓			TNF-α, NF-κB↓, Bcl-2↑	↓	
iNOS knockout and WIR	iNOS↓	C57BL/6 male rats	293 T cells	PUMA↓	↑	[55]
NAC and HIRI	eNOS↑, iNOS ↓	Male Wister albino rats	Liver	NOSTRIN, MDA, MPO activity↓	↓	[56]
TQ and HIRI	eNOS↑, iNOS ↓			NOSTRIN, MDA, oxidative stress, nitrosative stress↓, GSH↑	↓	
LA and HIRI	iNOS mRNA↓	Male Wistar rats	Liver	NF-κB p65, MIP-2 mRNA, GSH↓	↓	[57]
V and HIRI	NO, iNOS mRNA↓	Male Wistar rats	Lung and Liver tissue	Inhibit HIF-α/HGF/iNOS pathway	↓	[58]
Eupatilin and HIRI	iNOS↓	Male C57BL/6 mice	Embryonic liver BNL CL.2 cell	TLR2/4, p-IκB-a↓, Bcl-2↑	↓	[59]
N-SMase inhibitor and HIRI	iNOS	Male Wistar rats	Liver tissue	Protein nitration, nitrite/nitrate levels, HNE		[60]
Ad-eNOS and small-for-size liver transplatation	NO, eNOS↑	-	Human normal liver cell line L02	TNF-α↓, inhibit macrophage activation	↓	[61]
Ad-eNOS and HIRI	eNOS↑	Male inbred C57BL6 lean mice	Liver	ATP↓, bax, p53↑	↓	[62]

L-NAME: Nomega-nitro-L-arginine methyl ester; BDL: Bile duct ligation; WIR: Warm ischemia and reperfusion; LSEC: Rat liver sinusoidal endothelial cells; KLF2: Kruppel-like factor 2; VCAM-1: Vascular cell adhesion molecule-1; HIRI: Hepatic ischemia reperfusion injury; NF-κB: nuclear factor-kappaB; LTC4S: Leukotriene C4 synthase; AMPK: Adenosine monophosphate activated protein kinase; NAP:N-nitrosodiethylamine; LPS: Lipopolysaccharide; D-GalN: D-galactosamine; TNF-α: Tumor necrosis factor-α; Bax: BCL2-Associated X; Bcl-2: B-cell lymphoma-2; MAPK: Mitogen-activated protein kinase; IPC: Ischemic preconditioning; TM: Thrombomodulin; PI3K: Phosphoinositide 3-kinase; TLR4: Toll like receptor-4; Nrf2: Nuclear erythroid-related factor 2; MyD88: Myeloid differentiation primary-response protein 88; TBARS: Thiobarbituric acid reactive substances; AT1R: Angiotensin type 1 receptor; VE-cadherin: Vascular endothelial cadherin; JAK: Janus activated kinase 2; DCD: Donated after circulatory death; HMP: Hypothermic machine perfusion; TMZ: Trimetazidine; IGL-1: Institut georges lopez-1; SECs: Sinusoidal endothelial cells; EPO: Erythropoietin; TDF: Tadalafil; PTX: Pentoxifylline; SPF: Pathogen-free; BMMSCs: Bone marrow mesenchymal stem cells; NMP: Normothermic machine perfusion; L-NNA: Nomega-nitro-L-arginine; CDN: Cardamonin; PUMA: p53 up-regulated modulator of apoptosis; HIF-α: Hypoxia inducible factor 1α; HGF: Hepatocyte growth factor; MIP-2: Macrophage inflammatory protein-2, LA: Alpha-lipoic acid; V: Vildagliptin; MPO: Myeloperoxidase, MDA: Malondialdehyde; NAC: N-acetylcysteine; TQ: Thymoquinone; Ad-eNOS: Adenovirus-eNOS; N-SMase: Neutral sphingomyelinase; HNE: 4-hydroxynonenal; VEGFR: Vascular endothelial growth factor receptor; ICAM-1: Intracellular cell adhesion molecule-1; βcR: Common β receptor; NOSTRIN: Nitric oxide synthase trafficking.

liver, can activate autophagy and increase NO release during hepatic transplantation. This also indicates the possible connection between NO and autophagy[36].

NO decreases inflammatory cytokines and reduces ROS by inhibiting the mitochondrial respiratory chain

During reperfusion, the surge of inflammatory factors, cytokine liberation, neutrophil infiltration and ROS generation occurred, which led to hepatic injury. An increased level of NO can reduce cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1 which stimulate infiltration and

endothelial injury. Also, continuous NO production can reduce ROS and proinflammatory cytokine generation as well as neutrophil infiltration[37]. Inversely, NO deficiency can induce TNF-α expression as a result of ROS surging. Ragy *et al*[38] proved this by adding Nomega-nitro-L-arginine methyl ester (L-NAME) in IRI model rats treated with oxytocin. In this group, not only did the parameter damage increase but also the inflammatory factor such as TNF-α level increased compared with the control group.

ROLE OF ENOS IN HIRI

Activated eNOS produces NO to protect HIRI

eNOS performs various biological functions by promoting the production of NO, which is important for maintaining vascular tone and cardiovascular hemostasis, and inhibiting platelet activation and aggregation. It has been confessed that eNOS shows a hepatoprotective effect in HIRI by improving the production of NO (Figure 1).

There are two main regulation pathways for eNOS activation, one dependent on intracellular concentration of Ca^{2+} and the other independent. The increasing intracellular Ca^{2+} level can enhance the affinity of calmodulin binding to eNOS and activate enzymes to produce NO[39]. For the Ca^{2+}-independent regulation pathway, phosphorylation of the Ser1177 residue or dephosphorylation of the Thr495 residue activates it to produce NO[40].

Calcium-dependent eNOS activation

At the early stage of HIRI, the ischemia will lead to a shortage of oxygen and nutrients, which can decrease ATP availability. Without energy, ATP-dependent ion channels or transporters cannot work. The incompetence of the Na^+/K^+ pump leads to depolarization of the cell membrane, resulting in the influx of Ca^{2+}[41]. Besides, anaerobic glycolysis induces an increase in H^+, which activates intracellular proteases to increase cellular permeability. Furthermore, Na^+/Ca^{2+} exchange is activated due to a high concentration of H^+, leading to a further influx of Ca^{2+}. Consequently, eNOS is activated due to the increase of intracellular concentration to produce NO, carrying anti-HIRI activities at the initial stage.

Calcium-independent eNOS activation

Phosphoinositide 3-kinase/Akt pathway induced eNOS activation: The phosphoinositide 3-kinase (PI3K)/Akt signaling pathway is a cell survival pathway that regulates cell proliferation and apoptosis, as well as an endogenous negative feedback regulator that functions in anti-inflammation and anti-apoptosis effects in IR.

PI3K can activate Akt to act on the phosphorylation of eNOS. It has been proven that telluric acid has a hepatoprotective effect by elevating the expression of eNOS, which is accompanied with elevated expression of p-PI3K and p-Akt proteins. Besides, the activation of PI3K/Akt also inhibits NF-κB and activates nuclear erythroid-related factor-2, reducing pro-inflammation cytokine expression and inducing anti-oxidative effects[42]. Moreover, through the PI3K/Akt pathway, apelin preconditioning can increase the expression of eNOS and counteract the pathological effects of the angiotensin II/angiotensin II type 1 receptor system in HIRI[43]. Thus, the activation of the PI3K/Akt pathway leads to the phosphorylation of eNOS and continuous catalysation of NO production, which is essential to counteract HIRI.

KLF2 induced eNOS activation: There may exist other ways of influencing the eNOS activity during IR. It has been proven that WIR injury can decrease the expression of KLF2 in endothelial cells. Also, this reduction is accompanied by a decrease in phosphorylated eNOS (p-eNOS), one of the KLF2 targets. And the IR damage can be mitigated by pretreatment with simvastatin through a KLF2-dependent mechanism, upregulating the mRNA expression of *KLF2* and *eNOS* as well as the protein expression of KLF2 and p-eNOS[16,44]. Hu *et al*[45] also demonstrated that hypothermic machine perfusion inhibited NF-κB signaling and activated eNOS/NO signaling through KLF2 expression, thereby alleviating the inflammatory response and oxidative stress injury. It has demonstrated that KLF2 activators can be candidate therapeutic agents for HIRI.

AMPK induced eNOS activation: AMPK plays a key role in the regulation of cellular energy homeostasis. The activation of this kinase is a response to the stimulus. Mahfoudh *et al*[46] reported that repeated administration of trimetazidine protected against WIR injury by decreasing liver damage and oxidative stress. The underlying mechanism involves the activation of the AMPK/eNOS signaling pathway. In addition, similar mechanisms have been identified in the protective effect of Institut Georges Lopez 1 solution on cold-stored fatty liver grafts. The effect is mainly exerted through the activation of the AMPK pathway, which targets eNOS to produce NO, offsetting aggravated microcirculatory changes, and improving vascular resistance and function during IR[47,48].

Other pathways: SEW2871, a selective sphingosine-1-phosphate receptor 1 (S1PR1) agonist, can restore

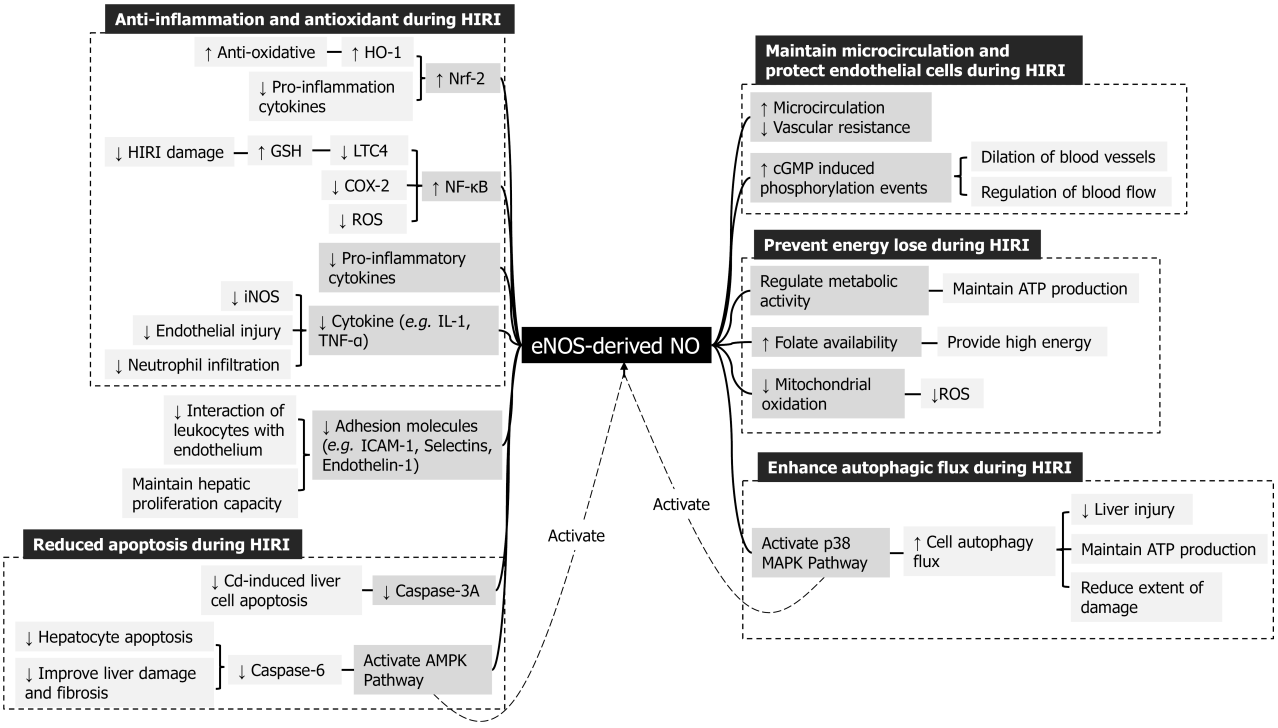

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Figure 1 Hepatoprotective effects of endothelial nitric oxide synthase-derived nitric oxide during hepatic ischemia-reperfusion injury and underlying mechanisms. Nrf2: Nuclear erythroid-related factor; HO-1: Heme oxygenate-1; IL-1: Interleukin-1; TNF-α: Tumor necrosis factor-α; NF-κB: Nuclear factor-κ-gene binding; LTC4: Leukotriene C4; GSH: Glutathione; COX-2: Cyclooxygenase 2; ROS: Reactive oxygen species; ICAM-1: Intracellular cell adhesion molecule-1; AMPK: Adenosine monophosphate activated protein kinase; MAPK: Mitogen-activated protein kinase; iNOS: Inducible nitric oxide synthase.

the expression of eNOS and vascular endothelial (VE) cadherin in sinusoidal endothelial cells during HIRI *in vivo* and does not influence the expression of p-Akt. Thus, there may be a regulation pathway between S1PR1 and eNOS[49]. And the expression of VE-cadherin is important for vascular integrity, which is the basis for eNOS expression[49].

Moreover, ischemia preconditioning (IPC) can protect HIRI through p38 MAPK activation, which induces eNOS-derived NO expression to enhance cell autophagy in HIRI[35]. However, pretreatment with 3,7-dimethyl-1-propargylxanthine, an adenosine A2 receptor (A2AR) antagonist, can repeal the protective effect induced by IPC. Therefore, it can be inferred that there may be a relationship between the A2AR and eNOS[50].

The study of Kebschull *et al*[51] showed that low-dose erythropoietin (EPO) treatment significantly increased hepatic NO bioavailability by up-regulating eNOS expression. EPO-mediated eNOS phosphorylation is promoted by EPOR-mediated activation of the Janus kinase 2/PI3K/Akt pathway and common β receptor (βcR)-dependent activation of AMPK. In addition to this, activation of the βcR2-vascular endothelial growth factor receptor-2 complex is also involved in the regulation, but its downstream signaling is currently unclear.

ROLE OF INOS IN HIRI

As mentioned above, iNOS-derived NO may have different functions in HIRI[15]. Although in most cases iNOS is considered to be harmful to the HIRI, it does not affect or even protects the HIRI in some conditions. In a study of models with liver ischemia and partial liver resection, *iNOS* mRNA expression was not found to be significantly altered compared to the sham group. While during 6 to 8 h after hepatectomy, iNOS expression and NO production were promoted by cytokines, thereby improving liver microcirculation and preventing cell apoptosis[52]. The protective effect of iNOS has only been demonstrated in a few specific experiments and lacks widespread validation. Due to differences in experimental subjects, measurement criteria, and experimental time constraints, iNOS-derived NO exhibits a more complex and unclear role than eNOS.

iNOS aggravates HIRI

Hide *et al*[14] found a surge of NO in WIR in aged livers, which was mainly induced by iNOS

production. The surge of NO derived from iNOS can increase the expression of reactive nitrogen species (RNS) and inflammatory cytokines, resulting in cytotoxic damage in hepatocytes. Besides, the damage from iNOS is also confirmed in other studies. As intrahepatic macrophages, KCs are activated in early IRI, producing excessive amounts of iNOS-derived NO and leading to massive production of pro-inflammatory factors, cytokines, and ROS, which are key links to impaired microcirculation in the liver and deteriorate HIRI[5,53].

At the late phase of HIRI, the function of iNOS will be at a prominent stage. Excess NO derived from iNOS has cytotoxic effects that induce inflammation and excessive oxidation, and performs many deleterious functions in HIRI. Increased iNOS expression is associated with increased TNF-α and NF-κB, which leads to increased expression of pro-inflammatory genes, inflammatory mediators, and regulatory enzymes[54]. They are both important to trigger inflammation reactions and may have deleterious effects on IRI. Besides, in studying the role of iNOS/NO in the interferon regulatory factor-1 (IRF1) signaling pathway of primary human hepatocytes, Du *et al*[55] found the existence of a positive-feedback loop between iNOS and IRF1. The IRF1 and p53 can upregulate the p53 up-regulated modulator of apoptosis (PUMA), which is a modulator of apoptosis, resulting in hepatocyte death and further damage to hepatic IRI.

REGULATING INOS AND ENOS EXPRESSION TO PROTECT HIRI

The extent and intensity of eNOS and iNOS in HIRI are both higher than those in the normal state, while excess NO will produce peroxynitrite to aggravate IR damage. These can be reduced by using high doses of tadalafil and pentoxifylline to mitigate the deterioration of nitrosative stress and endothelial cell injury[53].

Iwasaki *et al*[6] demonstrated that L-NAME, an NOS inhibitor, attenuated liver damage in IRI of cholestatic livers by inhibiting the NO production. Comparing the expression of iNOS and eNOS with L-NAME treatment, they found that this kind of protection was mainly mediated by the inhibitory effects of iNOS. It also prevented the increase of asymmetric dimethylarginine, which is an exogenous inhibitor of eNOS, to protect against IRI at the early stage.

Bone marrow mesenchymal stem cell (BMMSC) transplantation can regulate NOS synthesis by increasing eNOS expression as well as inhibiting iNOS expression and excessive NO production to protect HIRI and reduce hepatocyte apoptosis. Its regulations are closely related to the inhibition of NOS-induced macrophage activation, the suppression of large amounts of iNOS and NO synthesized by macrophages, and the amelioration of endothelial damage. And the combined use of BMMSCs and normothermic machine perfusion can increase the balance of ET/NO ratio[5].

Besides, the eNOS traffic inducer (NOSTRIN) can significantly inhibit NO release by decreasing the enzymatic activity of eNOS. Pretreatment with N-acetylcysteine or thymoquinone can up-regulate eNOS along with NO production and down-regulate iNOS and NOSTRIN expression to attenuate HIRI injury, showing the protective effect of increasing eNOS and NO levels and inhibiting iNOS expression against IRI in rat liver[56].

Inhibiting iNOS to protect HIRI

After reperfusion, the expression of inflammatory factors such as macrophage inflammatory protein-2 and iNOS increase with the activation of NF-κB, leading to a series of inflammation reactions. Alpha-lipoic acid can reduce the formation of excess NO during reperfusion by decreasing the expression of *iNOS* mRNA and reduce cellular damage from NO-forming NOS superoxide and peroxide anions[57]. Beyond that, in a study of vildagliptin function in lung injury after hepatic IRI, significant inhibition of *iNOS* mRNA expression and NO was observed by the involvement of the hypoxia-inducible factor (HIF)-α/hepatocyte growth factor/iNOS pathway. The evaluated HIF-α can increase iNOS expression in various models. Therefore, targeting HIF-α expression can reduce tissue damage[58]. Furthermore, hepatic IR-induced iNOS protein expression can be diminished by eupatilin, which also suppresses the Toll-like receptor 2/NF-κB pathway to ameliorate inflammation response[59]. In addition, neural-sphingomyelinase (N-SMase) can produce ceramide, which is a mediator of iNOS expression. Inhibition of N-SMase leads to a decrease in iNOS levels, along with a decrease in protein nitrification and nitrite/nitrate levels in WIR[60].

Inhibiting overexpression of and eNOS

Some studies have demonstrated the hepatoprotective effect of genetic eNOS overexpression in small-for-size liver transplantation and illustrated the importance of promoting eNOS expression for hepato-protection[61]. However, there is insufficient evidence for a protective effect of eNOS overexpression, and evidence that eNOS overexpression is detrimental to HIRI[62]. The dual effect of eNOS in HIRI remains controversial.

The fact is that the expression of eNOS will be deteriorated by oxidative stress and endothelial damage during the progression of ischemia, while the function of iNOS will be stimulated by oxidative stress during reperfusion and aggravate the liver injury. The imbalance of eNOS and iNOS can also

aggravate IRI.

THERAPEUTIC PERSPECTIVES

NOS drugs as well as drugs for the regulation of NOS enzymes may be the way forward for liver protection. However, more in-depth studies are still needed. Not only do drugs need to be stable, but they also need to avoid the harm that NO and NOS can cause to reduce side effects. Besides, despite a number of experimental studies demonstrating the beneficial effects of NO-releasing compounds and some drugs that promote NO release in ameliorating hepatic IRI, the results of trials and evaluations in the clinical setting are still lacking. Perhaps more randomised controlled clinical trials should be strengthened in the future to obtain more therapeutic results.

In a nutshell, increasing or decreasing NO availability in the hepatic tissue may both be ways to prevent and treat HIRI and identifying ways to balance the expression of eNOS and iNOS is important to protect IR and can be a promising direction for clinical research.

CONCLUSION

In general, NO along with eNOS and iNOS can play complex roles in HIRI. NO can down-regulate LTC4S by inhibiting the NFκB pathway, inhibit apoptotic related genes such as *Bax* and *Bcl-2*, enhance autophagic flux, decrease inflammatory cytokines, and reduce ROS by inhibiting the mitochondrial respiratory chain. Furthermore, NO induced by different NOS results in a duality of action in HIRI. NO derived by eNOS prefers to protect endothelial cells and attenuate liver injury in HIRI. However, iNOS promotes the production of NO in response to stimuli, thus exacerbating liver damage. But their role is not set in stone. Overexpression of eNOS also worsens HIRI, whereas iNOS has also been reported to have a protective effect against HIRI. Actually, these views remain controversial, and the underlying mechanisms are urgently needed to be clarified.

FOOTNOTES

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MINIREVIEWS

Renal manifestations of hepatitis E among immunocompetent and solid organ transplant recipients

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Abstract

Hepatitis E virus (HEV) infections are generally self-limited. Rare cases of hepatitis E induced fulminant liver failure requiring liver transplantation are reported in the literature. Even though HEV infection is generally encountered among developing countries, a recent uptrend is reported in developed countries. Consumption of unprocessed meat and zoonosis are considered to be the likely transmission modalities in developed countries. Renal involvement of HEV generally holds a benign and self-limited course. Although rare cases of cryoglobulinemia are reported in immunocompetent patients, glomerular

manifestations of HEV infection are frequently encountered in immunocompromised and solid organ transplant recipients. The spectrum of renal manifestations of HEV infection include pre-renal failure, glomerular disorders, tubular and interstitial injury. Kidney biopsy is the gold standard diagnostic test that confirms the pattern of injury. Management predominantly includes conservative approach. Reduction of immunosuppressive medications and ribavirin (for 3-6 mo) is considered among patients with solid organ transplants. Here we review the clinical course, pathogenesis, renal manifestations, and management of HEV among immunocompetent and solid organ transplant recipients.

Key Words: Hepatitis E; Acute kidney injury; Glomerular disorders; Kidney biopsy; Solid organ transplant; Kidney transplant

Core Tip: Hepatitis E virus (HEV) infection is infrequently associated with significant mortality and morbidity. HEV infection is not only restricted to developing countries, but is also identified among developed nations and predominantly holds zoonotic transmission. Renal manifestations of HEV infection range from acute tubular necrosis to immune-mediated glomerular injury. Conservative approach is routinely employed in management of acute kidney injury from HEV. Ribavirin and reduction of immunosuppression are considered among patients with solid organ transplants as they are prone to develop chronic hepatitis E infection. Plasma exchange and pulse steroids are sometimes used in management of crescentic glomerular nephritis associated with HEV infection.

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INTRODUCTION

Hepatitis E virus (HEV) has a pronounced worldwide distribution. It is a spherical, single-strand RNA virus consisting of three partially overlapping open reading frames (ORF) ORF1, ORF2, and ORF3[1]. HEV belongs to hepeviridae family, and eight genotypes of HEV (HEV1 to HEV 8) have been identified [2,3]. Genotypes HEV1 and HEV2 are routinely encountered in developing countries and are transmitted through fecal-oral route. HEV3 and HEV4 are associated with sporadic autochthonous infection among western countries and are predominantly transmitted through animal reservoirs and ingestion of uncooked meat[4-6]. Additionally, HEV genome 3 related infection is associated with solid organ transplant recipients and immunocompromised patients. Other uncommon modalities of transmission could occur through blood products and solid organ transplants[7,8]. Transfusion-related transmission is not common in the United States, but is reported in countries like China and Japan[9, 10]. Lastly, vertical transmission of HEV infection from mother to fetus could be up to 100%, as reported by Kumar *et al*[11] and is associated with fatal outcomes.

CLINICAL COURSE

HEV infection commonly holds a benign, self-limiting course, and the case-fatality rate in developing countries is estimated to be 0.5%-4%[12,13]. Clinical presentation of HEV infection is similar to that of hepatitis A. Majority of the infected patients sustain mild and asymptomatic course. Acute HEV infection is accompanied by jaundice, icteric eyes, malaise, anorexia, and abdominal discomfort. Severe infection is usually reported among patients with underlying chronic liver disease and is associated with increased mortality[14]. Additionally, solid organ transplant recipients encounter a more sustained course[15]. Among such patients, HEV antibody production could be delayed, often leading to sustained viremia with progression to chronic hepatitis and cirrhosis[16,17].

Pregnant women can suffer a complicated course with fulminant HEV infection and sustain higher mortality rates compared to non-pregnant cohorts. It is estimated that fatality rates reach 10%-40% among pregnant women[11,18]. Both obstetric and non-obstetric complications are encountered. Non-obstetric complications include fulminant hepatic failure, acute liver failure, acute cerebral edema and

obstetric complications include pre-term delivery, antepartum hemorrhage, intrauterine fetal demise[19-21].

RENAL MANIFESTATIONS OF HEV INFECTION

Non-glomerular manifestations

Renal manifestations of hepatitis B and hepatitis C (HBV, HCV) infection are well described. The association between HEV infection and kidney is established as the HEV particles are isolated from the urine of infected patients[22,23]. Additionally, when urine of infected monkeys was induced into healthy animals, the development of HEV infection was well appreciated and confirmed the infectious nature of the viral particles shed in the urine[23]. HEV-associated renal manifestations include prerenal or intrinsic renal disorders. Among intrinsic renal conditions, glomeruli and tubules are the affected sites[24,25].

HEV infection is less commonly associated with the progression of kidney disease in immunocompetent patients. Chronic HEV infection and subsequent development of decompensated liver cirrhosis are frequently encountered among solid organ transplant recipients. Hepatorenal physiology secondary to increased circulating vasoactive agents like nitric oxide is often noted. Similar to other cirrhotic patients, HEV-associated liver dysfunction patients could have increased vasodilatory mediators released secondary to shear stress on the portal vasculature, leading to splanchnic vasodilatation, portosystemic shunting, and bacterial translocation. Additionally, reduction in effective arterial blood volume perpetuates decrease in renal perfusions that ultimately leads to renal vasoconstriction[26]. Urine sodium levels remain low, indicating prerenal failure. However, prolongation of renal hypoperfusion contributes to ischemic injury of the proximal tubule with manifestations of acute tubular necrosis[13].

Bile cast nephropathy, also called cholemic nephrosis, is typically encountered among patients with cholestasis secondary to advanced cirrhosis or acute liver failure. Nayak *et al*[27] reported a case of cholemic nephrosis secondary to acute HEV infection. Historically, the diagnosis is made by kidney biopsy with the presence of bile cast obstructing distal tubules. The pathogenesis of cholemic nephrosis is not completely understood, however, it is hypothesized secondary to intraluminal obstruction of the bile cast along with direct tubular toxicity[28,29].

Cases of hemolysis and subsequent renal failure are reported with HEV infection. Karki *et al*[30] reported a case of massive hemolysis in a patient with glucose-6-phosphate dehydrogenase (G6PD) deficiency, heme pigment causing direct proximal tubular toxicity. Development of hemoglobin cast further leads to intratubular obstruction and subsequent development of acute kidney injury. It is hypothesized that the liver dysfunction secondary to acute HEV leads to accumulation of toxins along with the depletion of antioxidants like glutathione. Additionally, if patients have underlying G6PD deficiency, massive hemolysis, and acute kidney injury are encountered[31] (Figure 1).

Glomerular manifestation

Glomerular manifestations of HEV infection are reported among solid organ transplant recipients associated with HEV genotype 3. However, it is unclear if renal manifestations and presentation differ among various organ transplant recipients. While glomerular manifestations are commonly noted among immunocompromised patients[32,33], autochthonous HEV-induced membranoproliferative glomerular pattern was reported in an immunocompetent individual[33].

Study by Kamar *et al*[34] evaluated the renal function of patients with HEV infection in solid organ transplants recipients. Out of total 51 cases of genotype 3 HEV infections, 43.2% were cleared of the virus spontaneously within 6 mo of infection, whereas 56.8% progressed to chronic hepatitis. Among 36 kidney and kidney-pancreas-transplant patients, glomerular filtration rate (GFR) significantly decreased from baseline of 52.9 ± 17.7 mL/min at four-month median before HEV infection to 48.8 ± 18.7 mL/min during acute HEV infection (P = 0.04). Acute rejection episode, infection, modification in immunosuppressant type or dose, and functional renal insufficiency were ruled out, and the GFR decline is attributed to acute HEV infection. Proteinuria levels significantly increased in four kidney-transplant patients at HEV diagnosis, which subsequently improved with improvement in renal functions and HEV clearances.

Kidney biopsy performed during acute phase revealed patterns of membranoproliferative glomerulonephritis, cryoglobulinemia II and III types, and IgA nephropathy[34]. Additionally, among patients who developed chronic hepatitis, 12 patients who received anti-viral therapy with ribavirin for three months had clearances of HEV with subsequent improvement in GFR at 6 mo follow up. Interestingly, In the subgroup who received anti- viral therapy, cryoglobulinemia was detected in 70% of patients before therap, eventually became undetectable in all patients after viral clearance. Renal manifestations of the reported cases of HEV infection among immunocompetent and solid organ recipients are summarized in Table 1.

Table 1 Renal manifestations of the reported cases of hepatitis E virus infection among immunocompetent and solid organ recipients

Case study	Status	Age	Sex	Country	Serum creatinine/eGFR	Renal manifestations	Treatment	Follow up	Outcomes
Karki *et al*[30]	I.C	48 yr	M	India	8.1 mg/dL	ATN(Hemoglobin Cast)	Hemodialysis; Supportive care	3 mo	Improved kidney function
Verschuuren *et al*[13]	I.C	34 yr	F	Netherlands	10 mg/dL	ATN	Hemodialysis; Supportive care	3 wk	Complete kidney function recovery
Biliotti *et al*[51]	I.C	57 yr	M	Italy	44 mL/min	NR	Sofosbuvir; Ribavarin	3 wk	Patient died from MRSA infection
Guinault *et al*[33]	I.C	48 yr	M	France	3.6 mg/dL	MPGN	Steroids	4 mo	
Kamar *et al*[34]	K.T	33 yr	M	France	2.1 mg/dL	MPGN	Steroids	16 mo	Improved kidney function
Kamar *et al*[34]	K.T	26 yr	M	France	2.4 mg/dL	IgAN	Ribavarin 3 mo	9 mo	Stable kidney function
Kamar *et al*[34]	K.T	40 yr	M	France	2.1 mg/dL	IgAN	Change in IS + Rituximab	3 mo	
Kamar *et al*[34]	K.T	24 yr	M	France	2.3 mg/dL	MPGN	Rituximab	3 yr	Renal replacement therapy
Kamar *et al*[52]	K.T	28 yr	M	France	2.4 mg/dL	ATN	None	3 mo	Serum creatinine returned to baseline
Del Bello *et al*[32]	K.T	46 yr	M	France	2 mg/dL	MPGN	Ribavarin 30 mo	12 mo	Improved serum creatinine

NR: Not reported; eGFR: Estimated glomerular filtration rate; I.C: Immunocompromised; K.T: Kidney transplant; M: Male; F: Female; ATN: Acute tubular necrosis; MPGN: Membranoproliferative glomerulonephritis; IgAN: IgA nephropathy.

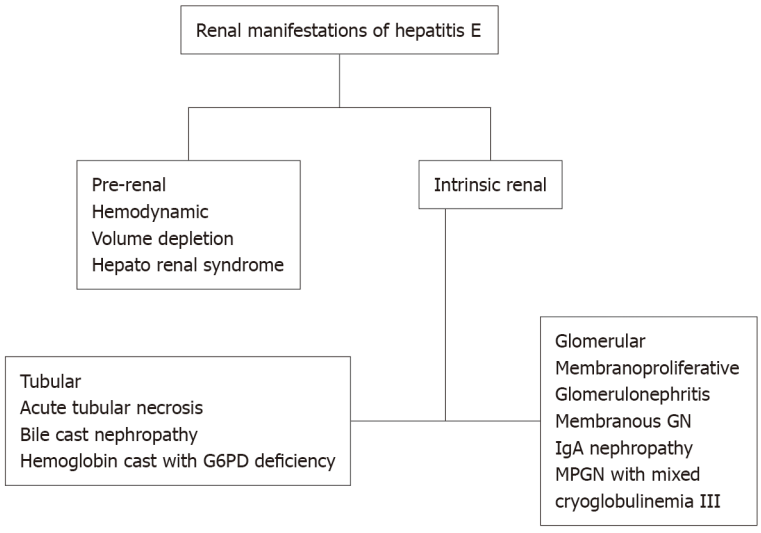

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Figure 1 Causes of acute kidney injury in acute hepatitis E virus-infected patients.

PATHOPHYSIOLOGY OF HEV-INDUCED RENAL INJURY

Pathophysiology of HEV-induced kidney injury is not completely known. HEV-mediated renal manifestations were thought to be a result of direct cytopathic injury due to the viral infection per se or related to immune-mediated mechanisms. Similar to HBV and HCV, it is hypothesized that HEV plays a role in precipitating glomerular injury through immune complex-mediated mechanisms[35]. The study by El- Mokhtar *et al*[36] assessed the role of immune-mediated mechanisms in HEV-induced renal dysfunction. CD10 and CD13 positive proximal tubular epithelial cells were isolated and challenged *in vitro* with HEV inoculum. HEV infection minimally upregulated inflammatory markers in the absence

of peripheral blood mononuclear cells, and no measurable changes were noted in lactate dehydrogenase (LDH) levels, kidney injury molecules, or transcription of chemokines. However, when the HEV infected proximal tubular cells were inoculated with peripheral blood mononuclear cells, there was upregulation of inflammatory molecules, kidney injury markers, and LDH levels, indicating that HEV infection per se might not be completely responsible for glomerular injury. Thus, it is the intersection between immune cells, HEV infection, and proximal tubular epithelial cells that contribute to renal injury[36].

MANAGEMENT OF RENAL MANIFESTATIONS OF HEV INFECTION

Diagnostics

Over the recent years, HEV laboratory testing has been refined drastically. Two main methods for testing HEV currently are indirect and direct serological tests. With regards to indirect studies, there are commercially available kits for serological testing for the presence of anti-HEV IgM and anti-HEV IgG that relies on the presence of antibodies in the serum to detect infection[37]. In addition, indirect studies rely heavily on patient's immune response to HEV infection, decreasing sensitivity in immunocompromised patients to some degree[38]. Direct testing predominately uses more advanced nucleic acid testing, that works *via* detecting the presence of viral genetic material in the form of nucleic acid sequences (HEV RNA) to determine the presence or absence of infection along with detection of viral capsid antigens[39,40].

In Immunocompetent patients, it is advised to check anti-HEV IgM initially for suspected HEV infection[41]. A negative test rules out the disease, however, if the test is positive, HEV RNA analysis is needed. On the other hand, among immunocompromised patients, it is recommended to test HEV-RNA even with negative anti-HEV IgM in blood and in stool before ruling out HEV infection[37]. Urine studies and electrolytes give subtle clues in identifying various causes of AKI. Urine microscopy adds an additional advantage to diagnose patients with acute tubular necrosis in the presence of muddy brown granular cast. Kidney biopsy remains the gold standard diagnostic testing for glomerular disorders and tubular obstructions, including bile cast nephropathy, while evaluating renal manifestations of HEV. Patients with acute or chronic hepatitis with new-onset proteinuria should be considered for kidney biopsy[42].

Treatment

Management of HEV-associated renal manifestations depends on the clinical presentation. Treatment is predominantly based on a conservative approach given benign course of the disease. Acute infection with HEV usually does not require anti-viral therapy. In patients with severe acute infection or acute on chronic liver disease, ribavirin therapy is considered[42]. For patients with acute kidney injury secondary to acute tubular necrosis or bile cast nephropathy, routine care to maintain mean arterial pressures, avoid nephrotoxic agents, and further insults are recommended. Indications for initiation of renal replacement therapy are similar to routine indications of dialysis initiation. Management of HEV-associated glomerular disorders should be based on underlying pathology. Guinault *et al*[33] reported a case of HEV–induced cryoglobulinemic glomerulonephritis in an immunocompetent patient with serum monoclonal IgG k light chain type II cryoglobulin. Renal biopsy results were consistent with lobular membranoproliferative exudative glomerulonephritis with fibrinoid necrosis and cellular crescents with a ruptured Bowman capsule. The patient was subsequently treated with seven sessions of plasma exchange along with pulse steroids with improvement in HEV RNA titers and cryoglobulinemic levels. Occasionally acute HEV infection follows a fulminant course as reported in pregnant individuals and could manifest as acute cerebral edema, seizures, acute fatty liver and are associated with increased mortality[43].

While managing patients with solid organ transplants, benefits of treatment need to be weighed against risks of rejection. Reduction of immunosuppression is considered the first-line approach[44], allowing HEV clearance in about one-third of patients. Ribavirin, an anti-viral agent, is considered in patients with severe acute or acute on chronic liver failure[45,46]. It has also been postulated that ribavirin acts by inhibiting HEV viral replication and increases the expression of interferon stimulating genes leading to immune modulation[47]. In a study done by Kamar *et al*[34], patients who received anti-viral therapy with ribavirin, cryoglobulinemia was detected in 70% of patients before therapy and became undetectable in all patients after viral clearance. Ribavirin is also used successfully to treat HEV-associated membranoproliferative glomerulonephritis in a solid organ transplant recipient[32] (Figure 2).

In a multicenter retrospective study by Karmer *et al*, solid-organ transplant recipients were treated with ribavirin at a median dose of 600 (range, 29-1200) mg/d for three months. Similar virological remission was observed in patients who received ribavirin for three months as compared to those who were treated for more than three months. In patients with detectable HEV RNA in the serum and/or in the stool, at the end of three months, ribavirin monotherapy can be continued for an additional three months[48] Hence it is indicated to treat with ribavirin initially for three months and evaluate the

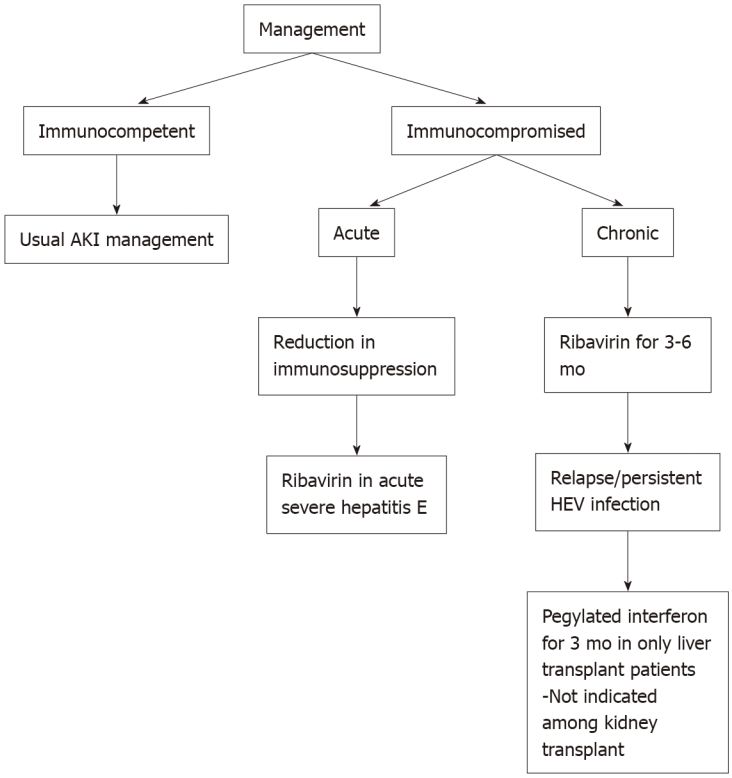

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Figure 2 Management of acute kidney injury in acute hepatitis E infected patients.

response. With non-sustained virological remission, ribavirin is recommended to be continued for a total of 6 mo. Among liver transplant recipients, interferon (IFN) α has shown to achieve sustained virological remission among patients with HEV after liver transplant. However, the use of IFNα is not recommended among other solid organ transplant recipients due to the risk of graft rejection (Table 1).

Sofosbuvir, a nucleotide analog, is evaluated along with ribavirin in patients who failed ribavirin monotherapy. Wezel *et al*[49] evaluated two solid organ transplant recipients who failed ribavirin monotherapy and observed that sofosbuvir showed variable antiviral activity in chronic HEV patients. Sofosbuvir was ineffective in achieving sustained virological response. Pegylated IFNα has shown efficacy in achieving a sustained virological response in patients with hemodialysis and liver transplants [50]. However, given the concern of interference with graft and risk of acute rejection, interferon α is contraindicated in patients with other solid organ transplants[47].

CONCLUSION

HEV infection is a global health concern and is uncommonly associated with mortality and morbidity. HEV infection is restricted not only to developing countries, but is increasingly identified among developed countries. Renal manifestations of HEV range from prerenal failure, acute tubular necrosis, glomerular disorders, and intratubular obstruction form bile cast nephropathy. Similar to HBV and HCV infections, immune-mediated mechanisms are hypothesized in development of HEV-associated glomerular diseases. Conservative approach is routinely employed in cases of renal involvement from acute hepatitis in immunocompetent patients. Among solid organ transplant recipients, ribavirin is considered in patients with chronic HEV infection for a duration of 3-6 mo along with reduction of immunosuppression. IFNα has shown to achieve sustained virological remission among patients with HEV after liver transplant. However, the use of IFNα is not recommended among other solid organ transplant recipients secondary to the risk of graft rejection. In patients who failed monotherapy with ribavirin, sofosbuvir has been evaluated in conjunction with ribavirin with variable anti-viral effects. Plasma exchange, in addition to pulse steroids is occasionally used in management of crescentic glomerular nephritis associated with HEV infection.

FOOTNOTES

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MINIREVIEWS

Safety of direct acting antiviral treatment for hepatitis C in oncologic setting: A clinical experience and a literature review

Anna Maria Spera

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Abstract

With a globally estimated 58 million people affected by, chronic hepatitis C virus (HCV) infection still represents a hard challenge for scientific community. A chronic course can occur among patients with a weak innate ad adaptive response with cirrhosis and malignancies as main consequences. Oncologic patients undergoing chemotherapy represent a special immunocompromised population predisposed to HCV reactivation (HCVr) with undesirable changes in cancer treatment and outcome. Aim of the study highlight the possibility of HCVr in oncologic population eligible to chemotherapy and its threatening consequences on cancer treatment; underline the importance of HCV screening before oncologic therapy and the utility of direct aging antivirals (DAAs). A comprehensive overview of scientific literature has been made. Terms searched in PubMed were: "HCV reactivation in oncologic setting" "HCV screening", "second generation DAAs". Pharmacokinetic and Pharmacodynamics characteristics of DAAs are reported, along with drug - drug interactions among chemotherapeutic drug classes regimens and DAAs. Clinical trials conducted among oncologic adults with HCV infection eligible to both chemotherapy and DAAs were analyzed. Viral eradication with DAAs in oncologic patients affected by HCV infection is safe and helps liver recovery, allowing the initiation of cancer treatment no compromising its course and success.

Key Words: Hepatitis C Virus; Direct aging antivirals; Drug interactions; Pharmacodynamic; Pharmacokinetic; Pre-emptive therapy

Core Tip: Chronic hepatitis C virus (HCV) infection is a hard clinical challenge, especially regarding oncologic patients eligible to chemotherapy. HCV reactivation in this setting of population is due to iatrogenic immunosuppression and can impair cancer treatment and outcome. Several specialists still do not prescribe direct aging antivirals to oncologic patients affected by HCV infection, because no univocal guidelines on HCV treatment in oncologic setting are available. The review highlights the importance of screening HCV infection before starting oncologic treatment, the safety of direct aging antivirals treatment under chemotherapy and the utility of treating HCV infection in oncologic setting no compromising chemotherapy course and success.

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INTRODUCTION

Hepatitis C is a viral infection due to a single-stranded RNA enveloped virus, with a mainly hepatic trophism. Eight genotypes of hepatitis C virus (HCV) along with several different subtypes have been identified[1,2]. Since its discovery, in 1989, 184 million patients with hepatitis C have been reported worldwide[3], and 40% of hepatic transplantations performed until 2009 were due to HCV-based liver cirrhosis[1]. According to the World Health Organization, an estimated 58 million people worldwide live with chronic HCV infection in 2021, with approximately 1.5 million new infections occurring per year, and approximately 400000 people died from hepatitis C, mostly from cirrhosis and hepatocellular carcinoma, in 2019[3].

BIOLOGICAL COURSE OF HCV INFECTION

The interplay between viral replication and a patient's immune response determines the biological course of HCV infection[4], considering that viral immune tropism secondary to hepatocyteinfection activates the innate and adaptive immune systems.

The typical outcome of primary infection in immunocompetent subjects is a self-limited illness with spontaneous resolution after an acute phase, characterized by host-protective antibody production. Otherwise, a chronic course of hepatitis has been often described in exposed patients, with weak innate and adaptive immune responses determining an insufficient reduction in viral load, despite concomitant liver function recovery[4]. Consequences of chronicity are cirrhosis and hepatocarcinogenesis[5,6], along with haematologic malignancies, including B cell non-Hodgkin's lymphoma[7], intrahepatic cholangiocarcinoma and other solid tumours, such as head and neck, colorectal, renal, and pancreatic cancers[7,8,9]. Any kind of immune central reconstitution after immunosuppressive medication can trigger viral reactivation in this chronic setting of HCV, with diversified clinical manifestations ranging from asymptomatic flares of transaminases to severe liver damage[4].

Approximately two weeks before hepatitis flares, an increase in viral RNA often occurs[4]. Hepatitis C reactivation is therefore defined by an increase in HCV-RNA > 1 Log IU/mL over baseline, while the detection of anti-HCV antibodies cannot help in distinguishing between acute and chronic infection but can determine only the occurrence of an infection[10]. Early identification of HCV infection and/or its reactivation can be merely ensured only by liver function testing and anti-HCV and viral load level surveillance.

HCV REACTIVATION IN AN ONCOLOGIC SETTING

According to Rung Li *et al*[11], HCV reactivation (HCVr) in an oncologic setting is promoted by immunosuppression due to chemotherapy, often resulting in deleterious changes in the cancer treatment plan and its outcomes. HCVr prevalence rates in cancer patients receiving chemotherapy range from 1.5% to 32% worldwide[12]. Although less fearful than HBV reactivation, HCVr is challenging for oncologists and HCV treating physicians, who often avoid administering antiviral treatment to patients under chemotherapy because of a lack of data about the safety of this treatment combination[12,10]. The multicentre, prospective cohort study performed by Ramsey and colleagues[13] among more than 5000 new oncologic patients found an observed infection rate of 2.4% (95%CI: 1.9% to 3.0%) for HCV, with a substantial proportion of patients being unaware of their viral status at the time

of cancer diagnosis (31%) and having no identifiable related risk factors (32.4%). Finally, according to this cohort study, therapeutic decisions were changed in 8% of patients because of their viral status[4, 13]. In an observational study conducted at MD Anderson Cancer Center, an HCVr rate of 23% was estimated among patients with cancer (36% in haematologic and 10% in solid tumour settings), with a more frequent recurrence in patients with prolonged lymphopenia (median 95 *vs* 22 d, $P < 0.001$) and in patients receiving rituximab (44% *vs* 9%), bendamustine (22% *vs* 0%), high-dose steroids (57% *vs* 21%) and purine analogues (22% *vs* 5%). The study also showed an unanticipated discontinuation or dose reduction of chemotherapy for 26% (6 of 23) of oncologic patients with HCVr[4]. In both studies, it was concluded that the early identification and treatment of chronic HCV hepatitis prevent HCVr after iatrogenic immunodepression and the remodulation of chemotherapy itself. Thus, screening for HCV infection before cancer treatment appears to be useful and advisable. Figure 1 shows an HCV screening recommendation flowchart for oncologic patients eligible for chemotherapy.

HCV INFECTION THERAPEUTIC STRATEGIES

HCV infection therapeutic strategies have changed over time[2]. The first therapeutic combination employed against HCV infection in 1990 was based on interferon (IFN) plus ribavirin, which was associated with suboptimal response rates and short- and long-term toxicity even related to drug-to-drug interactions with other medications taken[14]. Moreover, because of intrinsic contraindications for each element of the compound, patients with unbalanced mood unbalanced or anaemia were excluded from the treatment[14]. The first direct-acting antivirals (DAAs), boceprevir and telaprevir, were approved in 2011; since then, the HCV cure rates have markedly improved, and they have been added to the classic dual therapy represented by IFN + ribavirin[15]. After the introduction of the combined regimens based on glecaprevir/pibrentasvir [Glecaprevir (GLE)/Pibrentasvir (PIB)], sofosbuvir/velpatasvir [(SOF)/Velpatasvir (VEL)] with, or without voxilaprevir (VOX), and elbasvir/grazoprevir [Elbasvir (EBR)/Grazoprevir (GZR)], summarized in Table 1, the majority of chronic HCV-infected patients have been treated since 2015, achieving sustained virologic response (SVR)[16-20].

PHARMACOKINETIC CHARACTERISTICS OF CURRENTLY USED DAAS

In relation to the pharmacokinetic characteristics of currently used DAAs, the time to maximal plasma concentration (tmax), maximal plasma concentration (cmax), area under the concentration time curve (AUC) and minimal plasma concentration (cmin) are considered with regard to absorption, while the apparent volume of distribution (Vd/L) and percentage of protein binding are considered in relation to distribution. Metabolism is described in terms of the type of substrate elicited by DAAs and excretion as the elimination half-life (T ½)[1].

The pharmacokinetic characteristics of currently used DAAs are summarized in Table 2.

EBR/ GZR

Absorption: EBR is a substrate of P-gp, with a median tmax of 3 h and a range of 3-6 h. The bioavailability is estimated approximately 32%. Absorption (AUC 11% and Cmax 15%) can be decreased by a high-fat meal (900 kcal; 500 kcal fat). GZR acts as a substrate for P-gp and has a median tmax of 2 h with a range of 0.5-3 h. The absolute bioavailability varies from 15 to 27% after a single dose and from 20 to 40% after multiple doses. Absorption (AUC 50% and Cmax 108%) can be increased by a high-fat meal (900 kcal; 500 kcal fat). HCV-infected patients have increased exposure (approximately 2-fold) compared with healthy individuals. Steady state is reached at approximately the sixth day of administration[21, 22].

Distribution: EBR and GZR are highly bound to albumin for > 99.9% and to α1-acid glycoprotein for > 98.8%[23,24]. The estimated Vd/L values for EBR and GZR are 680 and 1250 L, respectively. The hepatic transporter OATP1B1/3 actively transports GZR[25]. EBR inhibits P-gp. EBR and GZR inhibit BCRP[21, 22].

Metabolism: EBR and GZR are metabolized by CYP3A4, but no circulating metabolites can be found in plasma. CYP3A4 is weakly inhibited by GZR[21,22].

Excretion: EBR and GZR are excreted mainly by liver; more than 99% of the excreted dose can be found in faeces. The apparent t1/2 of EBR and GZR is 24 and 31 h[21,22].

SOF/VEL

Absorption: The SOF Cmax after administration is 0.5-1 h. The AUC∞ of SOF can be increased by 60% and 78% by a moderate- and high-fat meal, respectively. However, the SOF Cmax is not affected by food [23,24]. The VEL median tmax is estimated around 3 h, while the AUC and Cmax values are lower in healthy volunteers (41% and 37%), when compared to those of HCV-infected subjects. The AUC of VEL

Table 1 Currently used direct aging antiviral characteristics

Trade name	Compound	Year of FDA/EMA approval	Mechanism of action	Pharmaceutical form	Dose	Genotypes
Zepatier	Elbasvir/grazoprevir	2016	NS5A inhibitor/protease inhibitor	Film-coated tablet	50 mg/100 mg qd	1a, 1b, 4
Epclusa	Sofosbuvir/velpatasvir	2016	NS5B inhibitor/ NS5A inhibitor	Film-coated tablet	400 mg/100 mg	Pangenotypic
Maviret	Glecaprevir/pibrentasvir	2017	Protease inhibitor/NS5A inhibitor	Film-coated tablet	100 mg/40 mg qd	Pangenotypic
Vosevi	Sofosbuvir/velpatasvir/voxilaprevir	2018/2017	NS5B inhibitor /NS5A inhibitor/protease inhibitor	Film-coated tablet	400 mg/100 mg/100 mg	Pangenotypic

DAA: Direct aging antiviral; FDA: Food and drug administration; EMA: European Medicines Agency.

Table 2 Pharmacokinetics of currently used direct aging antivirals

DAAs		Absorption				Distribution		Metabolism	Excretion
Tradename	Compound	*Tmax* (h)	*Cmax* (ng/mL)	*Cmin* (ng/mL)	AUC (ng·h/mL)	Vd/F	Protein binding (%)	Substrate of	T ½, (h)
Zepatier	Elbasvir	3	121	48.4	1920	680	> 99.9	P-gp	31
	Grazoprevir	2	165	18.0	1420	1250	> 98.8	P-gp	24
Epclusa	Sofosbuvir	0.5-1/3	566/868	NR	1260/13970	NR	61-65 minim	P-gp and BCRP	0.5/25
	Velpatasvir	4	311	NR	2970	NR	> 99.5	P-gp, OATP1B, and BCRP	15
Maviret	Glecaprevir	5.0	597	NR	4800	NR	97	P-gp	6-9
	Pibrentasvir	5.0	110	NR	1430	NR	> 99.9	P-gp	23-29
Vosevi	Sofosbuvir	2/4	678/744	NR	1665/12,834	NR	61-65 minim	P-gp and BCRP	0.5/29
	Velpatasvir	4	311	NR	4041	NR	> 99	P-gp, OATP1B1/3, and BCRP	17
	Voxilaprevir	4	192	47	2577	NR	> 99	P-gp and BCRP	33

DAAs: Direct aging antivirals; NR: Data not reported and/or available; T ½: Elimination half time.

can be increased after a moderate- (600 kcal; 30% fat) and high-fat (800 kcal; 50% fat) meals, while the Cmax increases by only 34% and 5%, respectively. The solubility of VEL is pH-dependent: In fact the increase of pH determines a reduction in solubility and absorption[23,24].

Distribution: Circulation proteins highly protein bind VEL (> 99.5%), regardless of the concentration range 0.09-1.8 μg/mL of the drug. SOF acts as a substrate of BCRP and P-gp. VEL acts as a substrate of BCRP, P-gp and OATP1B[25,26]. Plasma proteins that are not dose-dependent (1-20 μg/mL) bind SOF at 61%-65%[23,24].

Metabolism: VEL is metabolized by CYP2B6, CYP2C8, and CYP3A4, but > 98% of the parent drug can be found in the blood after a single dose. VEL inhibits P-gp, BCRP, and OATP1B1/3[23,24]. Refers to the SOF/VEL/VOX paragraph for SOF metabolism.

Excretion: The clearance of VEL is mainly hepatic, VEL is retrieved in faeces for > 94% and in urine for 0.4%. The t1/2 of VEL is approximately 15 h[25,26]. SOF is mainly excreted by kidneys (80%) as GS-331007 (78%). The t1/2 of SOF is 0.5 h, while the t1/2 of GS-331007 is 25 h[23,24].

GLE/PIB

Absorption: The tmax of GLE/PIB is about 5 h. Fat meals (moderate and high) can increase the absorption of GLE/PIB: The exposure of GLE after a meal is increased 83%-163% and the exposure of PIB is increased 40%-53%. Both drugs are P-gp substrates[25,26].

Distribution: Plasma proteins highly bind 97.5% to GLE and > 99.9% to PIB, both of drugs are actively

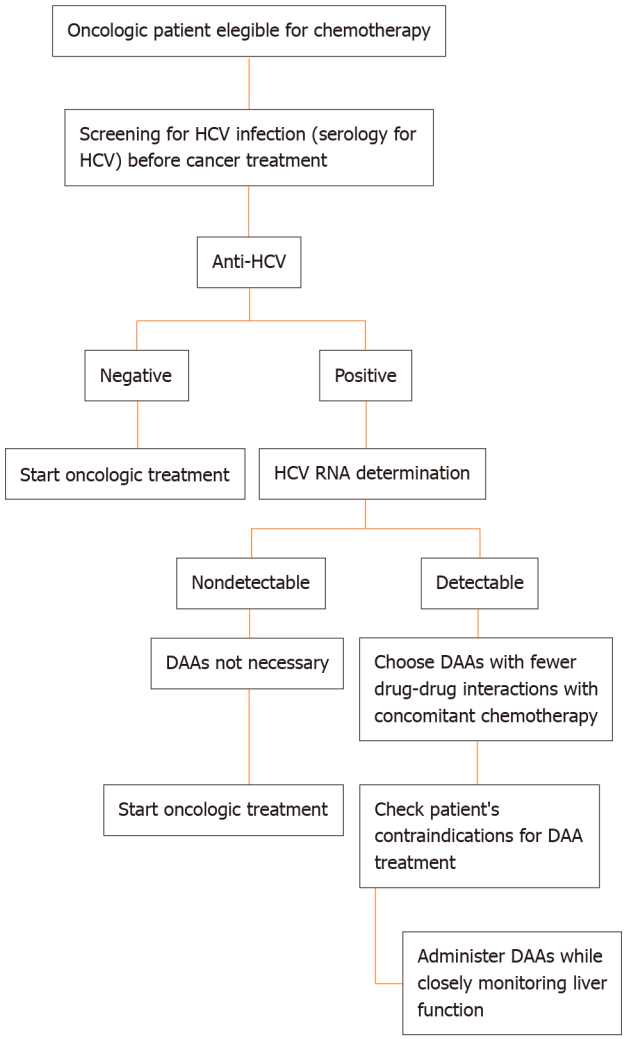

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Figure 1 Hepatitis C virus screening flowchart for oncologic patients eligible for chemotherapy. HCV: Hepatitis C virus; DAA: Direct aging antiviral.

transported by BCRP. GLE constitutes also a substrate of OATP1B1/3[25,26].

Metabolism: GLE is metabolized by CYP3A4, and PIB does not undergo biotransformation[25,26].

Excretion: GLE is primarily excreted by the liver; in fact, 92.1% of a radioactive dose is retrieved in faeces. The t1/2 is 6-9 h at steady state. PIB is also primarily found in stool (96.6%), with a t1/2 of 23–29 h [25,26].

SOF/VEL/VOX

Absorption: The Cmax of VOX, VEL, and a major metabolite of SOF, namely, GS-331007 is reached after approximately 4 h; the Cmax of SOF is reached after 2 h. The AUC and Cmax of VEL are 41% and 39% decreased in patients, respectively, while the AUC and Cmax of VOX are both elevated by 260% when comparing HCV-infected individuals and healthy volunteers[27,28]. The AUC∞ and Cmax of SOF increasefrom 64 to 114% and 9% to 76%, respectively, after a meal. The Cmax of GS-331007 after a meal decreases (19%-35%). The AUC∞ and Cmax of VEL increase (40%-166% and 37%-187%, respectively). The AUC of VOX increases from 112% to 435%, while the Cmax of VOX increases from 147% to 680% [27,28].

Distribution: Plasma proteins highly bind to SOF, VEL, and VOX (61%-65%, > 99%, and > 99%, respectively), with a concentration independent pharmacokinetics (ranging from 1 to 20 and 0.09 to 1.8 μg/mL, respectively) for SOF and VEL. SOF acts as a substrate of P-gp and BCRP, while VEL acts as a substrate of P-gp, OATP1B1/3, and BCRP. Finally, VOX acts as a substrate of P-gp and BCRP[27,28].

Metabolism: VOX is a substrate of CYP3A4. VOX is an inhibitor of P-gp, BCRP, and OATP1B1/3[27, 28]. The metabolism of SOF and VEL is reported in the paragraph on SOF/VEL combination therapy

above.

Excretion: SOF is excreted by the kidneys (80%), mainly in the form of GS-331007 (78%). The t1/2 of SOF is 0.5 h and the t1/2 of GS-331007 is 29 h[28,29]. The clearance of VEL is mainly hepatic. The t1/2 of VEL is approximately 17 h (27). The excretion is mainly biliary[27,28].

PHARMACODYNAMICS OF CURRENTLY USED DAAS

Intended as the balance between the effect (reduction of HCV-RNA under therapy) and toxicity (adverse effects), the pharmacodynamics of currently used DAAs consist of the duration of therapy, safety profile and estimated adverse effects.

EBR/GZR is efficacious for subjects affected by genotypes 1 and 4 HCV infection treated for 12 wk. EBR/GZR is approved for patients with renal insufficiency and compensated cirrhosis. This combination is approved in the fixed dose combination of 50 mg/100 mg once daily. The favourable safety profile with low discontinuation rates (< 5%) makes this compound suitable for HCV-infected patients with genotypes 1 and 4. The most frequent adverse effects are fatigue, headache, asthenia, nausea, rash, and an increase in ALT/AST and ALP[1,21].

SOF/VEL combination for 12 wk is valid in HCV pangenotypic patients treatment-experienced and/or treatment-naïve. Mild described adverse events are headache, fatigue, nausea and insomnia. Combination therapy with ribavirin leads to anaemia in over 10% of patients[1,24].

GLE/PIB is a pangenotypic regimen that is highly effective when administered for 8 to 12 wk once daily at doses of 100 mg/40 mg. Naïve and experienced patients with or without cirrhosis can be treated with this compound, whichhas a mild toxicity profile, in which headache, fatigue, nasopharyngitis and nausea can arise[1,25].

Finally, the pangenotypic highly effective SOF/VEL/VOX combination is licenced for patients who fail to respond to IFN/riba and DAAs and those with or without compensated cirrhosis. The adverse effects described are headache, diarrhoea, fatigue, nausea and constipation[1,27].

The pharmacodynamic properties of currently used DAAs are summarized in Table 3.

DRUG TO DRUG INTERACTIONS

Drug-drug interactions are challenging in the course of cotreatment with chemo-therapy and DAAs because most of these compounds are substrates and inhibitors of drug transporters and CYP enzymes [7]. Consulting the HEP drug interaction website can be extremely useful for clinical decision-making [29]: A report listing the summaries of potential interactions (*i.e.*, "red", "amber" and “yellow” classifications) for the drugs considered can be downloaded to guide the choice on a case-by-case basis. Potential interactions between currently used DAAs and the following drug classes of chemotherapy regimens are reported in this review: Platinum-containing agents (cisplatin, carboplatin, oxaliplatin), folate antagonists (methotrexate, pemetrexed), pyrimidine compounds (fluorouracil, capecitabine, cytarabine, gemcitabine, decitabine), purine analogues (mercaptopurine, fludarabine, cladribine, clofarabine), alkylating agents (cyclophosphamide, ifosfamide, melphalan, bendamustine, busulfan), anthracyclines (daunorubicin, doxorubicin, epirubicin, idarubicin, bleomycin), topoisomerases (topotecan, etoposide, irinotecan), cytidine analogues (azacytidine, decitabine), immunosuppressants (tacrolimus, cyclosporine), immunomodulatory drugs (lenalidomide, thalidomide), mitotic inhibitors (paclitaxel, docetaxel, vinblastine, vincristine), hormonal therapies (tamoxifen), targeted therapies other than rituximab (*e.g.*, cetuximab, bortezomib, alemtuzumab). Interactions between DAAs and the main oncologic therapeutic categories considered in this review are summarized in Table 4.

According to the Liverpool HEP chart, drugs that absolutely should not be coadministered (RED interactions) are as follows: Elbasvir/grazoprevir + immunosuppressants (cyclosporine): Concomitant use of elbasvir/grazoprevir with OATP1B inhibitors, such as cyclosporine, is contraindicated. The coadministration of multiple doses of elbasvir/grazoprevir and a single dose of cyclosporin increases the grazoprevir AUC by 15-fold. The risk of ALT elevations may be increased due to the significant increase in grazoprevir plasma concentrations caused by OATP1B1/3 inhibition[29].

Sofosbuvir/velpatasvir/voxilaprevir + folate antagonists (methotrexate): Coadministration has not been studied but would not be recommended due to increased exposure tomethotrexate due to BCRP inhibition by voxilaprevir[29]. Sofos-buvir/velpatasvir/voxilaprevir + immunosuppressants (cyclosporine): Coadministration has been studied with sofosbuvir, velpatasvir or voxilaprevir, and coadministration with sofosbuvir/velpatasvir/voxilaprevir is not recommended. Concentrations of voxilaprevir increased by 19.0-fold due to OATP1B1 inhibition by cyclosporine. The safety of this increase has not been established[29].

According to the Liverpool HEP chart, potential clinically significant interactions-likely to require additional monitoring and an alteration of drug dosage or the timing of administration (AMBER

Table 3 Pharmacodynamics of currently used direct aging antivirals

Trade name	Compound	Efficacy	Toxicity
Zepatier	Elbasvir/grazoprevir	Effective regimen used for 12 wk against HCV genotype 1 and 4. Approved for patients with renal insufficiency and compensated cirrhosis. Fixed dose combination of 50 mg/100 mg once daily. Favourable safety profile with low discontinuation rates (< 5%)	Fatigue, headache, asthenia, nausea, rash, ALT/AST and ALP increase
Epclusa	Sofosbuvir/velpatasvir	Treatment for 12 wk highly effective in both treatment-experienced and treatment-naïve HCV pangenotypic patients	Fatigue, headache, nausea and insomnia. Combination therapy with ribavirin led to anaemia in over 10% of patients
Maviret	Glecaprevir/pibrentasvir	Pangenotypic highly effective regimen. Administered for 8 to 12 wk once daily at doses of 100 mg/40 mg. Naïve and experienced patients with or without cirrhosis	Headache, fatigue, nasopharyngitis and nausea
Vosevi	Sofosbuvir/velpatasvir/voxilaprevir	Pangenotypic, highly effective, licenced for patients in whom IFN/riba and DAAs *failed*	Headache, diarrhoea, fatigue, nausea and constipation

ALT: Alanine transaminase; AST: Aspartate transaminase; DAAs: Direct aging antivirals; HCV: Hepatitis C virus; IFN: Interferon.

Table 4 Chemotherapy drug classes employed

Chemotherapy drug classes	Examples
Platinum-containing agents	(Cisplatin, carboplatin, oxaliplatin)
Folate antagonists	(Methotrexate, pemetrexed)
Pyrimidine compounds	(Fluorouracil, capecitabine, cytarabine, gemcitabine, decitabine)
Purine analogues	(Mercaptopurine, fludarabine, cladribine, clofarabine)
Alkylating agents	(Cyclophosphamide, ifosfamide, melphalan, bendamustine, busulfan)
Anthracyclines	(Daunorubicin, doxorubicin, epirubicin, idarubicin, bleomycin)
Topoisomerases	(Topotecan, etoposide, irinotecan)
Cytidine analogues	(Azacytidine, decitabine)
Immunosuppressants	(Tacrolimus, cyclosporine)
Immunomodulatory drugs	(Ienalidomide, thalidomide)
Mitotic inhibitors	(Paclitaxel, docetaxel, vinblastine, vincristine)
Hormonal therapies	(Tamoxifen)
Targeted therapies other than rituximab	(*e.g.*, cetuximab, bortezomib, alemtuzumab)

interactions)-are described among the following: Elbasvir/grazoprevir + folate antagonists (methotrexate): Coadministration has not been studied. Methotrexate is a substrate of BCRP, and concentrations could increase due to inhibition by elbasvir/grazoprevir. No a priori dose alteration is recommended, but patients should be closely monitored[29]. Sofosbuvir/velpatasvir + folate antagonists (methotrexate): Coadministration has not been studied. Methotrexate is a substrate of BCRP, and concentrations may increase due to inhibition by sofosbuvir/velpatasvir. Although no a priori dose alteration is required, close monitoring is recommended[29]. Glecaprevir/pibrentasvir + immunosuppressants (cyclosporine): Concomitant use of glecaprevir/pibrentasvir with cyclosporine requires close monitoring of doses, as concentrations of glecaprevir/pibrentasvir may increase due to the inhibition of OATP1B. The coadministration of gleca-previr/pibrentasvir and cyclosporine (100 mg) increased glecaprevir/pibrentasvir concentrations within acceptable parameters (glecaprevir Cmax, AUC and Cmin by 30%, 37% and 34%, respectively; no change in pibrentasvir Cmax and AUC, but Cmin increased by 26%). However, at higher doses of cyclosporine (400 mg), glecaprevir concentrations increased significantly (Cmax 4.51-fold, AUC 5.08-fold). Glecaprevir/pibrentasvir is not recommended for use in patients requiring stable cyclosporinedosesat 100 mg/d[29]. Glecaprevir/pibrentasvir + anthracyclines (doxorubicin): Coadministration has not been studied. Doxorubicin is metabolized by CYP enzymes and is a substrate for P-gp. Since gleca-previr/pibrentasvir inhibits P-gp and is a mild inhibitor of CYP3A4, there is the potential for increased doxorubicin exposure,and a clinically significant interaction has to be considered[29]. Glecaprevir/pibrentasvir + folate antagonists

(methotrexate): Coadministration has not been studied. Methotrexate is a substrate of BCRP, and concentrations could increase due to the inhibition of BCRP by gleca-previr/pibrentasvir. Patients should be closely monitored for methotrexate-associated toxicities[29]. Glecaprevir/pibrentasvir + immunosuppressants (tacrolimus): The coadministration of glecaprevir/pibrentasvir with systemic tacrolimus (1 mg single dose) increased tacrolimus Cmax and AUC by 1.5-fold and 1.45-fold, respectively. There was no change in the Cmax, AUC or Cmin of glecaprevir or pibrentasvir. As tacrolimus is a narrow therapeutic index drug, it should be used with caution. Therapeutic blood monitoring should be performed[29]. Sofos-buvir/velpatasvir/voxilaprevir + immunosuppressant (tacrolimus): Coadministration with sofosbuvir/velpatasvir/voxilaprevir has not been studied. No clinically significant drug interactions were observed with sofosbuvir and tacrolimus. The coadministration of tacrolimus (5 mg single dose) and sofosbuvir (400 mg single dose, n = 16) decreased tacrolimus Cmax by 27% and increased AUC by 9%; sofosbuvir Cmax decreased by 3% but AUC increased by 13%. No effect of velpatasvir or voxilaprevir is expected. However, in the absence of data, the monitoring of tacrolimus concentrations should be considered[29]. Elbasvir/grazoprevir + mitotic inhibitors (paclitaxel): Coadministration has not been studied. Paclitaxel is primarily metabolized by CYP2C8 and to a lesser extent by CYP3A4. Grazoprevir is a weak inhibitor of CYP3A4 and could potentially increase paclitaxel exposure. Paclitaxel-induced toxicity should be monitored[29]. Glecaprevir/pibrentasvir + mitotic inhibitors (paclitaxel): Coadministration has not been studied. Paclitaxel is primarily metabolized by CYP2C8 and to a lesser extent by CYP3A4. Glecaprevir is a weak inhibitor of CYP3A4 and could potentially increase paclitaxel exposure. Paclitaxel-induced toxicity should be monitored[29]. Elbasvir/grazoprevir + immunosuppressants (tacrolimus): The coadministration of elbasvir/grazoprevir with systemic tacrolimus increased tacrolimus AUC by 43% (due to weak inhibition of CYP3A4 by grazoprevir) but had no effect on the concentrations of grazoprevir and elbasvir. Frequent monitoring of tacrolimus whole-blood concentrations, changes in renal function, and tacrolimus-associated adverse events upon the initiation of coadministration is recommended[29].

According to the Liverpool HEP chart, potentially weak interactions-for which additional action/monitoring or dosage adjustment is unlikely to be required (YELLOW interactions)-are described among the following: Sofosbuvir/velpatasvir + hormonal therapies (tamoxifen): Coadministration has not been studied. Tamoxifen is mainly metabolized by CYP3A4 and CYP3A5, which are not affected by sofosbuvir/velpatasvir. However, tamoxifen induces CYP3A4 and could potentially decrease the concentrations of velpatasvir, although to a moderate extent. Coadministration with food is suggested if tamoxifen is coadministered with sofos-buvir/velpatasvir as this increases exposure to velpatasvir[29]. Sofosbuvir/ velpatasvir/voxilaprevir + hormonal therapies (tamoxifen): Coadministration has not been studied. Tamoxifen is mainly metabolized by CYP3A4 and CYP3A5, which are not affected by sofosbuvir/velpatasvir/voxilaprevir. However, tamoxifen induces CYP3A4 and could potentially decrease theconcentrations of velpatasvir and voxilaprevir, although to a moderate extent. Coadministration with food is suggested if tamoxifen is coadministered with sofosbuvir/ velpatasvir/voxilaprevir as this increases exposure tovelpatasvir and voxilaprevir[29].

Some comedications with a green classification may require dose adjustment due to hepatic impairment.

MANAGEMENT OF CHRONIC HCV INFECTION IN PATIENTS WITH CANCER

HCV-infected oncologic patients represent a special population needing guided treatment[12]: The updated guidelines provided by the AASL and IDSA[4] for the first time address treatment in this setting, supporting that the virologic and hepatic benefits of DAA treatment in oncologic patients with HCV infection overcome the risk of no treatment[7,30,31]. In fact, the quick eradication of chronic HCV infection prior to cancer therapy helps liver recovery, normalizes liver enzymes and avoids potentially decompensating hepatitis flares; in other words, it allows the initiation of cancer treatment that could be hampered by persistent elevated ALT levels due to HCV virus infection[12]. The eradication of HCV in oncologic patients can also diminish the risk of HCVr, allow patients to participate in experimental oncologic clinical trials based on new drug strategies against cancer, reduce the risk of the development of HCV-associated cancers[4], minimize drug-induced hepatotoxicity and avoid detrimental dose reduction.

DAA-based therapy can also promote liver disease progression[12]. In clinical practice, the temporary suspension of cancer treatment during DAA-based therapy has often been observed to avoid overlapping toxicities and DDIs. However, the present review proves that when cancer treatment cannot be interrupted, currently used DAAs can be simultaneously administered under close comonitoring by oncologists and hepatologists, especially during the first month of this dual therapy, since serious observed adverse events most usually appear within the first 2-4 wk of concomitant treatment.

CONCLUSION

Economides *et al*[12] stated that DAA therapy in cancer patients was efficacious and durable in terms of SVR, and few drug-drug interactions were observed. Otherwise, prospective data on HCV in oncologic patients remain limited.

This review, in the absence of current specific available guidelines for the use of DAA therapy in HCV-infected cancer patients, tried to clarify that treatment with DAAs for oncologic patients undergoing chemotherapy affected by HCV infection is safe and favourably impacts oncologic outcomes.

Finally, given that cancer treatment can negatively impact untreated chronic HCV-related liver disease, it appears clear that pre-emptive antiviral therapy in the oncologic setting is necessary to pursue chemotherapy without risking the progression of viral liver disease.

FOOTNOTES

Author contributions: Spera AM studied conception and design; data collection; analysis and interpretation of results; draft manuscript preparation; Spera AM finally reviewed the results and approved the final version of the manuscript.

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ORIGINAL ARTICLE

Basic Study

Fertaric acid amends bisphenol A-induced toxicity, DNA breakdown, and histopathological changes in the liver, kidney, and testis

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Abstract

BACKGROUND

Bisphenol A (BPA) is present in many plastic products and food packaging. On the other hand, fertaric acid (FA) is a hydroxycinnamic acid.

AIM

To investigate the effect of FA on BPA-related liver, kidney, and testis toxicity, DNA breakdown, and histopathology in male rats.

METHODS

Thirty male albino rats were divided into five equal groups (6 rats/group): Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control and paraffin oil groups were administered orally with 1 mL distilled water and 1 mL paraffin oil, respectively. The FA-, BPA-, and FA+ BPA-treated groups were administered orally with FA (45 mg/kg, bw) dissolved in 1 mL distilled water, BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, and FA (45 mg/kg, bw) followed by BPA (4 mg/kg, bw), respectively. All these treatments were given once a day for 6 wk.

RESULTS

BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3β-hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited the degradation of liver, kidney, and testis DNA content. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels.

CONCLUSION
FA ameliorates BPA-induced liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes.

Key Words: Bisphenol A; Fertaric acid; Liver; Kidney; Testis; Toxicity; DNA

Core Tip: BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3β-hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited DNA degradation in the liver, kidney, and testis. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels. Therefore, FA ameliorates BPA-induced liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes.

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INTRODUCTION

Industrial pollutants such as bisphenol A (BPA), octylphenols, and nonylphenols are known as endocrine-disrupting compounds[1]. BPA is present in many consumer plastic products, food packaging, and in the dentistry for the manufacturing of resin materials[2]. The burning of dumped waste in an open air transfers BPA from plastic waste into the environment. The human and animal exposure to BPA is rapid and continuous[3]. The world production of BPA was 1 million tons in the 1980s[4], which increased to more than 2.2 million tons in 2009[5] and became 3.6 million tons of BPA-derived chemicals in 2015[6]. BPA is released into the surrounding environment by pre-consumer and post-consumer leakage. The pre-consumer leakage into the environment is directly from staining manufacturers, coat, and plastics. The post-consumer BPA is from wastewater treatment plants, agriculture irrigation pipes, ocean-borne plastic trash, and papers or materials recycling companies[7]. BPA affects reproduction, growth, and development of aquatic invertebrates, amphibians, reptiles, and fish at lower doses (1μg/L to 1 mg/L)[8]. BPA is a precursor to important plastics such as plastic bottles including baby bottles, water bottles, and food storage containers. BPA is a monomer that is part of polycarbonates and epoxy resins. However, it can improve the properties of other plastics, which is why it is found in many objects. BPA is similar in its structure to estrogen. Therefore, it interacts with estrogen receptors (in the cell membrane and in the cytoplasm/nucleus). It plays an important role in cardiovascular physiology and diseases such as hypertension[9]. BPA weakened liver function by increasing alkaline phosphatase, aspartate and alanine aminotransferases, triglyceride, cholesterol, globulin, and total bilirubin levels. BPA caused kidney damage by increasing blood urea nitrogen and serum creatinine levels. Histology study exhibited damages of the liver and kidney. The apoptosis of liver and kidney cells was increased by exposure to BPA[10]. BPA decreased sperm quality and serum testosterone (Ts) level. Exposure to a low dose of BPA (0.2 μg/mL) impaired mouse sperm quality by damaging germ cell proliferation, leading to declined male fertility[11]. The dose used in this study (4 mg/kg/d) is not a high dose because the US Environmental Protection Agency (EPA) has calculated its human acceptable daily-intake level, known as the reference dose, by dividing the rodent "lowest effect" level of 50 mg/kg/d by 1000. This calculation is based on the assumption that humans are 10 times more sensitive than rodents to BPA exposure and a sensitive human is 10 times more sensitive than a typical human[12,13]. That is mean oral administration of 4 mg/kg/d in rats = oral administration of 4 μg/kg/d in human. Furthermore, BPA has been in use commercially for over 50 years, and workers producing BPA and its products (such as epoxy resins) have been exposed to an average air levels of 10 mg over decades[13], which is equal to double and half the dose used in this research.

Therefore, it becomes a challenging responsibility to find a safe and effective way to overcome the BPA toxicity in regions where BPA is already present in water bottles and food packaging and people are therefore exposed to BPA toxicity day and night. The use of herbal plants in the medicine has been known for a long time and today it has made a comeback in all over the world. This is because of their

minor side effects and good therapeutic effects. A large number of secondary metabolites derived from natural sources are currently undergoing evaluation in clinical trials. Fertaric acid (FA) is a hydroxycinnamic acid found in grapefruit[14]. It is formed by the binding of ferulic acid with tartaric acid. FA publications are very rare. Maier *et al*[15] developed a method for the isolation of FA as well as caftaric and coutaric acids from grape pomace. The purities of FA, caftaric acid, and coutaric acid were 90.4%, 97.0%, and 97.2%, respectively. Moreover, Koriem and Arbid[16] proved that FA ameliorated liver function, antioxidants, and inflammatory cytokines in the 4-tert-octylphenol-induced toxicity. In addition, Wetchakul *et al*[17] stated that Thai traditional preparation (Jatu-Phala-Tiga [JPT]; FA is a major constituent in JPT) exhibited strong antioxidant activities. Thus, FA is a promising agent for anti-aging and oxidative stress prevention. Furthermore, Lukić *et al*[18] used liquid chromatography with mass spectrometry method to determinate FA in 173 wines made from 4 red and 6 white grape varieties. Moreover, Abdallah *et al*[19] isolated FA with a protective effect in ameliorating liver function and antioxidants in t-BHP-induced HepG2 hepatic carcinoma cells. Additionally, FA occurs in vine seeds (*Vitis vinifera* L.) and it has antioxidant activity. FA is among 14 antioxidant components in grape seeds [20].

The aim of this study was to investigate the protective effect of FA in ameliorating oral BPA-induced toxicity, DNA breakdown, and histopathological changes in liver, kidney, and testis tissues in male rats.

MATERIALS AND METHODS

Materials

The kits used for the detection of liver function were obtained from Stanbio Laboratory, United States. The kidney function and serum electrolytes (sodium, potassium, and chloride) were measured with analytical kits from Bio-Diagnostics, United Kingdom. Ts, luteinizing hormone (LH), follicle stimulating hormone (FSH), and dehydroepiandrosterone sulfate (DHEA-SO_4) kits were purchased from BioSource Co., Nivelles, Belgium. The sex hormone binding globulin (SHBG), γ-glutamyl transpeptidase (γ-GT), glucose-6-phosphate dehydrogenase (G6PD), and 3β-hydroxysteroid dehydrogenase (3βHSD) kits were obtained from IBL Co., Hamburg, Germany. BPA (purity = 99%) was obtained from Sigma-Aldrich, United States, while FA (purity = 98.2%) was purchased from Riven International PVT, LTD, India.

Animals

The animal house of the National Research Centre (NRC), Giza, Egypt provided the necessary animals for this study. This study included male albino adult rats of *Spargue Dawley* strains (10 wk old, 120 ± 10g). These rats were preserved in plastic polycarbonate (without bisphenol A) cages [special cages were manufactured without PBA in Faculty of Agriculture, Cairo University, Giza, Egypt]. The rats were maintained with ordinary food and tap water. This research was started after the approval form was received from the ethical committee of NRC, Giza, Egypt and in accordance with the regulations for the suitable care and use of laboratory animals (NIH Publication No 85:23, revised 1985). The experimental conditions included 12 h light and 12 h dark cycle, laboratory temperature of 27-30 °C, and experimental room humidity of 40%-70%.

Experimental design

Thirty male albino rats were divided into six equal groups (6 rats/ group) as follows: Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control group was administered orally with 1 mL of distilled water once a day for 6 wk. The paraffin oil group was administered orally with 1 mL of paraffin oil once a day for 6 wk. Paraffin oil was chosen because this oil had no antioxidant activity in contrast to corn oil, olive oil, and safflower oil which contain vitamin E with an antioxidant effect. The FA-treated group was administered orally with FA (45 mg/kg body weight [bw])[16] dissolved in 1 mL of distilled water once a day for 6 wk. The BPA-treated group was administered orally with BPA (4 mg/kg, bw) [21] dissolved in 1 mL of paraffin oil once a day for 6 wk. The 4 mg/kg of BPA is equivalent to 10% of the LD_{50} of BPA; the median lethal dose (LD_{50}) of BPA is 40 mg/kg[22] and 10% of the LD_{50} is a safe dose[23,24]. The FA+ BPA-treated group was initially orally administered with FA (45 mg/kg, bw) dissolved in 1 mL of distilled water. After 1 h, the rats were administered orally with BPA (4 mg/kg, bw) dissolved in 1 mL of paraffin oil. Both FA and BPA were administered orally once a day for 6 wk.

The animals were observed daily for any clinical symptoms or animal death. During the experimental period, the food ingestion, water drinking, and body weight were calculated and recorded daily until the end of this study.

Determination of urine volume

The urine volume was determined according to the method of Kau *et al*[25], with minor modifications where urine of each rat was collected daily throughout the whole experiment and urine volume was calculated.

Blood sampling and handling

After 6 wk of the research, the blood samples were collected from the retro-orbital plexus of the animals. Then, the blood samples were transferred to capillary tubes. After the coagulation of the blood samples, the samples were centrifuged at 4000 rpm for 15 min to obtain the serum. These serum samples were stored at -80 °C for detection of liver and kidney function and male sex hormones.

Liver, kidney, and testicular tissue preparation

The next step following blood collection was the execution of the animals by cervical dislocation in this study. Liver, kidney, and testis tissues were collected from each group for histological and genetic analyses. Briefly, liver, kidney, and testis organs were taken and washed with saline solution. The filter papers were used to obtain dry liver, kidney, and testis organs. These organs were homogenized in a homogenizer apparatus for 30 min and the resulting liver, kidney, and testis homogenates were stored at -80 °C for the detection of liver, kidney, and testis DNA.

Biochemical investigation

Serum transaminases (AST and ALT) were determined according to Reitman and Frankel[26]. Serum alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined as described by Kind and King[27]. Serum γ-glutamyl transferase (γ-GT) activity was measured according to the method of Szasz [28]. Serum lactate dehydrogenase (LDH) activity was estimated according to the method of Weisshaar *et al*[29]. Serum total bilirubin determination was performed according to the method of Walter and Gerard[30]. Serum urea was calculated according to the method of Patton and Crouch[31]. Serum creatinine was determined by the kinetic method as described by Houot[32]. Serum uric acid was measured according to the method of Kabasakalian *et al*[33]. Blood urea nitrogen was estimated according to the method of Zhu *et al*[34]. Serum electrolytes (sodium, potassium, and chloride) were analyzed colorimetrically according to the methods of Jooste and Strydom[35], Wang *et al*[36], and Hassan *et al*[37], respectively. Urinary and testicular proteins were determined according to the method of Gornall *et al*[38]. Urinary albumin was measured using the method of Drupt[39]. Serum Ts was determined according to the method of Maruyama *et al*[40]. Serum LH was calculated using the method of Knobil[41]. Serum FSH was estimated according to the method of Odell *et al*[42]. Serum DHEA-SO_4 was obtained according to the method of De-Peretti and Forest[43]. Serum SHBG was evaluated according to the method of Selby[44]. Testicular G6PD was determined according to the method of Chan *et al*[45]. Testicular 3βHSD was calculated using the method of Talalay[46]. Testicular cholesterol level was estimated according to the method of Kim and Goldnerg[47].

Determination of DNA content in liver, kidney, and testis

Feulgen-stained slides were prepared for the nuclear DNA analysis using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, UK). The system was calibrated before each measurement session using the calibration slides provided with the system at high power magnification (400×). The optical density of the selected nuclei in each microscopic field was measured and automatically converted by the system into DNA content. The DNA fields were selected by the desired number of nuclei (100-150). The results are presented as a frequency histogram on the monitor by plotting the DNA content against the number of nuclei calculated. The DNA histograms were divided according to Danqu *et al*[48], Darzynkiewicz *et al*[49], Darzynkiewicz *et al*[50], and El-Gamal[51] into: (1) Diploid (DNA index ranging from 0.9-1.1), (2) tetraploid (DNA index ranging from 1.8-2.2), and (3) aneuploid (when at least 10% of the total events showed distinct abnormal peak outside the 2c or 4c) based on the amount of DNA related to the normal control. Liver, left kidney, and left testis tissues were used in DNA determination.

Histopathological investigation

The liver, right kidney, and right testis tissues were fixed in 10% formalin solution and then processed for routine technique by embedding in paraffin. The tissue blocks were sectioned (5 μm thick) and then stained with hematoxylin and eosin for histopathological examination under a light microscope.

Statistical analysis

The results obtained are expressed as the mean ± standard deviations (SD). Data distribution was tested by the Kolmogorov-Smirnov test. Statistical analyses were calculated through one-way analysis of variance (ANOVA) using SPSS program, followed by a *post-hoc* test using Tukey's analysis. A P value ≤ 0.05 was considered statistically significant.

RESULTS

Protective effect of FA on body weight, food and water intake, urine volume, and urinary protein, albumin, and albumin/protein ratio in rats exposed to BPA

The effect of FA on body weight, food and water intake, urine volume, and urinary protein and albumin in the BPA-treated group is shown in Table 1. BPA induced significant decrease in body weight, food intake, and water consumption while causing a significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. On the other side, FA oral administration with BPA administration increased body weight, food intake, and water consumption, but decreased urinary volume, protein, albumin, and albumin/globulin ratio in the BPA-treated group to approach the control levels. Furthermore, paraffin oil and FA oral administration showed an insignificant impact on body weight, food intake and water consumption, urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. There was not any edema, hair loss, death, or other clinical symptoms observed in animals throughout the experimental period of the study.

Protective effect of FA on liver, kidney, and testis toxicity following BPA exposure

The protective effect of FA on liver toxicity in BPA-treated rats is shown in Table 2. It is clear from the data in this table that the oral administration of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum AST, ALT, ALP, ACP, γGT, LDH, and bilirubin levels. On the contrary, the oral administration of BPA caused a highly significant decrease in serum ALP and ACP but a highly significant increase in serum AST, ALT, γGT, LDH, and bilirubin compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused an increase in serum ALP and ACP levels and a decrease in serum AST, ALT, γGT, LDH, and bilirubin levels compared to these liver parameters in the BPA-treated group.

The protective effect of FA on kidney toxicity and serum electrolytes in BPA-treated rats is shown in Table 3. It is clear from the data in this table that the oral intake of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum urea, creatinine, uric acid, sodium, potassium, and chloride levels, as well as blood urea nitrogen. On the contrary, the oral administration of BPA caused a highly significant increase in serum urea, creatinine, uric acid, and blood urea nitrogen but a highly significant decrease in serum sodium, potassium, and chloride levels compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused a decrease in serum urea, creatinine, uric acid, and blood urea nitrogen levels and an increase in serum sodium, potassium, and chloride levels compared to these kidney parameters in the BPA-treated group.

The protective effect of FA on male sex hormones in BPA-treated rats is shown in Table 4. It is clear from the data in this table that the oral administration of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum Ts, LH, FSH, DHEA-S, and SHBG, as well as testicular G6PD, 3βHSD, cholesterol, and protein levels. On the contrary, the oral administration of BPA caused a highly significant decrease in serum Ts, DHEA-S, G6PD, 3βHSD, and protein levels but a highly significant increase in serum LH, FSH, SHBG, and cholesterol levels compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused an increase in serum Ts, DHEA-S, G6PD, 3βHSD, and protein levels and a decrease in serum LH, FSH, SHBG, and cholesterol levels compared to these testicular parameters in the BPA-treated group.

Protective effect of FA on liver, kidney, and testis DNA content after BPA exposure

The data presented in Table 5 exhibit the liver content in male rats. It is clear from the data in this table that control rats revealed 65.77% of diploid cells (2c), 11.71% of triploid cells (3c) (medium proliferation index), 0.90% of tetraploid cells (4c), and 0.0% of aneuploid cells (> 5c) (diploid-medium proliferation index). In BPA-treated rats, the liver tissue displayed 22.64% of diploid cells, 9.43% of triploid cells (low proliferation index), 31.13% of tetraploid cells, and 36.79% of aneuploidy cells (aneuploid-low proliferation index). In rats administered with FA before BPA exposure, the liver tissue presented 33.65% of diploid cells, 15.89% of triploid cells (high proliferation index), 40.19% of tetraploid cells, and 10.28% of aneuploid cells (diploid-high proliferation index).

The data presented in Table 6 display the kidney content in male rats. It is clear from the data in this table that control rats demonstrated 72.90% of diploid cells, 14.95% of triploid cells (medium proliferation index), 0.0% of tetraploid cells, and 0.0% of aneuploid cells (diploid-medium proliferation index). In the BPA-treated group, the kidney tissue exhibited 19.81% of diploid cells, 31.13% of triploid cells (high proliferation index), 28.30% of tetraploid cells, and 20.76% of aneuploidy cells (tetraploid-high proliferation index)]. In rats treated with FA before BPA exposure, the kidney tissue exhibited 57.80% of diploid cells, 29.36% of triploid cells (medium proliferation index), 5.51% of tetraploid cells, and 7.34% of aneuploid cells (diploid-medium proliferation index).

The data presented in Table 7 show the testis content in male rats. It is clear from the data in this table that control rats displayed 66.37% of diploid cells, 12.39% of triploid cells (medium proliferation index), 0.89% of tetraploid cells, and 0.0% of aneuploid cells (diploid-medium proliferation index)]. In the BPA-treated group, the testis tissue revealed 23.85% of diploid cells, 11.01% of triploid cells (high proliferation index), 27.52% of tetraploid cells, and 37.62% of aneuploidy cells (tetraploid-high prolif-

Table 1 Protective effect of fertaric acid on body weight, food and water intake, urine volume, and urinary protein, albumin, and albumin/protein ratio in rats exposed to bisphenol A

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Initial body weight (g)	132.5 ± 14.5	133.1 ± 15.6	131.8 ± 14.2	134.8 ± 13.9	135.0 ± 15.3
Final body weight (g)	185.0 ± 16.8	187.1 ± 17.2	186.3 ± 18.64	87.8 ± 12.5[d]	183.75 ± 16.7[b]
Initial food consumption (g/d)	11.5 ± 1.3	11.3 ± 1.0	11.4 ± 1.2	11.7 ± 1.1	11.6 ± 1.4
Final food consumption (g/d)	14.1 ± 1.1	14.3 ± 1.4	14.2 ± 1.3	5.7 ± 1.0[d]	14.0 ± 1.2[b]
Initial water intake (mL/d)	12.2 ± 1.5	12.4 ± 1.3	12.1 ± 1.2	12.3 ± 1.4	12.5 ± 1.0[b]
Final water intake (mL/d)	15.4 ± 1.6	15.2 ± 1.3	15.3 ± 1.0	6.8 ± 0.9[d]	15.1 ± 1.2[b]
Urine volume (mL/100 g/8 h)	0.98 ± 0.07	0.96 ± 0.09	0.99 ± 0.08	1.26 ± 0.15[c]	1.01 ± 0.08[a]
Urinary protein excretion (g/dL)	4.06 ± 0.24	4.04 ± 0.21	4.07 ± 0.26	5.29 ± 0.19[c]	4.08 ± 0.25[a]
Urinary albumin excretion (g/dL)	2.30 ± 1.4	2.28 ± 1.1	2.32 ± 1.3	4.13 ± 1.5[d]	2.31 ± 1.2[b]
Urinary albumin/protein excretion ratio	0.57 ± 0.05	0.56 ± 0.03	0.57 ± 0.04	0.78 ± 0.02[c]	0.57 ± 0.03[a]

[a]$P \leq 0.05$ compared to bisphenol A (BPA).
[b]$P \leq 0.01$ compared to BPA.
[c]$P \leq 0.05$ compared to control.
[d]$P \leq 0.01$ compared to control. Number of animals = 6 rats/group. Initial body weight, food consumption, and water intake = body weight, food consumption, and water intake at the first day (day 0) of the experiment. Final body weight, food consumption, and water intake = body weight, food consumption, and water intake at the final day of the experiment. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A.

Table 2 Protective effect of fertaric acid on liver toxicity in rats exposed to bisphenol A

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum AST (U/L)	121.8 ± 2.64	122.4 ± 3.26	124.0 ± 2.73	175.2 ± 0.72[d]	130.4 ± 4.17[b]
Serum ALT (U/L)	60.9 ± 2.37	61.9 ± 2.18	63.1 ± 2.59	86.7 ± 1.96[d]	65.1 ± 2.9[b]
Serum ALP (U/100 mL)	14.9 ± 1.27	14.7 ± 1.46	16.2 ± 1.56	5.65 ± 0.82[d]	13.7 ± 1.22[b]
Serum ACP (U/100 mL)	17.0 ± 2.79	16.2 ± 2.48	15.8 ± 2.75	6.86 ± 2.01[d]	12.15 ± 2.34[b]
Serum γGT (U/L)	8.42 ± 1.32	8.52 ± 1.25	9.90 ± 1.37	13.6 ± 2.30[d]	10.2 ± 1.57[b]
Serum LDH (U/L)	261.0 ± 43.6	257.9 ± 35.8	257.4 ± 36.8	755.9 ± 53.17[d]	277.8 ± 52.8[b]
Serum bilirubin (mg/dL)	0.53 ± 0.08	0.55 ± 0.09	0.58 ± 0.06	0.82 ± 0.05[d]	0.54 ± 0.04[b]

[a]$P \leq 0.05$ compared to bisphenol A (BPA).
[b]$P \leq 0.01$ compared to BPA.
[c]$P \leq 0.05$ compared to control.
[d]$P \leq 0.01$ compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ACP: Acid phosphatase; γ-GT: γ-Glutamyl transferase; LDH: Lactate dehydrogenase.

eration index). In rats treated with FA and then BPA, the testis tissue revealed 36.94% of diploid cells, 22.52% of triploid cells (medium proliferation index), 28.83% of tetraploid cells, and 11.71% of aneuploid cells (diploid-medium proliferation index)].

Protective effect of FA on liver, kidney, and testis histopathology after BPA exposure

Figure 1 exhibits the histology of liver tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the hepatocytes were large in size, rounded, and bounded by a distinct nuclear envelope. The structure of the liver in the control, paraffin oil, and FA-treated groups showed normal hepatocytes, vascular sinusoids, and centro-lobular vein (Figure 1A-C). The oral administration of BPA caused rim edema in

Table 3 Protective effect of fertaric acid on kidney toxicity and serum electrolytes in rats exposed to bisphenol A

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum urea (mg/dL)	26.5 ± 2.83	27.2 ± 2.54	25.8 ± 2.59	34.0 ± 2.59[c]	28.2 ± 2.74[a]
Serum creatinine (mg/dL)	0.75 ± 0.08	0.76 ± 0.06	0.74 ± 0.07	0.98 ± 0.09[c]	0.76 ± 0.09[a]
Serum uric acid (mg/dL)	8.21 ± 0.43	8.16 ± 0.52	8.28 ± 0.64	17.65 ± 0.82[d]	9.16 ± 0.58[b]
Blood urea nitrogen (mg/dL)	16.25 ± 1.54	16.47 ± 1.39	16.18 ± 1.52	34.42 ± 1.80[d]	17.18 ± 1.71[b]
Serum sodium (mmol/L)	154.60 ± 3.29	155.28 ± 3.61	153.81 ± 3.50	110.24 ± 4.16[d]	144.9 ± 3.28[a]
Serum potassium (mmol/L)	5.62 ± 0.19	5.71 ± 0.15	5.59 ± 0.24	2.71 ± 0.28[d]	5.43 ± 0.18[a]
Serum chloride (mmol/L)	102.83 ± 2.19	103.20 ± 2.34	102.36 ± 2.75	81.47 ± 1.85[d]	101.26 ± 2.39[a]

[a]$P \leq 0.05$ compared to bisphenol A (BPA).
[b]$P \leq 0.01$ compared to BPA.
[c]$P \leq 0.05$ compared to control.
[d]$P \leq 0.01$ compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A.

Table 4 Protective effect of fertaric acid on testicular toxicity in rats exposed to bisphenol A

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum Ts (ng/mL)	5.98 ± 0.42	6.00 ± 0.51	5.96 ± 0.62	3.14 ± 0.49[c]	5.89 ± 0.68[a]
Serum LH (mIU/mL)	18.28 ± 1.85	18.31 ± 1.64	17.96 ± 1.93	33.30 ± 2.21[d]	18.15 ± 5.14[b]
Serum FSH (mIU/mL)	1.05 ± 0.13	1.03 ± 0.19	0.98 ± 0.15	2.32 ± 0.22[d]	1.02 ± 0.18[b]
Serum DHEA-SO_4 (μg/dL)	197.50 ± 23.29	198.25 ± 26.12	195.74 ± 21.84	154.25 ± 13.56[c]	193.72 ± 19.71[a]
Serum SHBG (nmol/L)	6.65 ± 0.49	6.67 ± 0.62	6.63 ± 0.51	9.06 ± 1.84[c]	6.71 ± 4.95[a]
Testis G6PD (U/g tissue)	11.92 ± 0.66	11.94 ± 0.86	11.89 ± 0.73	5.64 ± 0.43[d]	10.86 ± 1.52[b]
Testis 3βHSD (U/g tissue)	4.46 ± 0.86	4.48 ± 0.75	4.43 ± 0.62	2.15 ± 0.36[d]	4.35 ± 0.88[b]
Testis Chol (mg/g tissue)	130.67 ± 8.16	132.17 ± 6.90	128.86 ± 7.48	192.53 ± 8.44[d]	129.21 ± 7.51[b]
Testis protein (mg/g tissue)	290.6 ± 14.23	288.9 ± 16.51	292.19 ± 13.64	187.60 ± 15.18[d]	289.45 ± 11.59[b]

[a]$P \leq 0.05$ compared to bisphenol A (BPA).
[b]$P \leq 0.01$ compared to BPA.
[c]$P \leq 0.05$ compared to control.
[d]$P \leq 0.01$ compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A; Ts: Testosterone; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; DHEA-SO_4: Dehydroepiandrosterone sulfate; SHBG: Sex hormone binding globulin; G6PD: Glucose-6-phosphate dehydrogenase; 3βHSD: 3β-hydroxysteroid dehydrogenase; Chol: Cholesterol.

the periportal area, which compressed the surrounding hepatocytes. Intra-cytoplasm vacuolation was also found after BPA oral administration (Figure 1D). The oral administration of FA in BPA-treated rats resulted in preserved hepatic lobular architecture and normal structure of the hepatocytes and dilated hepatic sinusoids where the hepatocytes were within normal limits and preserved their plate pattern (Figure 1E).

Figure 2 displays the histology of kidney tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the glomeruli showed a normal size with normal tubules (Figure 2C). Figure 2D reveals that the BPA-treated group showed widespread coagulated necrosis with dilatation, vacuolar degeneration, epithelial desquamation, and intraluminal cast formation. Figure 2E shows that the FA + BPA-treated group revealed marked improvement in the histological picture which was comparable to that of the control group.

Figure 3 reveals the histology of testis tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the testis tissue revealed well-layered seminiferous tubules with germ cells (Figure 3C). In the BPA-treated group, the testis tissue showed disrupted basement membrane and tubular epithelium (Figure 3D). The

Table 5 Liver DNA content in different groups

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	111	100.0%	1.000	All	106	100.0%	2.374	All	107	100.0%	1.923
5cER	0	0.0%	-	5cER	39	36.79%	2.948	5cER	11	10.28%	2.815
< 1.5c	24	21.62%	0.679	< 1.5c	0	0.0%	-	< 1.5c	0	0.0%	-
1.5c-2.5c	73	65.77%	1.031	1.5c-2.5c	24	22.64%	1.045	1.5c-2.5c	36	33.65%	1.181
2.5c-3.5c	13	11.71%	1.359	2.5c-3.5c	10	9.43%	1.558	2.5c-3.5c	17	15.89%	1.595
3.5c-4.5c	1	0.90%	1.779	3.5c-4.5c	33	31.13%	2.033	3.5c-4.5c	43	40.19%	2.273

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid cells containing two copies of DNA; 3c: Proliferation index (S-phase cells containing three strands of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4c: Cells with more than 4c DNA content; < 1.5c: Cells containing less than 1.5c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

Table 6 Kidney DNA content in different groups

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	107	100.0%	1.000	All	106	100.0%	1.628	All	109	100.0%	1.136
5cER	0	0.0%	-	5cER	22	20.76%	2.716	5cER	8	7.34%	-
< 1.5c	13	12.15%	0.650	< 1.5c	0	16.04%	0.623	< 1.5c	0	8.26%	0.663
1.5c-2.5c	78	72.90%	0.984	1.5c-2.5c	21	19.81%	0.984	1.5- 2.5c	63	57.80%	1.008
2.5c-3.5c	16	14.95%	1.364	2.5c-3.5c	33	31.13%	1.364	2.5c-3.5c	32	29.36%	1.411
3.5c-4.5c	0	0.0%	-	3.5c-4.5c	30	28.30%	1.988	3.5c-4.5c	6	5.51%	1.842

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid (cells containing two copies of DNA; 3c: Proliferation index (S-phase cells containing three copies of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4 c: Cells with more than 4c DNA content; < 1.5 c: Cells containing less than 1.5 c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

FA + BPA-treated group (Figure 3E) exhibited normal seminiferous tubules with germ cells.

DISCUSSION

BPA is an environmental pollutant that belongs to the endocrine disrupting chemicals. BPA is present in many consumer plastic products, such as water bottles and food packaging, and in the dentistry for the manufacturing of resin materials[2]. The burning of dumped waste in an open air transfers BPA from plastic waste into the environment and consequently the human and animal exposure to BPA is rapid and continuous[3]. On the other hand, FA is a hydroxycinnamic acid found in grapefruit.

The aim of this study was to evaluate the protective effect of FA on the oral BPA-induced toxicity, DNA breakdown, and histopathological changes of the liver, kidney, and testis induced.

BPA induced a significant decrease in body weight, food intake, and water consumption while causing a significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. On the other side, oral administration of FA increased the body weight, food intake, and water consumption while decreasing urinary volume, protein, albumin, and albumin/globulin ratio in BPA-treated rats to approach the control levels. These results are in agreement with that of Kazemi *et al*[52] who found that oral administration with 5, 25 and 125 μg/kg of BPA for 35 d decreased the body weight of rats and this weight loss was more evident at doses of 25 and 125 μg/kg. On the other hand, oral administration of FA in BPA-treated rats led all the above mentioned parameters to approach the normal levels and these effects are similar to the effect of FA (45

Table 7 Testis DNA content in different groups

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	113	100.0	1.000	All	109	100.0	2.614	All	111	100.0	1.951
5cER	0	0.0	-	5cER	41	37.62	2.953	5cER	13	11.71	2.726
< 1.5c	23	20.35	0.662	< 1.5c	0	0.0	-	< 1.5c	0	0.0	-
1.5c- 2.5c	75	66.37	1.071	1.5c- 2.5c	26	23.85	1.805	1.5c- 2.5c	41	36.94	1.250
2.5c- 3.5c	14	12.39	1.412	2.5c- 3.5c	12	11.01	1.741	2.5c- 3.5c	25	22.52	1.803
3.5c- 4.5c	1	0.89	1.503	3.5c- 4.5c	30	27.52	2.019	3.5c- 4.5c	32	28.83	1.948

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid cells containing two copies of DNA; 3c: Proliferation index (S-phase cells contained three copies of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4 c: Cells with more than 4c DNA content; < 1.5 c: Cells containing less than 1.5 c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

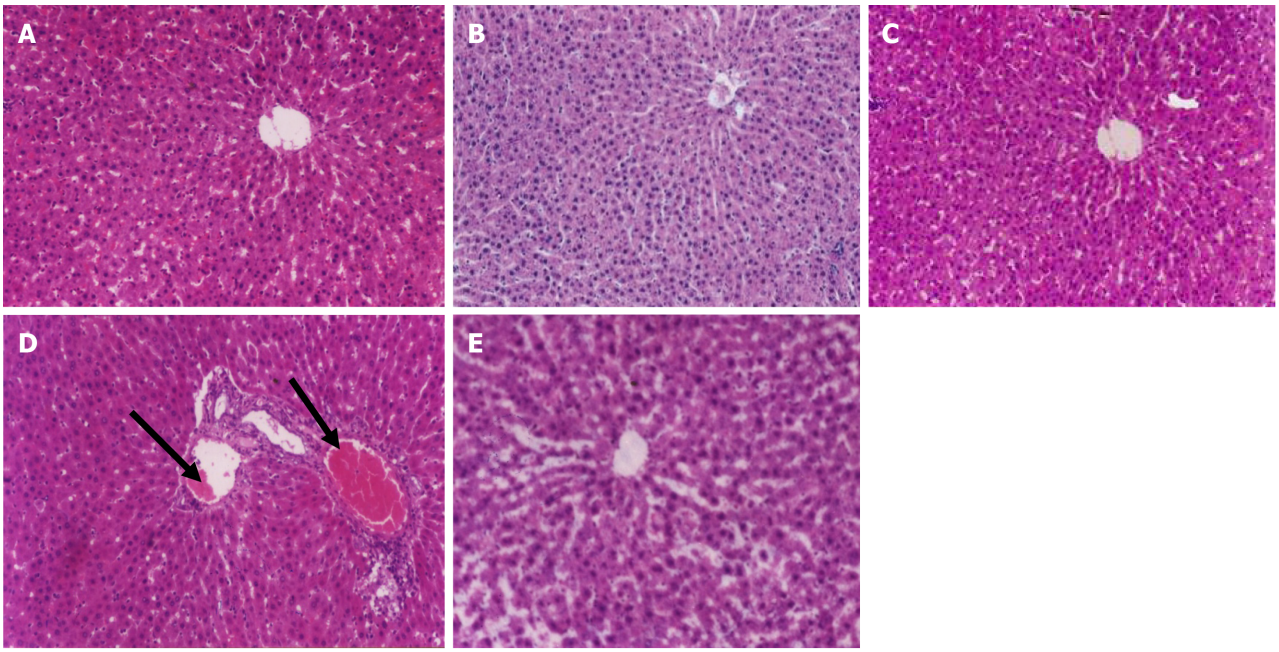

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Figure 1 Pathological changes in the liver after treatment. A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA + BPA-treated group. The control, paraffin oil, and FA-treated rats (A, B, and C; H&E staining, 200 ×) showed a normal hepatic architecture with preserved hepatic architecture. On the contrary, in BPA-treated rats (D), there was rim edema in the periportal area (black arrows) which compressed the surrounding hepatocytes. Intra-cytoplasm vacuolation was noted. FA + BPA-treated rats (E) had a preserved hepatic lobular architecture.

mg/kg) to increase food consumption, water intake, and body weight in endocrine disrupting chemicals exposed rats[16].

The liver is the main site of toxicity disposal or degradation in the human body. Therefore, any changes in the liver transaminases (AST and ALT) are indicators of liver dysfunction[53] and hepatic toxicity[54]. In this study, both AST and ALT activities showed a highly significant increase in BPA-treated rats. Thus, the oral intake of BPA changed the hepatocytes and liver metabolism and liver toxicity occurred. Moreover, all liver enzymes such as serum ALP, ACP γ-GT, LDH, and bilirubin were increased in this study, which indicated hepatic toxicity[55,56]. These observations are in agreement with that of Sun *et al*[57] who found that BPA induced an increase in liver enzymes (AST, ALT, and γ-GT), inflammatory cell infiltration, and hepatocyte necrosis. The authors of that paper[57] used 500 mg/kg BPA, which was higher than the dose of BPA in the present study (4 mg/kg), but Kazemi *et al*[52] used oral doses of 5, 25, and 125 μg/kg of BPA (induced liver toxicity in adult rats), which were lower than our dose. Moreover, Sun *et al*[57] found an increase in ALP as a result of liver toxicity after

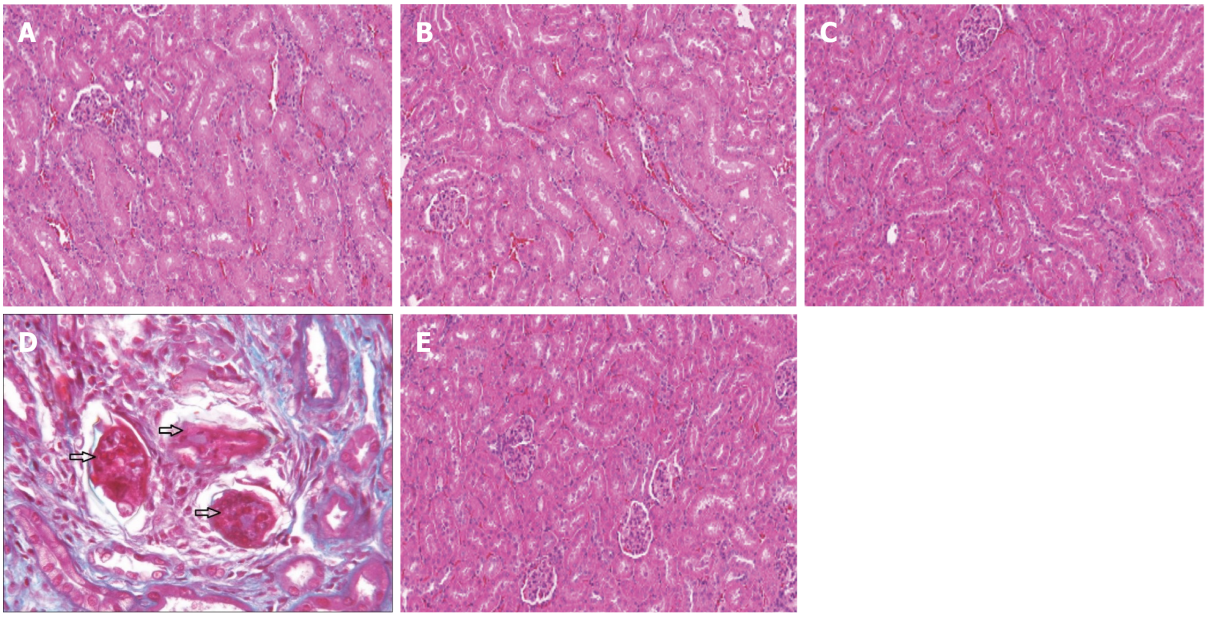

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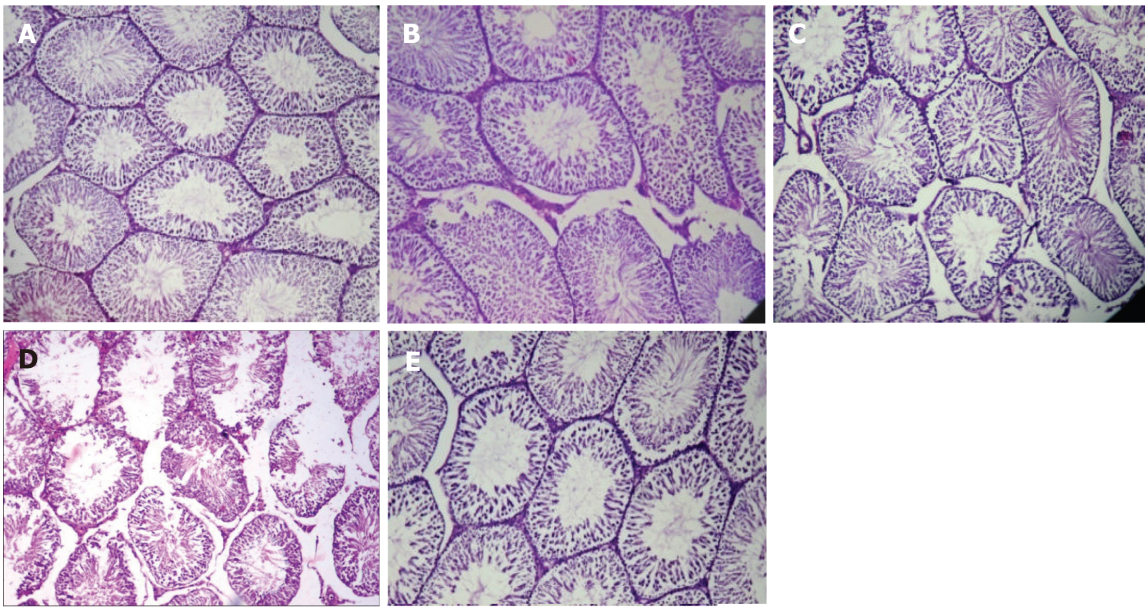
Figure 2 Pathological changes in the kidney after treatment. A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA + BPA-treated rats. It is clear from these figures (H&E staining, 200 ×) that control, paraffin oil-, and FA-treated rats showed a normal size of glomeruli with normal tubules (A-C). BPA-treated rats (D) showed widespread coagulated necrosis with dilatation, vacuolar degeneration, epithelial desquamation, and intraluminal cast formation. FA + BPA-treated rats (E) revealed marked improvement in the histological picture which is comparable to that of the control group.



DOI: 10.4254/wjh.v14.i3.535

Figure 3 Pathological changes of the testis after treatment. A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA+BPA-treated group. It is clear from these figures that control, paraffin oil-, and FA-treated rats revealed well-layered seminiferous tubules with germ cells (A-C). In BPA-treated rats, testis tissue showed disrupted basement membrane and tubular epithelium (D). FA + BPA-treated rats (E) exhibited normal seminiferous tubules with germ cells.

BPA oral administration but Kazemi *et al*[52] reported a decrease in ALP level after oral administration of lower doses (5, 25, and 125 μg/kg) of BPA, and these observations are in parallel to our result. Also, Akçay *et al*[58] found that BPA is a reason of liver steatosis, which leads to the formation of metabolic syndrome. Further, Elswefy *et al*[59] found that BPA induced hepatic damage and fibrosis. On the contrary, the oral administration of FA in the BPA-treated group returned all the above mentioned liver function to approach the control levels and this effect was related to the ability of FA to protect the liver against the harmful effects of BPA. Such results are in agreement of that of Koriem and Arbid[16] who

proved that FA at a dose of 45 mg/kg ameliorated liver function, antioxidants, and inflammatory cytokines in the endocrine-disrupting chemical 4-tert-octylphenol-induced toxicity. The authors proved that FA ameliorated serum AST, ALT, γ-GT, LDH, ALP, ACP, and bilirubin. Also, Sochorova *et al*[20] found that FA had antioxidant activity, and it therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to fight against the harmful effects of BPA.

The kidney excretes many of waste products produced by metabolism into the urine. These include the nitrogenous wastes urea (from protein catabolism) and uric acid (from nucleic acid metabolism). The kidney participates in human homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. Therefore, any clinical and diagnostic changes are associated with the changes in kidney function (serum urea, creatinine, and uric acid) as mentioned by Martin and Friedman [55] and Plaa and Hewitt [56]. Thus, the increase in kidney function parameters (serum urea, creatinine, uric acid, and blood urea nitrogen) levels and the decrease in serum electrolytes (sodium, potassium, and chloride) levels in BPA-treated rats suggested an indication of kidney toxicity caused by BPA exposure. Such observation is in accordance with Jiang *et al*[60] who found that BPA induced kidney toxicity in rats after 5 wk of treatment. Also, Esplugas *et al*[61] found that BPA (25 μg/kg bw) caused renal and liver damage evidenced by oxidative stress in mice. Furthermore, Ola-Davies and Olukole[62] found that oral administration of BPA at 10 mg/kg for 14 d in male rats increased renal reactive oxygen species and declined the antioxidant system. BPA induced significant increases in serum urea and creatinine in BPA-treated rats. Lesions of the kidney including inflammation, vascular congestion, and erosion of epithelial cells were also observed in BPA-treated rats.

BPA-exposed rats revealed renal dysfunction and histopathological abnormalities, oxidative stress, apoptosis, mitochondrial functional impairment, mitochondrial dynamic changes, and mitophagy disproportion. Sodium, chloride, and potassium are electrolytes that work together to regulate nutrients within the cells and regulate body fluids. Potassium is the main electrolyte in the fluid inside of cells, while sodium is the principal electrolyte in the fluid outside of cells. Chloride is an electrolyte that is important in keeping the suitable amount of fluids inside and outside the cells. The drastic decline of serum sodium, potassium, and chloride electrolytes after BPA exposure in this research was related to BPA exposure-stimulated accumulation of more sodium in the small intestine in male rats[63], which in turn decreased serum sodium and consequently both serum potassium and chloride decreased to keep sodium/potassium pump in normal state and to sustain body homeostasis of electrolytes. On the contrary, the decline in the levels of kidney function parameters and the increase in serum electrolytes in the FA + BPA-treated group indicated the ability of FA to protect the kidney against the harmful effect of BPA. Such observation is in accordance with that of Koriem and Arbid[16] who proved that FA at a dose of 45 mg/kg ameliorated serum and liver antioxidants such as serum and hepatic superoxide dismutase, glutathione peroxidase, and catalase. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to fight against BPA-related oxidative stress.

The testis is a male reproductive organ. The function of the testis is to produce both sperm and androgens (Ts). Testosterone is controlled by LH but sperm production is controlled both by and Ts. The testis is well known to be very sensitive to injury, especially from endocrine disturbing chemicals such as BPA. These are because endocrine disturbing chemicals such as BPA can affect the size and function of the testis. The oral administration of BPA in this study caused a decrease in serum Ts, DHEA-S, G6PD, 3βHSD, and protein levels but an increase in serum LH, , SHBG, and cholesterol levels. The decrease of Ts is attributed to: (1) The inhibitory effect of BPA on human chronic gonadotropin-stimulated Ts biosynthesis by both cultured rat precursor and immature Leydig cells[64]; or (2) the ability of BPA to convert cholesterol to androstenedione through inhibiting 17-α-hydroxylase and 3β-hydroxysteroid dehydrogenase-isomerase steps[65]. The decrease of 3β-hydroxysteroid dehydrogenase activity in this study was accompanied with an increase LH and levels in BPA-treated rats. The increase in LH and levels following BPA exposure is related to: (1) LH-induced Leydig cell secretion of Ts (which participated in the regulation of spermatogenesis by targeting androgen receptors in the germinal epithelium); and (2) targeting of receptors inside Sertoli cell to control spermatogenesis by stimulating many Sertoli cell factors. The decrease of testicular cholesterol and protein in this study was linked to testicular dysfunction. On the contrary, FA oral administration in BPA-treated rats increased the number of Leydig cells, ameliorated Ts levels, and consequently restored testicular function[66]. Such observations are in agreement with that of Koriem and Arbid[16] who proved that FA at a dose of 45 mg/kg ameliorated serum and liver antioxidants as well as inflammatory cytokines in endocrine disturbing chemical-exposed rats. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to protect against the harmful effects of BPA.

In this study, content in the liver, kidney, and testis was determined in BPA-treated rats and FA + BPA-treated rats. BPA caused a very high increase in breakdown in these organs. Such observation is in agreement with that of Akram *et al*[67] who found that BPA increased damage in liver, kidney, and brain tissues. The very low concentrations of BPA caused toxic effects *via* affecting the physiological and biochemical parameters in multiple tissues of fish. Also, Panpatil *et al*[68] found that the BPA-treated groups exhibited significantly higher mean levels of damage in the liver and kidney as compared to the untreated control group. Furthermore, Pan *et al*[69] found that BPA declined sperm chromatin integrity

while increased damage in mouse spermatogenic cells. On the contrary, the oral administration of FA in BPA-treated rats resulted in the return of the content in liver, kidney, and testis tissues to the normal diploid level. These observations were recorded due to the antioxidant activity of FA. These results are in accordance with those of Koriem and Arbid[16] and Sochorova *et al*[20] who found that FA had antioxidant activity, which increased the antioxidant activity in the liver, kidney, and testis of BPA-treated rats. These results are in agreement with that of Koriem and Arbid[16] who proved that FA at a dose of 45 mg/kg counteracted the inhibitory action on the gene expression of liver proteins induced by the endocrine-disrupting chemical 4-tert-octylphenol, where FA prevented the degradation of liver , and consequently reformation occurred. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to protect against the harmful effects of BPA.

The mechanism sustaining the protective effect of FA against BPA-induced liver, kidney, and testis-related toxicity, DNA breakdown, and histopathological changes depends on the antioxidant effect of FA. Therefore, FA increases serum and tissue superoxide dismutase, glutathione peroxidase, and catalase in BPA-treated rats. This will stop the BPA-related side effects such as liver, kidney, and testicular toxicity, DNA breakdown, and histopathological changes [16,20].

The implication of the results of this research to the human population is that daily oral administration of FA protects against the harmful effect of low-dose exposure to BPA. The significant impact of this research is that FA is available, very cheap, and without any side effects to protect against the toxicity related to daily exposure of babies, children, young, and elderly people to BPA. The FA dose used in this research is very useful to babies, children, and elderly people because these human groups are very susceptible to lower doses of BPA caused by daily exposure to cumulative amounts of BPA doses through foods, drinks, and inhalation.

CONCLUSION

In conclusion, this study proved that FA can be used as a protective agent in ameliorating the BPA-induced toxicity, DNA breakdown, and histopathology of the liver, kidney, and testis, which suggests the use of this acid in preventing the toxicity of BPA that is present in plastic industry such as water bottles and food packages.

ARTICLE HIGHLIGHTS

Research background

Bisphenol A (BPA) is present in many plastic products and food packaging. On the other hand, fertaric acid (FA) is a hydroxycinnamic acid.

Research motivation

It is a challenging responsibility to find a safe and effective way to overcome the toxicity of BPA toxicity in regions where BPA is already present in water bottles and food packaging and people are therefore exposed to BPA toxicity day and night. The use of herbal plants in the medicine has been known for a long time ago and today it has made a comeback in all over the world. This is because of their minor side effects and good therapeutic effects.

Research objectives

To investigate the effect of FA on BPA-related liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes in male rats.

Research methods

Thirty male albino rats were divided into five equal groups (6 rats/group); Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control and paraffin oil groups were administered orally with 1 mL distilled water and 1 mL paraffin oil, respectively. The FA-, BPA-, and FA+ BPA-treated groups were administered orally with FA (45 mg/kg, bw) dissolved in 1 mL distilled water, BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, and FA (45 mg/kg, bw) followed by BPA (4 mg/kg, bw), respectively. All these treatments were given once a day for 6 wk.

Research results

The results showed that BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3β-hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ-glutamyl

transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited the degradation of liver, kidney, and testis DNA content. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels.

Research conclusions

This study for the first time proposed that FA can amend the bisphenol A-induced toxicity, DNA content, and histopathological changes in the liver, kidney, and testis.

Research perspectives

The direction of the future research is to apply FA in clinical study and it will be interesting to prove that FA can amend the BPA-induced toxicity clinically.

FOOTNOTES

Author contributions: Koriem KMM designed the study, conceived of the manuscript, wrote and edited the first and final versions of the manuscript, conducted the literature search, and read and approved the final manuscript.

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ORIGINAL ARTICLE

Case Control Study

Prevalence of hypothyroidism and effect of thyroid hormone replacement therapy in patients with non-alcoholic fatty liver disease: A population-based study

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is currently considered as the most common cause of chronic liver disease worldwide. Risk factors for NAFLD have been well-described, including obesity, type 2 diabetes mellites (T2DM), dyslipidemia (DLP) and metabolic syndrome. Hypothyroidism has been identified as an independent risk factor for the development of NAFLD, although the literature is inconsistent

AIM

To evaluate the prevalence of hypothyroidism in patients with NAFLD, assess if it is an independent risk factor and explore the effect of thyroxine replacement therapy.

METHODS

Our cohort's data was obtained using a validated, large, multicenter database (Explorys Inc, Cleveland, OH, United States) aggregated from pooled outpatient and inpatient records of 26 different healthcare systems, consisting of a total of 360 hospitals in the United States, and utilizing Systematized Nomenclature of Medicine-Clinical Terms for coding. We evaluated a cohort of patients with

hypothyroidism and NAFLD. Multivariate analysis was performed to adjust for confounding risk factors including hypertension (HTN), T2DM, DLP, obesity and metabolic syndrome. SPSS version 25, IBM Corp was used for statistical analysis, and for all analyses, a 2-sided *P* value of < 0.05 was considered statistically significant. Exclusion criteria were limited to age < 18 years.

RESULTS

Among the 37648180 included individuals in this database who are above the age of 18 years, there were a total of 2320 patients with NAFLD (6.16 per 100000) in the last five years (2015-2020), amongst which 520 patients (22.4%) had hypothyroidism. Baseline characteristics of patients in this database are described in Table 1. Patients with NAFLD were also more likely to have obesity, T2DM, DLP, HTN, and metabolic syndrome (Table 2). While males and females were equally affected, patients in the age group 18-65 years as well as Caucasians seem to be at a higher risk. There was an increased risk of NAFLD among patients with hypothyroidism (OR = 1.587). Furthermore, thyroid hormone replacement was not associated with a decreased risk for developing NAFLD (OR = 1.106, C = 0.952-1.285, *P* = 0.303).

CONCLUSION

Hypothyroidism seems to be an independent risk factor for the development of NAFLD. Thyroid hormone replacement did not provide a statistically significant risk reduction. Further studies are needed to evaluate the effect of thyroid hormone replacement and assess if being euthyroid while on thyroid replacement therapy affects development and/or progression of NAFLD.

Key Words: Hypothyroidism; Non-alcoholic fatty liver disease; Thyroid hormone replacement therapy; Independent risk factor

Core Tip: One of the largest population-based case-control studies screening more than 37 million patients to study the inconsistent relationship between hypothyroidism and non-alcoholic fatty liver disease (NAFLD), and -to the best of our knowledge- the first paper investigating the theoretical role of thyroid hormone replacement in preventing NAFLD among hypothyroidism patients.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is currently considered as the most common cause of chronic liver disease worldwide and the second most common indication for liver transplantation in the United States after chronic hepatitis C with a histological disease spectrum ranging from steatosis to non-alcoholic steatohepatitis (NASH) and eventually cirrhosis. Its international prevalence is steadily increasing (15% in 2005 to 25% in 2010), and it is expected to emerge as the leading cause of end-stage liver disease in the near future[1]. Several genetic and environmental risk factors for NAFLD have been described in the literature, including obesity, unhealthy eating habits, low physical activity levels, type 2 diabetes mellitus (T2DM), dyslipidemia (DLP), hypertension (HTN) and metabolic syndrome[1-3].

Thyroid hormone plays a major role in regulating the metabolism of lipids and carbohydrates which are affected in patients with NAFLD. Furthermore, hypothyroidism in particular shares similar risk factors to those of NAFLD including insulin resistance, DLP, obesity and metabolic syndrome[4,5]. Liangpunsakul *et al*[4] was the first to describe the potential relationship between hypothyroidism and NAFLD, and found a significantly higher hypothyroidism prevalence among patients with NAFLD. This association was further replicated in later retrospective studies[5]. However, these studies were limited by the smaller sample size and the inconsistency of the literature to some degree. Our aim is to conduct a population-based study to estimate the prevalence of hypothyroidism in patients with NAFLD, and statistically adjust for all known confounders to assess whether hypothyroidism is an independent risk factor for NAFLD, and to further assess the effect of thyroid hormone replacement therapy.

MATERIALS AND METHODS

Database

Our cohort's data was obtained using a validated, multicentered and daily-updated database (Explorys Inc, Cleveland, OH, United States) developed by IBM Corporation, Watson Health[6]. Explorys consists of electronic health records of 26 different healthcare systems across the United States and a total of 360 hospitals with more than 50 million patients. Explorys utilizes Systematized Nomenclature of Medicine Clinical Terms (SNOMED-CT) for the definition of the diseases and pools large outpatient and inpatient daintified data that can be formulated into numerous cohorts according to the clinical element being studied. Explorys further allows for the identification of the timeline of events in reference to the index clinical event of interest, and hence the ability to study the temporal relationship between different variables. The Institutional Review Board approval is not required since Explorys is a Health Insurance Portability and Accountability Act-compliant platform.

Methodology and patient selection

We retrospectively evaluated an initial cohort of patients with a SNOMED-CT of "Hypothyroidism" between the years 2015 to 2020. Our exclusion criteria were limited to patients less than 18 years old. Baseline characteristics of patients with hypothyroidism are shown in Table 1. A second cohort of patients with a SNOMED-CT of "Non-Alcoholic Fatty Liver" was identified. Age, gender and race-based data were collected. Potential confounders that were analyzed included: hypothyroidism, HTN, T2DM, DLP, obesity and metabolic syndrome. Among those with hypothyroidism, weather the patient was on thyroxine replacement therapy was also analyzed.

Statistical analysis

Demographics and related diseases were characterized by descriptive statistics. The overall prevalence of NAFLD was calculated by dividing the total number of individuals with NAFLD by the total number of individuals in the database (2015-2020), hence making sure that all patients in the denominator had an equal opportunity of being diagnosed with NAFLD. Multivariate analysis was performed to adjust for the confounders in the later cohort (Table 2). SPSS version 25, IBM Corp was used for statistical analysis, and for all analyses, a 2-sided P value of < 0.05 was considered statistically significant.

RESULTS

Baseline characteristics of patients in this database are described in Table 1. Among the 37648180 included individuals in this database who are above the age of 18 years, there were a total of 2320 patients with NAFLD in the period from 2015 to 2020. The 5-year period prevalence rate of NAFLD was 6.16 per 100000. Amongst those with NAFLD, 520 patients (22.4%) had hypothyroidism. Patients with NAFLD were also more likely to have obesity (OR, 3.616, 95%CI: 3.318-3.940), type 2 diabetes mellitus (OR, 2.178, 95%CI: 1.994-2.379), dyslipidemia (OR, 2.346, 95%CI: 2.121-2.596), hypertension (OR, 1.326, 95%CI: 1.201-1.465), and metabolic syndrome (OR, 4.782, 95%CI: 4.782-5.460) (Table 2). Males (OR, 1.008, 95%CI: 0.934-1.088) and females were equally affected, but the results were statistically insignificant. Patients in the age group 18-65 years (OR, 1.658, 95%CI: 1.524-1.804) as well as Caucasians (OR, 1.63, 95%CI: 1.489-1.799) seem to be at a higher risk. There was an increased risk of NAFLD among patients with hypothyroidism (OR, 1.587, 95%CI: 1.388-1.815). Furthermore, thyroid hormone replacement was not associated with a decreased risk for developing NAFLD (OR, 1.106, 95%CI: 0.952-1.285, $P = 0.303$). Characteristics of patients with NAFLD and hypothyroidism are shown in Figure 1.

DISCUSSION

Discussion and review of literature

Over the last couple of decades, NAFLD has emerged as one of the most common causes of chronic liver disease, including cryptogenic cirrhosis across the globe[7-9]. Risk stratification for NAFLD has become a focus of research because of the close relationship with different metabolic syndromes like T2DM, DLP, obesity, polycystic ovarian syndrome, and thyroid disorders. Albeit the overlap of complex metabolic pathophysiology of NAFLD and thyroid function remains controversial, many studies have suggested a strong association between the two[10-12].

The underlying pathophysiological mechanism of NAFLD has not been well explained. Still, the most commonly accepted theory implicates insulin resistance as the central role in developing hepatic steatosis and perhaps steatohepatitis[13,14]. Thyroid hormone has a vital role in cell metabolism and energy hemostasis. Thyroid dysfunction is associated with many diseases, for instance, cardiovascular disease, obesity, dementia, fracture, and recently NAFLD[15]. Thyroid hormones impact various metabolic pathways, and evidence corroborates the association of thyroid dysfunction and the

Table 1 Baseline characteristics of patients with hypothyroidism in explorys database

Parameter		Hypothyroidism	
		Present (%)	Absent (%)
Age (yr)	18-65	1335370 (48.3)	21097850 (60.5)
	> 65	1402550 (50.7)	6951210 (19.9)
Gender	Female	2087040 (75.5)	18562590 (53.2)
Race	Caucasian	2267940 (82.0)	20165960 (57.8)
	African-American	196720 (7.1)	4120940 (11.8)
	Asian	40710 (1.5)	539190 (1.5)
Comorbidities	HTN	1665090 (60.2)	7441760 (21.3)
	T2DM	790680 (28.6)	3114700 (8.9)
	Dyslipidemia	1716240 (62.1)	6469880 (18.5)
	Obesity	753060 (27.2)	3391060 (9.7)
	Metabolic syndrome	54440 (2.0)	2709750 (7.8)

HTN: Hypertension; T2DM: Type 2 diabetes mellites.

Table 2 Multivariate analysis for risk factors in individuals with non-alcoholic fatty liver disease

Parameter	Odds ratio	95%CI	*P* value
Age (18-65)	1.658	1.524-1.804	< 0.0001
Male	1.008	0.934-1.088	0.841
Caucasian	1.636	1.489-1.799	< 0.0001
Obesity	3.616	3.318-3.940	< 0.0001
T2DM	2.178	1.994-2.379	< 0.0001
Dyslipidemia	2.346	2.121-2.596	< 0.0001
Hypertension	1.326	1.201-1.465	< 0.0001
Metabolic syndrome	4.782	4.782-5.460	< 0.0001
Hypothyroidism	1.587	1.388-1.815	< 0.0001
Hypothyroidism on Thyroxine replacement therapy	1.106	0.952-1.285	0.188

T2DM: Type 2 diabetes mellites.

pathogenesis of NAFLD. The two most telltale signs of the NAFLD disease spectrum are insulin resistance and hepatic lipid dysregulation[16]. Thyroid hormones (T3 and T4) use intracellular receptor signaling pathways in the liver to induce lipid metabolism. Even though molecular pathways leading to insulin resistance are complex and have not been completely elucidated, the association between thyroid dysfunction, both overt and subclinical hypothyroidism, and NAFLD has been extensively reported.

For example, a population-based study by Chung *et al*[12] showed that the prevalence of NAFLD and elevated liver enzymes were higher in a patient with hypothyroidism (OR: 1.38; 95%CI: 1.17-1.62) and confirmed a relevant dose-dependent clinal relationship between NAFLD and thyroid hormones. Moreover, thyroid hormones level has been shown to exert an effect in all the spectrum of steatosis. For instance, the exciting case-control comparative study by Pagadala *et al*[5] for the prevalence of hypothyroidism in NAFLD and NASH showed that hypothyroidism was more common in patients with NASH than patients with NAFLD (25% *vs* 12.8%, $P = 0.03$).

Another study from the western region of India by Parikh *et al*[17] reported a prevalence of 16.8% hypothyroidism in NAFLD patients with a strong clinically significant association amongst two diseases (OR, 14.94, 95%CI: 3.5-62.6). Authors also concluded that steatohepatitis was found to be more common in hypothyroid individuals as compared to controls (OR 3.9, 95%CI: 1.2-11.1). Ludwig *et al*[18] did a

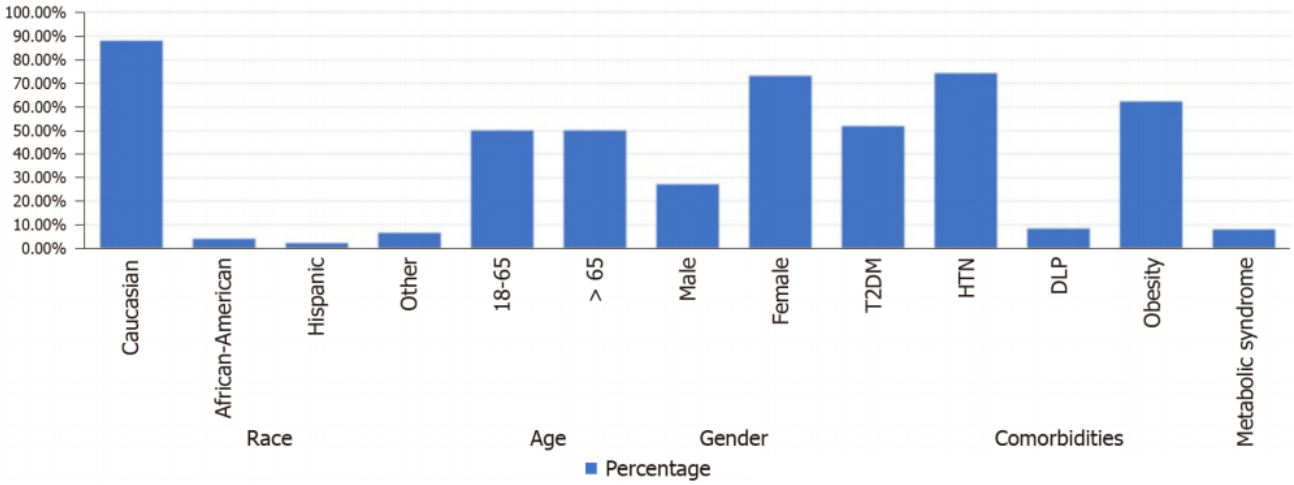

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Figure 1 Characteristics of patients with non-alcoholic fatty liver disease and hypothyroidism. HTN: Hypertension; T2DM: Type 2 diabetes mellitus; DLP: Dyslipidemia.

population-based cross-sectional study of 1276 participants which showed an increased prevalence of hepatic steatosis in subjects with reduced thyroid hormones ($P = 0.0143$; $P \leq 0.0001$).

Since hypothyroidism and NAFLD share numerous characteristics, including weight gain, whether hypothyroidism is a risk factor for NAFLD remains difficult to answer in retrospective studies (AA1). To provide stronger evidence of the causality relationship, Bano *et al*[19] conducted a prospective cohort study of 9419 patients followed over ten years and observed the effects of hypothyroidism in NAFLD patients, and found a 1.24-fold higher NAFLD risk (95%CI: 1.01-1.53) in patients with hypothyroidism. Another recent descriptive cross-sectional study by Martínez-Escudé *et al*[20] reported a significantly higher prevalence of NAFLD and liver fibrosis in subjects with TSH ≥ 2.5 (μIU/mL). Also, in a comparative study of 1773 euthyroid participants, both TSH and levels Free T3 Level were found to be positively associated with the risk of NAFLD when diagnosed by ultrasound and fatty liver index, respectively[21]. Finally, a recent metanalysis found that overall hypothyroidism has a positive association with the risk of NAFLD[22].

Along with these well-crafted studies, some substantial evidences have questioned the exact association between NAFLD and thyroid regulation. A recent Spanish study reported no association between hypothyroidism and NAFLD[23]. The authors observed that thyroid hormone level was not associated with a higher prevalence of NAFLD. Similarly, in a study by Lee *et al*[23], the authors found no relationship of increased incidence of NAFLD in patients with the subclinical or overt types of hypothyroidism.

Many of the studies describing the relationship between these two entities were largely limited by the sample size. To fill this gap, we conducted one of the largest nationwide multicenter studies which screened 37648180 individuals, among which 520 individuals had concomitant NAFLD and hypothyroidism. Our retrospective cohort study has shown that hypothyroidism is an independent risk factor for NAFLD, and that about 1 in every 5 patients with NAFLD have concomitant hypothyroidism (22.4%). Overall, this is one of the highest prevalence rates for NAFLD in hypothyroidism patients. Secondly, the effect of thyroid hormone replacement in hypothyroidism patients and its effect on NAFLD prevention has not been well explored. In a post hoc analysis of a randomized controlled trial for patients with subclinical hypothyroidism, the prevalence of NAFLD was reduced from 48.5% to 24.2% ($P = 0.041$) after 15 mo of thyroid hormone replacement, whereas the prevalence of NAFLD remained stable in the untreated group[24], however; this trial was limited by the small sample size of ~360 patients (AA2). Moreover, those who received thyroid hormone replacement therapy had higher weight loss, which can itself explain the prevalence change in the treated population. Our study failed to show a statistically significant NAFLD risk reduction among patients with hypothyroidism who are placed on thyroid hormone replacement (OR, 1.106, 95%CI: 0.952-1.285, $P = 0.303$) but the weight changes were difficult to assess. Without adequately powered prospective trials that also adjusts for weight changes, the question whether thyroid hormone replacement has a direct protective effect against NAFLD remains difficult to answer, and the appropriate duration for effective replacement therapy and the goals of treatment remain unclear (AA3).

Limitations

One of the limitations in our study is that we could not analyze the diagnostic method used for assessing NAFLD and set cut-off values for diagnosing hypothyroidism, since these are SNOMED-CT coded diagnoses on identified patient's charts. We also could not specify the exact degree at which

hypothyroidism becomes a NAFLD risk factor (AA4). Also, we could not evaluate for how long have these patients with hypothyroidism been on thyroid hormone replacement therapy, and whether they have achieved the euthyroid state or not. More prospective trials are needed to answer this question.

CONCLUSION

Hypothyroidism seems to be an independent risk factor for the development of NAFLD demonstrated in retrospective and prospective studies. Some studies have suggested that thyroid hormone replacement can potentially prevent or reverse NAFLD, which is potentially caused by weight loss. However, our study showed that thyroid hormone replacement did not provide a statistically significant risk reduction. Further prospective studies are needed to assess the role of thyroid hormone replacement therapy in patients with NAFLD, the duration for effective treatment and the treatment goals.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide, and hypothyroidism has been identified as an independent risk factor. The available data are limited by small sample size and the effect of thyroid hormone replacement therapy is not well studied.

Research motivation

The main topics of this article is to give a focused analysis on the hypothyroidism and to assess whether it is an independent risk factor for the development of NAFLD by filling the small sample size gap in the literature, provide a review of the current medical literature in this field, and -most importantly- to evaluate the role of thyroid hormone replacement therapy in the prevention of the disease.

Research objectives

The objective of this case control study is to assess whether hypothyroidism is an independent risk factor for the development of NAFLD, to review the updated medical literature, and to assess the role of thyroid hormone replacement therapy in the prevention of the disease.

Research methods

We used a validated multicenter database (Explorys Inc.) from pooled outpatient and inpatient records of 26 different healthcare systems, consisting of a total of 360 hospitals in the United States to collect our data. We evaluated a cohort of patients with hypothyroidism and NAFLD. Multivariate analysis was performed to adjust for confounding risk factors including hypertension (HTN), type 2 diabetes mellites (T2DM), dyslipidemia (DLP), obesity and metabolic syndrome. We evaluated a cohort of patients with hypothyroidism and NAFLD. Multivariate analysis was performed to adjust for confounding risk factors including HTN, T2DM, DLP, obesity and metabolic syndrome.

Research results

Among 37648180 in the database who are above the age of 18 years, a total of 2320 patients with NAFLD in the period from 2015 to 2020 were included. NAFLD prevalence was 6.16 per 100000, among which 520 patients (22.4%) had hypothyroidism. Patients with NAFLD were also more likely to have obesity, type 2 diabetes mellitus, dyslipidemia, hypertension, and metabolic syndrome. Males and females were equally affected, but the results were statistically insignificant. Patients in the age group 18-65 years as well as Caucasians seem to be at a higher risk. There was an independent increase in the risk of NAFLD among patients with hypothyroidism, and thyroid hormone replacement was not associated with a decreased risk for developing NAFLD. Prospective studies are needed to better delineate the role of thyroid hormone replacement therapy in these individual.

Research conclusions

There was an independent increase in the risk of NAFLD among patients with hypothyroidism, and thyroid hormone replacement is not associated with a decreased risk for developing NAFLD. Other studies have shown a potential protective effect of thyroid hormone replacement therapy. Based on the conflicting results with the existing literature, further studies are needed to better investigate the relationship between thyroid hormone replacement therapy and NAFLD.

Research perspectives

Future research should focus on assessing the degree of hypothyroidism that leads to NAFLD, and the role of thyroid hormone replacement therapy including the duration of treatment and the end-point goals.

FOOTNOTES

Author contributions: Almomani A and Kumar P wrote the manuscript; Hitawala AA designed the study; Alkhayyat M performed the statistical analysis; Alqaisi S, Alshaikh D collected the data; Asaad I is the corresponding author.

Institutional review board statement: No Institutional Review Board approval was required for this study since the database is de-identified.

Conflict-of-interest statement: No conflict of interest.

Data sharing statement: No additional data are available.

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Standards of liver cirrhosis care in Central Australia

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Abstract

BACKGROUND

Liver cirrhosis and hepatocellular carcinoma (HCC) are highly prevalent in Australia's Northern Territory. Contributing factors include high levels of alcohol consumption, viral hepatitis and metabolic syndrome. Rural Aboriginal residents form a significant proportion of the Central Australian population and present a challenge to traditional models of liver care. HCC surveillance and variceal screening are core components of liver cirrhosis management.

AIM

To assess participation in HCC and variceal surveillance programmes in a Central Australian liver cirrhosis patient cohort.

METHODS

Retrospective cohort study of patients with liver cirrhosis presenting to Alice Springs Hospital, Australia between January 1, 2012 and December 31, 2017. Demographic data, disease severity, attendance at hepatology clinics, participation in variceal and/or HCC surveillance programmes was recorded. Regression analyses were conducted to assess factors associated with two independent outcomes: Participation in HCC and variceal surveillance.

RESULTS

Of 193 patients were identified. 82 patients (42.4%) were female. 154 patients (80%) identified as Aboriginal. Median Model for End-stage Liver Disease Score at diagnosis was 11. Alcohol was the most common cause of cirrhosis. Aboriginal patients were younger than non-Aboriginal patients (48.4 years *vs* 59.9 years, $P < 0.001$). There were similar rates of excess alcohol intake (72.6% *vs* 66.7%, $P = 0.468$)

and obesity (34.5% *vs* 38.4%, P = 0.573 across non-Aboriginal and Aboriginal cohorts. 20.1% of patients took part in HCC surveillance and 42.1% of patients completed variceal screening. Aboriginal patients were less likely to engage with either HCC surveillance (OR: 0.38, 95%CI: 0.16-0.9, P = 0.025) or undergo variceal screening (OR: 0.31, 95%CI: 0.14-0.65, P = 0.002).

CONCLUSION
HCC or variceal surveillance programmes had less uptake amongst Aboriginal patients. Greater emphasis needs to be placed on eliminating cultural obstacles to accessing hepatology services.

Key Words: Viral hepatitis; Cirrhosis; Hepatocellular carcinoma; Alcoholic liver disease; Central australia

Core Tip: Liver cirrhosis is prevalent in Australia's Northern Territory. Liver disease is a contributor to the mortality gap between Aboriginal and non-Aboriginal Australians. 20.1% of patients included in our study participated in hepatocellular carcinoma surveillance and 42.1% of patients underwent screening endoscopy in a rural Australian centre. Aboriginal patients were less likely to engage with screening programs despite their predominance in our study cohort.

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INTRODUCTION

Liver cirrhosis and hepatocellular carcinoma (HCC) constitute end-stage manifestations for a diverse range of pathological processes affecting the liver. Medical care of patients with cirrhosis is centred on treating underlying causative pathology, screening for HCC and preventing decompensation of liver disease[1]. Standards of care in liver cirrhosis are well established in hepatological literature and national guidelines[2,3]. The core measurable components of cirrhosis care pertain to surveillance programmes for gastro-oesophageal varices and HCC. These have been shown to improve survival and ameliorate healthcare costs of liver disease[4-6].

Escalating morbidity and mortality rates from liver disease in Australia are widely recognized[2]. This rising tide of liver disease has been fuelled by hazardous alcohol consumption, viral hepatitis and obesity[7]. The healthcare costs of liver disease were estimated at $50 billion *per* annum across Australia in 2012[2]. Geographical and socio-economic disparities in liver-related health service provision are a reality in Australia. The Northern Territory (NT) of Australia is afflicted by the highest *per* capita alcohol intake in Australia and one of the highest in the world[8]. Furthermore, liver disease has been identified as one of the major diseases contributing to the well cited mortality gap between Aboriginal and non-Aboriginal Australians[9-11]. Cross-sectional studies have demonstrated significantly higher prevalence of Hepatitis B in Aboriginal NT residents[12].

Contemporary healthcare models focus on the provision of centralized specialist cirrhosis care at tertiary hospitals in metropolitan areas. The Central Australian region is centred around the town of Alice Springs and spans a vast area encompassing parts of NT, South Australia and Western Australia. Central Australia is distinct from other parts of Australia given its remoteness and high proportion of Aboriginal constituents. This posits significant challenges for service providers in providing easily accessible culturally appropriate liver-related healthcare surveillance and interventions. Adherence with nationally agreed standards of care in liver cirrhosis in Central Australia has hitherto not been formally assessed.

Our study intends to outline the demographical and epidemiological charachteristics of patients presenting to Alice Springs Hospital with liver cirrhosis. We also examined the influence of these factors on participation in variceal and HCC surveillance programmes.

Study setting

The Central Australian healthcare model is best described as "hub and spoke" in nature. Alice Springs Hospital is 186-bed healthcare facility that serves as the sole referral centre for an area of approximately 577000 km squared with a population of just under 50000. Thus, conducting our study at ASH provides an insight into the standards of liver cirrhosis care for the wider Central Australian region.

MATERIALS AND METHODS

Case ascertainment

The primary data for this study includes information on all patients admitted to ASH with an underlying or new diagnosis of liver cirrhosis between January 1, 2012 and December 31, 2017. The study cohort was identified using ICD-10 codes. Lists of ICD-10 Codes used to identify potential cases included liver cirrhosis as Principal (98 episodes) or Additional diagnosis (789 episodes) and chronic liver disease as principal (246) or additional diagnosis (4728) (Codes K70, K71, K72, K73, K74, K75, K76, K77).

Case episodes were screened using electronic and paper medical records to identify eligible patients. Our study inclusion criteria required a confirmed diagnosis of liver cirrhosis and permanent residence in the Central Australian region. Diagnosis of liver cirrhosis was confirmed through assessment of available histology, biochemistry, radiography and documented clinical findings. Importantly, patients with probable diagnosis of cirrhosis based on either radiology or biochemistry but without documented clinical confirmation were not included in the analysis.

Data collated from medical records included demographic data, time of initial diagnosis, risk factors, aetiology of liver cirrhosis, Child-Pugh (CP) score, Model for End-stage Liver Disease (MELD) score at time of diagnosis, mode of initial presentation, referral to specialist liver clinic, participation in variceal and/or HCC surveillance programmes and development of HCC. From a residential perspective, the majority of non-Aboriginal residents of Alice Springs reside in registered domiciles whilst a significant proportion of Aboriginal residents live in distinct camps in the fringes of the city[13]. Residential status of participants was thus divided into three entities: Alice Springs town, Alice Springs camps or rural.

Aetiology of liver cirrhosis was confirmed retrospectively based on medical records. Case-notes of patients diagnosed with Alcohol related cirrhosis were reviewed to confirm current or previous hazardous alcohol intake. For the purposes of this study, hazardous alcohol intake was defined as > 14 standard units *per* week in line with National Health and Medical Research Council recommendations [14]. Presence of hepatitis C virus (HCV) and Hepatitis B was confirmed through analysis of HCV RNA levels and hepatitis B serological tests (HBsAg, HBsAb, HbcAb, HbeAg, HbeAb), retrospectively. Non-Alcoholic fatty liver disease (NAFLD) related cirrhosis was diagnosed in patients with metabolic risk factors (obesity, type 2 diabetes, hypercholesterolemia) in the absence of hazardous alcohol intake. Autoimmune and primary biliary cirrhosis were diagnosed on the basis of serological, histological and biochemical testing.

Our primary outcomes were participation in HCC and variceal surveillance programmes. Participation in HCC Surveillance was defined as undergoing 6-monthly ultrasound assessment over a minimum of 1 year. Completion of an index screening endoscopy at diagnosis was used as a surrogate marker for adherence with variceal surveillance. Internationally validated Baveno VI criteria only recommend screening in selected patients with cirrhosis based on platelet count and elevated liver stiffness measurements[4]. However, the absence of transient elastography services at ASH prohibited the use of Baveno criteria as a discriminating tool. Regression analyses were conducted to assess factors associated with two independent outcomes: Participation in HCC and variceal surveillance.

Statistical analysis

Descriptive statistics are presented for all patients in Table 1. Table 2 outlines a comparison of Aboriginal *vs* non-Aboriginal patients. Categorical variables were compared using Chi square or Fisher's Exact Test. Normally distributed variables were analysed using Independent t-test while Wilcoxon Rank Sum Test was utilised for non-normally distributed variables. Our secondary outcomes focused on assessing the demographic and clinical variables influencing participation in HCC and variceal surveillance programmes. Unadjusted and adjusted binary logistic regressions were performed for both HCC and variceal surveillance (in separate models). These analyses are presented in Tables 3 and 4. Confounders included in the adjusted models include age, gender, CP score.

RESULTS

A total of 5861 Case Episodes were identified using the coding criteria stated in our methodology. From a thorough analysis of these case episodes, we identified 193 patients with confirmed cirrhosis presenting to ASH from January 1, 2012 to December 31, 2017.

The discrepancy between case episodes and included patients was due to multiple factors. Firstly, the majority of case episodes identified with our extended search criteria involved non-cirrhotic patients. Secondly, most of our cohort presented to ASH on multiple occasions during the study period. Thirdly, patients with probable cirrhosis who had not undergone confirmatory testing were not included.

Of 57.5% of the study cohort were male. 154 patients (80%) of the study cohort were Aboriginal. The average age at diagnosis was 50.7 years old (SD 11.9). The median MELD Score was 10 (IQR: 8.18). 49% of the study cohort presented with CP Class A cirrhosis at the time of diagnosis. Of the remainder, 38% of patients initially presented with CP Class B cirrhosis and 12% with CP Class C. 31% of patients

Table 1 Descriptive statistics for all data and all variables in the study

Total number of patients	193
Age at diagnosis, years–mean ± SD	50.7 (11.9)
Gender	
Female	82 (42.5%)
Male	111 (57.5%)
Aboriginal	154 (79.8%)
Residence	
Alice Springs	58 (30.1%)
Alice Springs township	31 (16.1%)
Rural	104 (53.9%)
Risk factors	
IVDU	15 (7.9%)
Hazardous alcohol intake	137 (71.4%)
Obesity	63 (35.6%)
Child-Pugh score	
A	94 (50%)
B	71 (37.8%)
C	23 (12.2%)
MELD score–median (IQR)	10 (8, 18)
Decompensating event triggering admission	58 (30.4%)
Aetiology	
Alcohol	96 (49.7%)
Hepatitis B	22 (11.4%)
NAFLD	11 (5.7%)
Hepatitis C	9 (4.7%)
Cardiac cirrhosis	6 (3.1%)
Cryptogenic	6 (3.1%)
Autoimmune hepatitis	2 (1%)
Biliary diseases	2 (1%)
NAFLD + Alcohol	5 (2.6%)
Hepatitis C + Alcohol	11 (5.7%)
Hepatitis B + Alcohol	18 (9.3%)
Cardiac cirrhosis + NAFLD	3 (1.6%)
Hepatitis B + NAFLD	2 (1.0%)
Participation in variceal surveillance	75 (41.9%)
Participation in HCC surveillance	32 (20.3%)
Development of HCC during study period	29 (15.0%)
Review in specialist clinic	95 (49.5%)
Referral for liver transplantation	12 (6.4%)

NAFLD: Non-Alcoholic fatty liver disease; HCC: Hepatocellular carcinoma; MELD: Model for End-stage Liver Disease; IVDU: Intravenous drug use.

Table 2 Comparison of aboriginal *vs* non-aboriginal patients

	Aboriginal	Non-aboriginal	*P* value
Total number	154 (79.8%)	39 (20.2%)	
Age at diagnosis–mean ± SD	48.4 (11.1)	59.9 (10.9)	< 0.001
Gender–Female	76 (49.4%)	6 (15.4%)	< 0.001
Residence			< 0.001
Alice Springs	24 (15.6%)	34 (87.2%)	
Alice Springs camp	31 (20.1%)	0	
Rural	99 (64.3%)	5 (12.8%)	
Risk factors			
IVDU	2 (1.3%)	13 (33.3%)	< 0.001
Hazardous alcohol intake	111 (72.6%)	26 (66.7%)	0.468
Obesity	48 (34.5%)	15 (38.4%)	0.573
Child-Pugh score			0.091
A	69 (46.3%)	25 (64.1%)	
B	62 (41.6%)	9 (23.1%)	
C	18 (12.1%)	5 (12.8%)	
MELD score–median (IQR)	11 (8, 20)	10 (8, 12)	0.026
Decompensating event triggering admission	45 (29.4%)	13 (34.2%)	0.565
Aetiology			< 0.001
Alcohol	86 (55.8%)	10 (25.6%)	
Hepatitis B	20 (13.0%)	2 (5.1%)	
NAFLD	12 (7.8%)	2 (5.1%)	
Hepatitis C	1 (0.7%)	8 (20.5%)	
Cardiac cirrhosis	4 (2.6%)	2 (5.1%)	
Cryptogenic	4 (2.6%)	2 (5.1%)	
Autoimmune hepatitis	1 (0.7%)	1 (2.6%)	
Biliary diseases	0	2 (5.1%)	
Hepatitis B + Alcohol	18 (11.7%)	0	
NAFLD + Alcohol	5 (3.3%)	0	
Hepatitis C + Alcohol	1 (0.7%)	10 (25.6%)	
Hepatitis B + NAFLD	2 (1.3%)	0	
Variceal surveillance	24 (17.8%)	11 (34.4%)	0.002
HCC surveillance	21 (16.7%)	11 (34.4%)	0.038
Development of HCC	21 (13.6%)	8 (20.5%)	0.283
Review in specialist clinic	63 (41.2%)	32 (84.1%)	< 0.001
Referral for liver transplantation	5 (3.3%)	7 (18.9%)	< 0.001

NAFLD: Non-Alcoholic fatty liver disease; HCC: Hepatocellular carcinoma; MELD: Model for End-stage Liver Disease; IVDU: Intravenous drug use.

presented with decompensating events as the first clinical manifestation of liver cirrhosis. The most common decompensating events were acute on chronic liver failure and variceal haemorrhage. 54% of our cohort were residents of rural Central Australia. 30% of patients lived in Alice Springs whilst 16% were listed as residents of the surrounding town camps.

Table 3 Unadjusted and adjusted binary logistic models of hepatocellular carcinoma surveillance versus Aboriginal status

Risk factor	Surveillance participation		Comparison	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
	Yes	No		Univariate		Multivariable	
Aboriginal status-Yes	18	95	Yes *vs* No	0.31 (0.13, 0.77)	0.011	0.29 (0.10, 0.87)	0.028
Age-mean ± SD	51.7 (10.9)	49.4 (10.8)		1.02 (0.98, 1.06)	0.308	1.00 (0.95, 1.04)	0.866
Gender–Male	18	64	Female *vs* Male	0.80 (0.35, 1.84)	0.398	1.10 (0.45, 2.71)	0.838
Child-Pugh score					0.930		0.950
A	18	65	A *vs* B	1.18 (0.51, 2.74)	0.694	0.97 (0.40, 2.34)	0.942
B	11	47					
Residence					0.026		
Alice Springs	14	27	AS *vs* ASC	11.41 (1.39, 93.66)	0.023		
Alice Springs camps	1	22	AS *vs* R	2.37 (1.00, 5.64)	0.05		
Rural	14	64	AST *vs* R	0.21 (0.03, 1.67)	0.14		
See specialist liver clinic–Yes	26	52	Yes *vs* No	10.17 (2.91, 35.52)	< 0.001		

AS: Alice springs; ASC: Alice springs camps; AST: Aspartate aminotransferase.

Table 4 Unadjusted and adjusted binary logistic models of Variceal surveillance

Risk factor	Surveillance participation		Comparison	Odds ratio (95%CI)	*P* value	Odds ratio (95%CI)	*P* value
	Yes	No		Univariate		Multivariable	
Aboriginal Status-Yes	51	90	Yes *vs* No	0.31 (0.14, 0.65)	0.002	0.29 (0.12, 0.69)	0.005
Age-mean ± SD	51.9 (11.6)	49.2 (11.2)		1.02 (0.99, 1.04)	0.116	1.01 (0.98, 1.04)	0.621
Gender–Male	43	59	Female *vs* Male	1.00 (0.55, 1.82)	0.995	1.36 (0.70, 2.63)	0.358
Child-Pugh score					0.930		0.950
A	39	51	A *vs* B	1.13 (0.60, 2.15)	0.703	0.90 (0.45, 1.76)	0.750
B	27	40	A *vs* C	1.05 (0.39, 2.86)	0.922	0.96 (0.34, 2.71)	0.941
C	8	11	B *vs* C	0.93 (0.33, 2.61)	0.888	1.07 (0.37, 3.13)	0.900
Residence					0.002		
Alice Springs	33	20	AS *vs* ASC	4.03 (1.55, 10.47)	0.004		
Alice Springs camps	9	22	AS *vs* R	3.05 (1.52, 6.13)	0.002		
Rural	33	61	AST *vs* R	0.76 (0.31, 1.83)	0.535		
See specialist liver clinic–Yes	54	39	Yes *vs* No	4.22 (2.22, 8.02)	< 0.001		

AS: Alice Springs; ASC: Alice Springs camps; AST: Aspartate aminotransferase.

Alcohol related cirrhosis was the most common cause of cirrhosis in our study. Liver cirrhosis was attributed to alcohol in 71% of the study cohort. Viral hepatitis was also prevalent amongst our study cohort. 42 patients (22%) were identified as having chronic hepatitis B whilst 20 patients (10%) had hepatitis C. 11% of patients were deemed as having liver cirrhosis related to NAFLD. 5% of patients developed chronic congestive liver cirrhosis as a sequelae of underling cardiac failure. Six patients had cryptogenic cirrhosis (Table 1). 29 patients developed HCC as a complication of liver cirrhosis. These patients were predominately male (72%) and Aboriginal (72%).

Table 2 presents a comparison of epidemiological data between Aboriginal and non-Aboriginal patients. Aboriginal patients were significantly younger than their non-Aboriginal counterparts (48.4 years *vs* 59.9 years, $P < 0.001$). Non-Aboriginal patients were predominately male (85%) while there was an equal gender split for the Aboriginal cohort. The average MELD score for Aboriginal patients was 11 (IQR: 8.20) and 54% presented with CP Class B or C cirrhosis. The corresponding figures for non-Aboriginal patients were 10 (IQR: 8.12) and 36%, respectively. There were no observed differences in

rates of hazardous alcohol intake (72.6% *vs* 66.7%, $P = 0.468$) and obesity (34.5% *vs* 38.4%, $P = 0.573$) between Aboriginal and non-Aboriginal cohorts. Our Aboriginal cohort had significantly lower rates of intravenous drug use (1.3% *vs* 33.3%, $P < 0.001$). From a geographical perspective, Aboriginal patients were significantly more likely to be residents of rural communities or town camps ($P < 0.001$). Aboriginal patients were less likely to attend specialist liver clinics.

Given their association with Aboriginal ethnicity, place of residence and specialist clinic non-attendance were excluded from adjusted models examining factors influencing participation in surveillance programmes.

Adherence with variceal surveillance

Four patients were excluded as they died during their index admission and 11 patients were excluded on account of incomplete data. Thus, 178 patients were included in the primary analysis. Of the included patients, 75 (42.1%) received a screening endoscopy within six months of their diagnosis.

On univariate analysis, attendance at specialist liver clinics was associated with participation in variceal surveillance (OR: 4.22, 95%CI: 2.22-8.02, $P < 0.0001$). Patients residing in Alice Springs were more likely to participate than patients from town camps or rural communities (AS *vs* AST, OR: 4.03, 95%CI: 1.5-10.5, $P = 0.004$; AS *vs* R, OR: 3.05, CI: 1.52-6.13, $P = 0.002$). Conversely, Aboriginal ethnicity (OR: 0.31, 95%CI: 0.14-0.65, $P = 0.002$) was associated with non-completion of screening endoscopy in both unadjusted and adjusted models. Neither age, gender nor disease severity were found to be associated with variceal surveillance in either model.

Adherence with HCC surveillance

Overall, 141 patients were included in the analysis of HCC surveillance participation. 29 patients (20.6%) participated with regular sonographic surveillance. Patients were excluded on the basis of CP disease severity (18 patients), concurrent diagnosis of HCC with cirrhosis[9], absence of follow up data [10] and death within 12 mo of cirrhosis diagnosis[15]. In unadjusted models, review at specialist clinic was strongly associated with participation in HCC surveillance (OR: 10.17, 95%CI: 2.91-35.5, $P < 0.001$). Residence in Alice Springs was associated with better adherence to regular liver sonography in comparison to Alice Springs town camps and rural regions. Aboriginal patients were less likely to participate in both unadjusted (OR: 0.31, 95%CI: 0.13-0.77, $P = 0.01$) and adjusted models (OR: 0.29, 95%CI: 0.10-0.87, $P = 0.03$). Neither age, gender nor disease severity were found to be associated with HCC surveillance in either model.

DISCUSSION

With respect to overall participation in HCC surveillance, 20% of our cohort demonstrated sustained engagement with 6 moly ultrasound scans. Poor uptake limits the utility of surveillance as a means of ameliorating the morbidity, mortality and healthcare costs of HCC at a population level. This is rendered of greater significance by the heavy burden of HCC in the NT[15]. It is important to note that poor uptake of HCC surveillance is not an issue specific to Central Australia. Participation is limited even in more urban and resource-rich settings. A retrospective study in Melbourne of patients diagnosed with HCC between 2012-2013 demonstrated a 41% compliance rate with surveillance[16]. These statistics reflect the broader social and medical disenfranchisement of patients with cirrhosis as well as the demanding nature of regular surveillance sonography. Comparatively, variceal surveillance had greater uptake and this likely reflects the liberal definition used in our study as well as ease of access to endoscopy services during index admissions. In clinical practice, variceal surveillance requires further endoscopies with advancing severity of liver cirrhosis. However formal guidelines on screening intervals vary considerably and lack consensus.

Aboriginal ethnicity was strongly associated with non-participation in both HCC and variceal surveillance. This is rendered further significance as 80% of our study cohort was Aboriginal; a particularly noteworthy fact given that Aboriginal residents make up less than one quarter of the Central Australian population. This disproportionate prevalence of cirrhosis in Aboriginal patients correlates well with epidemiological data showing significantly higher incidence rates of HCC and liver disease in Aboriginal Territorians[17,18]. We demonstrated other points of departure between Aboriginal and non-Aboriginal cohorts. Aboriginal patients with cirrhosis presented at a younger age and with more advanced disease. This is in keeping with findings from a larger Australian retrospective cohort study comparing cirrhosis admissions between Aboriginal and non-Aboriginal populations over a 10-year period in Queensland[10]. Additionally, half of our Aboriginal cohort were women. This contrasts with the male predominance of the non-Aboriginal cohort. Extrapolating further, these results are also out of keeping with national statistics that demonstrate distributions of premature liver deaths and liver related hospitilisations skewed towards men[2].

This significant burden of liver disease needs to be understood within broader socioeconomic context for Aboriginal Central Australians. Liver disease, similar to other highly prevalent chronic diseases, is a corollary of social, political and economic disenfranchisement[19]. It is important for clinicians and

policy makers to recognise the root causes for poor health and liver cirrhosis. Socioeconomic factors predisposing to high-risk behaviours such as hazardous alcoholic intake also play a role in the poor engagement of Aboriginal patients with formal liver services as demonstrated in our study.

Language and culture are additional factors that represent major obstacles to engagement with liver services for Aboriginal patients in Central Australia[20]. In rural Central Australia, up to 80% of Aboriginal households predominately speak one of the 18 traditional languages. Proficiency in standard English is typically variable. This is in stark contrast with national census data showing that 83% of Aboriginal and Torres Strait Islanders speak English as a first language[19]. Language barriers have significant repercussions for healthcare provision at ASH where most of the workforce are non-Aboriginal. Medical and follow-up information is often poorly disseminated and vulnerable to misinterpretation by patients. An ASH based study investigating recorded self-discharge rates found that up to 80% of patients were unaware of medical diagnosis or proposed length of stay[20]. Similarly, achieving effective patient engagement is limited by other cultural factors. A study in nearby Mount Isa, Queensland found that patient perceptions of poor understanding or respect of Aboriginal culture on the part of medical practitioners was a major barrier to care[21]. Communication barriers and failures in achieving patient trust clearly remain impediments in engaging Aboriginal patients with formal liver services in Central Australia.

Specialist review and residence in Alice Springs were both associated with completion of screening endoscopy and HCC surveillance in unadjusted models. This may reflect the fact that patients with sufficient motivation to attend outpatient appointments and located closer to central services are more likely to engage with surveillance programmes. It is also important to acknowledge the mediating effects of specialist review and place of residence on the causal pathway between Aboriginal status and reduced participation in liver surveillance programmes. Aboriginal patients were significantly less likely to attend specialist liver clinics and more likely to live either rurally or in town camps. This mediating effect is seen when considering the influence of place of residence on surveillance participation. Non-Aboriginal patients from Alice Springs were more likely to participate in both HCC and Variceal surveillance than the exclusively Aboriginal patients residing in Alice Springs town camps. Furthermore, there were no statistically significance difference in surveillance participation between rural and camp based Aboriginal patients.

From an aetiological perspective, alcohol and viral hepatitis were the main drivers of liver cirrhosis. Alcohol was implicated in the aetiology of more than two thirds of our study cohort either alone or in combination with viral hepatitis. Contextually, NT has been identified as having the highest *per* capita alcohol intake in Australia and one of the highest in the world. Similar proportions of Aboriginal and Non-Aboriginal patients exceeded recommended weekly limits of alcohol intake. Despite this, 75% of Aboriginal patients were classified as having alcohol related cirrhosis whilst only 25% of non-Aboriginal patients were labelled with this diagnosis. This discrepancy may be explained by the non-linear relationship between hazardous alcohol intake and development of cirrhosis[22]. Data from the Australian Institute of Health And Welfare's National Drug Strategy Household Survey showed that while Aboriginal individuals were less likely to drink than non-Aboriginal counterparts, those that do are more likely to do so at hazardous levels[23].

However, it is impossible to discount potential elements of diagnostic bias especially when patients were not under the purview of specialists. The potential under-recognition of NAFLD in our study may support this view. Less than 10% of our cohort were deemed as having NAFLD as *per* available documentation. One would expect a higher prevalence of NAFLD in a Central Australian cohort given the above average rates of obesity and diabetes as well as the fact this condition is the most prevalent form of liver disease in Australia[2]. Another point of concern for patients with cirrhosis who were not reviewed by liver specialists was a propensity to label alcohol as the primary aetiological factor without completion of the full battery of screening tests. This is clinically significant given that heavy alcohol intake has been shown to accelerate the progression of liver inflammation in underlying chronic hepatitis B and C[24]. Furthermore, potentially erroneous labelling of alcohol related liver disease can perpetuate stigmatisation of Aboriginal patients. Several authors have highlighted stigma as a major limiting factor in the engagement of Aboriginal patients with formal healthcare services[25].

Our study has a few limitations which our study design was unable to eliminate. Firstly, accurately quantifying the prevalence of liver cirrhosis in Central Australia is beyond the scope of this study. Secondly, our focus on hospital inpatients may not be reflective of the general cirrhosis population. This cohort of patients tend to be from more disadvantaged socio-economic backgrounds and present with more severe liver disease. A natural consequence of this is the presence of a selection bias that may render the study cohort less representative. However, our study does serve to determine whether the current model of liver care adequately meets the need of the most vulnerable subset of cirrhotic patients in Central Australia. We endeavour that this study can also be used as a foundation for further research in the area of liver cirrhosis in the Central Australian region.

CONCLUSION

Aboriginal patients were strongly overrepresented in our study and were less likely to engage with HCC or variceal surveillance. Strategies devised to address the issue of liver disease in Central Australia will need to focus on eliminating cultural barriers to accessing care, expanding capacity for specialist review and ameliorating hazardous alcohol intake on a population level. We endeavour that this study can also be used as a foundation for further research in the area of liver cirrhosis in the Central Australian region.

ARTICLE HIGHLIGHTS

Research background

Northern Territory (NT), Australia has high rates of liver cirrhosis and hepatocellular carcinoma (HCC) as a consequence of harmful alcohol use, viral hepatitis and metabolic syndrome. Aboriginal persons constitute a significant proportion of the population in the Central Australian region of NT. Several challenges are faced in providing culturally appropriate liver care to the diverse Central Australian population.

Research motivation

Liver disease has been identified as a significant contributor to the well cited mortality gap between Aboriginal and non-Aboriginal Australians. Central Australia is unique within Australia given its high proportion of Aboriginal residents. Formal adherence with HCC or variceal screening programmes have not been specifically assessed in Central Australia.

Research objectives

Our first research objectives involves description of the baseline charachteristics of inpatients presenting to a Central Australian hospital. Our second research objective involves assessment of adherence with HCC surveillance as well as analysis of the factors associated with participation. Our third research objective involves assessment of adherence with HCC surveillance as well as analysis of the factors associated with participation.

Research methods

Our study methodology involved performing a retrospective cohort study. All idenitified patients presenting to inpatient departments at Alice Springs Hospital, NT, Australia between 2012 to 2017 were included in the study. We collected data including demographics, disease causation and severity (Child-Pugh Score), referral to hepatology clinics and adherence with variceal and/or HCC surveillance programmes. Regression analyses were conducted to assess factors associated with two independent outcomes: Adherence with HCC and variceal surveillance.

Research results

Aboriginal persons were over-represented and made up 80% of the study cohort. Aboriginal patients were younger and presented with more severe disease than non-Aboriginal counterparts. Overall 20.1% of our study cohort participated in HCC surveillance while 42.1% of patients underwent variceal screening. Aboriginal ethnicity was inversely associated with participation in HCC surveillance.

Research conclusions

This is the first study examining adherence with standards of liver cirrhosis care in Central Australia. Liver cirrhosis in Central Australia disproportionately affects Aboriginal communities as a corollary of adverse metabolic profiles, hazardous alcohol intake and viral hepatitis. The current centralised model of cirrhosis care does not adequately meet the need of Aboriginal Central Australians. Our study demonstrates the pressing need for interventions to improve participation of Aboriginal patients with cirrhosis in HCC screening in order to ameliorate the morbidity and mortality associated with delayed diagnosis. Language, geographical and cultural factors are important prisms through which to examine low participation rates among Aboriginal patients in Central Australia. This is compounded by limited utilisation of valuable primary care links. Correspondingly, interventions aimed at closing the gap in liver related health outcomes between Aboriginal and non-Aboriginal patients need to focus on addressing these factors.

Research perspectives

Future research should focus on piloting alternative models of cirrhosis care for Aboriginal patients with liver cirrhosis in Central Australia. Alternative care models should focus on expanding provision of telehealth services, enhancing utilisation of primary health care links and culturally tailoring care.

FOOTNOTES

Author contributions: Raja SS and Batey RG designed the research study; Raja SS applied for local Ethical Approval and wrote the manuscript; Raja SS and Aung HH performed data collection; Raja SS, Edwards S and Batey RG analyzed the data; all authors have read and approve the final manuscript.

Institutional review board statement: Approval for this study was given by the Central Australian Health and Research Ethics Committee (Ref: CA-19-3415) and Alice Springs Hospital, Central Australian Health Service (Ref:EDOC2019/0172321).

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at sreecanth.raja@sa.gov.au. Individual consent was not obtained but the presented data is de-identified without risk of identification.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Risk factors and prediction of acute kidney injury after liver transplantation: Logistic regression and artificial neural network approaches

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Abstract

BACKGROUND

Acute kidney injury (AKI) has serious consequences on the prognosis of patients undergoing liver transplantation. Recently, artificial neural network (ANN) was reported to have better predictive ability than the classical logistic regression (LR) for this postoperative outcome.

AIM

To identify the risk factors of AKI after deceased-donor liver transplantation (DDLT) and compare the prediction performance of ANN with that of LR for this complication.

METHODS

Adult patients with no evidence of end-stage kidney dysfunction (KD) who underwent the first DDLT according to model for end-stage liver disease (MELD) score allocation system was evaluated. AKI was defined according to the International Club of Ascites criteria, and potential predictors of postoperative AKI were identified by LR. The prediction performance of both ANN and LR was tested.

RESULTS

The incidence of AKI was 60.6% (n = 88/145) and the following predictors were identified by LR: MELD score > 25 (odds ratio [OR] = 1.999), preoperative kidney dysfunction (OR = 1.279), extended criteria donors (OR = 1.191), intraoperative arterial hypotension (OR = 1.935), intraoperative massive blood transfusion (MBT) (OR = 1.830), and postoperative serum lactate (SL) (OR = 2.001). The area under the receiver-operating characteristic curve was best for ANN (0.81, 95% confidence interval [CI]: 0.75-0.83) than for LR (0.71, 95%CI: 0.67-0.76). The root-mean-square error and mean absolute error in the ANN model were 0.47 and 0.38, respectively.

CONCLUSION

The severity of liver disease, pre-existing kidney dysfunction, marginal grafts, hemodynamic instability, MBT, and SL are predictors of postoperative AKI, and ANN has better prediction performance than LR in this scenario.

Key Words: Logistic regression; Liver transplantation; Acute kidney injury; Machine learning; Artificial neural network

Core Tip: This study aimed to identify the risk factors of acute kidney injury (AKI) after deceased-donor liver transplantation and compare the performance of artificial neural network (ANN) with that of logistic regression (LR) analysis to predict this complication. LR analysis revealed the following predictors of AKI: Previous kidney dysfunction, marginal grafts, intra-operative arterial hypotension, massive blood transfusion, and serum lactate. ANN prediction had better performance than LR in this scenario.

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INTRODUCTION

Among the possible complications of complex abdominal and liver procedures, acute kidney injury (AKI) should be considered a major cause of postoperative morbidity and mortality[1-6]. Updated data report a 0.9%-17.9% incidence of AKI after liver resection[7-9], and 4%-94% after LT[10,11], either living-donor (LDLT) or deceased-donor LT (DDLT). Although there is a lack of a reported standard definition of postoperative AKI[12] after DDLT, it is of fundamental importance to identify patients at risk for AKI after LT, ideally by the set of preoperative clinical evaluation, as well as by the complementary information of the intraoperative period, thus enabling the adoption of preventive measures or early therapies for AKI in the postoperative period.

There are many studies available based on deep learning models for different clinical purposes in distinct fields of medicine, such as for complex imaging acquisition and processing[13-17], and artificial neural network (ANN) as a deep learning modality is commonly used to solve complex problems, where the behavior of variables is not rigorously known. In the specific field of AKI after LT, along with other machine learning techniques (gradient boosting machine, random forest, decision tree, support vector machine, naïve Bayes, and deep belief network), ANN has already been compared to multivariable logistic regression (LR) regarding their prediction performance[18]. We hypothesized that ANN would be a feasible alternative with higher performance than the classic LR model, reinforcing the wide applicability of ANN and its ability to learn from input data with or without supervision.

The multifactorial origin of AKI after LT makes it complex to predict which candidate for the procedure has an increased risk of this complication, and in the face of this complexity, along with the classical LR, ANN would be a very reliable prognostic tool for AKI risk assessment, where the relative risk term is parameterized by an ANN instead of regression, enabling the application of deep learning, whereas comparative studies evaluating such a promising tool for predicting AKI following LT are scarce[19-20].

In face of this serious postoperative complication, this retrospective study of patients who underwent only-first DDLT aimed to identify the risk factors for postoperative AKI and compare the prediction performance of ANN with that of LR for this complication.

MATERIALS AND METHODS

Study design

A retrospective study was conducted on patients of both sexes, aged > 18 yr, diagnosed with liver cirrhosis and portal hypertension (platelets < 100000/mm^3, splenomegaly and/or esophageal varices), eventually associated with hepatocellular carcinoma (HCC), and undergoing the first DDLT at a tertiary referral hospital between September 2017 and June 2021. The patients were allocated according to Model for End-Stage Liver Disease (MELD) score, with no evidence of end-stage kidney disease. The MELD score was dichotomized at 25 points for statistical purposes according to Romano *et al*[21], and the minimum hospital stay was 7 d according to Wong *et al*[22] and the International Club of Ascites (ICA) definitions for the onset of AKI[23].

Renal dysfunction definitions

Kidney dysfunction (KD) subtypes were defined according to Wong *et al*[22] (Table 1) and the ICA definitions (Table 2)[23], and both the acute deterioration of renal function and the background CKD could be structural or functional in nature, including hepatorenal syndrome (HRS) types 1 and 2 (Table 3)[23]. Estimated glomerular filtration rate (eGFR) was calculated by the Modified Diet in Renal Disease 6 (MDRD6) formula: eGFR = 198 × [serum creatinine (mg/dL)$^{-0.858}$ × age$^{-0.167}$ × 0.822 if patient is female × 1.178 if patient is black] × [serum urea nitrogen concentration (mg/dL)]$^{-0.293}$ × [urine urea nitrogen excretion (g/d)]$^{0.249}$[3].

Graft definitions

Marginal liver grafts of extended criteria donor (ECD) were defined as grafts with three or more of the following donor features: > 60 yr, body mass index (BMI) > 27-30 kg/m^2, macrovesicular steatosis > 30%, intensive care unit (ICU) stay > 4 d, sustained arterial hypotension > 1 h, cold ischemia times (CIT) > 8 h, warm ischemia times (WIT) > 40-45 min, controlled sepsis, history of alcoholism, serum creatinine > 1.2 mg/dL, arterial hypotensive episodes < 60 mmHg for > 1 h, bilirubin > 2.0 mg/dL, alanine transaminase (ALT) > 170 U/L and aspartate transaminase (AST) > 140 U/L, the use of dopamine doses > 10 microg/kg per min, and peak serum sodium > 155 mEq/L[24-26].

Routine biopsy was performed on the donor allograft for all patients included in the study. Liver specimens were evaluated by hematoxylin and eosin staining using either frozen or permanent section. Macrovesicular steatosis was defined as a single vacuole larger than the nucleus, replacing most of the hepatocyte cytoplasm and displacing the nucleus to the cell membrane[27]. Macrosteatosis was categorized as no steatosis (< 5%), mild steatosis (10%-29%), moderate steatosis (30%-60%), and severe steatosis (> 60%)[28].

Hemodynamic status and monitoring

Fluid administration consisted of a baseline infusion of a balanced crystalloid (Plasmalyte, Baxter, Belgium) with or without 4% albumin (depending on patient conditions). Rapid infusers, perfusion heaters, and a Cell Saver (Haemonetics, Massachusetts, EUA) for blood recovery were ready for use prior to induction. In accordance to American Society of Anaesthesiologists (ASA) guidelines, Cell Saver has effectiveness in reducing the volume of allogeneic blood transfused[29].

A Flow Trac/EV1000 System (Edwards Lifesciences, Irvine, USA) was inserted and hemodynamic interventions were guided using continuous cardiac index (CCI), stroke volume index (SVI), mixed venous oxygen saturation (SvO_2), central venous pressure (CVP), and mean arterial pressure (MAP). Fluids were administered if SVI was < 30 mL/m^2 and/or CCI < 2 L/min/m^2 for compensation for blood loss *via* 250-500 mL fluid boluses of Plasmalyte, to strictly maintain MAP > 65 mmHg, avoiding hemodynamic instability as described elsewhere[30,31].

Blood loss monitoring consisted of visual assessment of the surgical field, including the extent of blood present, presence of microvascular bleeding, surgical sponges, clot size and shape, and volume in suction canister. In case of active hemorrhage, blood product administration was guided by using rotational thromboelastometry monitoring *via* ROTEM (Tem Innovations GmbH, Munich, Germany), hemoglobin/hematocrit monitoring, coagulation tests (international normalized ratio [INR]), activated partial thromboplastin time [aPTT], fibrinogen concentration [normal range: 200 to 400 mg/dL], and platelet count[29]. Whereas there is no clear evidence that ROTEM improved survival in LT patients, it was effective in reducing bleeding and fewer patients required both platelets and fresh frozen plasma (FFP) transfusion[32]. Monitoring for perfusion of vital organs included standard ASA monitoring, renal monitoring (urine output), and analysis of arterial blood gases and serum (SL) level (cutoff of 2.0 mmol/L)[29].

Massive blood transfusion (MBT) protocol for avoidance of dilutional coagulopathy was activated when hemorrhage was expected to be massive (anticipated need to replace 50% or more of blood volume within 2 h), or bleeding continued after the transfusion of 4 units of packed red blood cells (PRBC) within a short period of time (1-2 h), or systolic blood pressure (SBP) was below 90 mmHg and heart rate was above 120 beats per minute in the presence of uncontrolled bleeding[33]. According to the Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) study group recommendations,

Table 1 Diagnostic criteria for kidney dysfunction in cirrhosis (Wong *et al*[22], 2011)

Diagnosis	Definition
AKI	Rise in serum creatinine of > 50% from baseline or rise of sCr by > 26.4 mmol/L (> 0.3 mg/dL) in < 48 h; HRS type 1 is a specific form of AKI
CKD	eGFR of < 60 mL/min for > 3 mo calculated using MDRD6 formula; HRS type 2 is a specific form of CKD
ACKD	Rise in serum creatinine of > 50% from baseline or rise of sCr by > 26.4 mmol/L (> 0.3 mg/dL) in < 48 h in a patient with cirrhosis whose eGFR is < 60 ml/min for > 3 mo calculated using MDRD6 formula

AKI: Acute kidney injury; sCr: Serum creatinine; HRS: Hepatorenal syndrome; CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rate; ACKD: Acute on chronic kidney disease; MDRD6: Modification of Diet in Renal Disease 6.

Table 2 Definition and classification of acute kidney injury for patients with liver cirrhosis according to the International Club of Ascites (Angeli *et al*[23], 2015)

Baseline sCr	A sCr value obtained in 3 mo prior to hospital admission, with preference to the value dated the closest to hospital admission. In patients without a previous sCr value, the value on admission should be used
AKI definition	Increase in sCr ≥ 0.3 mg/dL (≥ 26.5 μmol/L) within 48 h; or the percentage increase in sCr ≥ 50%, which occurred in the last 7 d
Stage 1 AKI	Increase in sCr ≥ 0.3 mg/dL (26.5 μmol/L) or an increase of 1.5 to 2 times the baseline value
Stage 2 AKI	Increase of sCr 2 to 3 times the baseline value
Stage 3 AKI	Increase in sCr > 3 times the baseline or sCr ≥ 4.0 mg/dL (353.6 μmol/L), with acute increase in sCr ≥ 0.3 mg/dL (26.5 μmol/L) or onset of RRT

AKI: Acute kidney injury; ICA: International Club of Ascites. sCr: Serum creatinine; RRT: Renal replacement therapy.

Table 3 Diagnostic criteria and hepatorenal syndrome subtypes (Angeli *et al*[23], 2015)

Diagnostic criteria for HRS	HRS subtype
1) Presence of cirrhosis or ascites; 2) sCr > 1.5 mg/dL or 133 μmoles/L; 3) No improvement in sCr (below 1.5 mg/dL) after at least 48 h of diuretic withdrawal and volume expansion with albumin; 4) Absence of shock; 5) Has not undergone recent treatment with nephrotoxic drugs; 6) Absence of parenchymal kidney disease as indicated by proteinuria less than 500 mg/d, microhematuria (less than 50 erythrocytes/high-magnification field), and/or abnormal renal ultrasound findings	HRS type 1-Rapidly progressive renal failure defined as the doubling of initial serum creatinine to a level greater than 2.5 mg/dL or 220 μmoles/L in less than 3 wk, and associated with a very poor prognosis; HRS type 2-Moderate renal failure (sCr > 1.5 mg/dL or 133 μmoles/L), following a stable or slowly progressive course, often associated with refractory ascites

HRS: Hepatorenal syndrome; sCr: Serum creatinine.

blood transfusion of RBC, fresh frozen plasma (FFP), and platelets were at a 1:1:1 ratio[34].

Postreperfusion (PRS) was defined as a decrease in MAP > 30% below the baseline value, for at least 1 min, occurring during the first 5 min after reperfusion of the liver graft, asystole, or hemodynamically significant arrhythmias, or the need to start the infusion of vasopressors during the postreperfusion period[35]. Intraoperative arterial hypotension (IOAH) was defined as MAP less than 65-60 mmHg for at least 5 min, or any exposure to MAP less than 55-50 mmHg[31], irrespective of the cause: Prolonged surgery time, massive bleeding, PRS, and/or hemodynamic instability because of end-stage liver disease.

Statistical analysis

The baseline characteristics of the patients are expressed in absolute values, the mean ± SD, and percentages, when appropriate. The comparison between groups was performed for continuous variables using the Kruskal-Wallis test and the Mann-Whitney test. The assumptions were made to perform or not the parametric tests, and the categorical variables were compared using the chi-square test. Independent variables with significance in the univariate model was selected for the bootstrap classical LR model to assess the effect of bivariate independent variables (graft quality, patients characteristics, and intraoperative events) on the incidence of postoperative AKI. The results of the model are expressed by odds ratio (OR), together with the corresponding 95% confidence intervals [CIs], Nagelkerke R2 statistic, and Hosmer and Lemeshow goodness of fit test. P values < 0.05 were considered significant. A relationship map between the significant variables in the LR model was also

Table 4 Acute kidney injury stages according to International Club of Ascites criteria (*n* = 145)

Overall incidence (*n* = 88)	Stage 1 (*n* = 22)	Stage 2 (*n* = 36)	Stage 3/RRT (*n* = 30/12)
60.6%	15.1%	24.8%	20.6/8.7%

RRT: Renal replacement therapy.

constructed.

The explanatory variables selected in the LR model were used for the ANN machine learning. Before developing prediction models, our collected data were divided into 70% of training dataset cases and 30% of test dataset cases. The cases in the training dataset were used for developing machine learning models. The ANN method had its own hyperparameters (number of layers in multilayer perceptron ANN), with a 10-fold cross-validation. This cross-validation process was used for developing the model, and performance was evaluated. The activation function of the hidden layer was made by hyperbolic tangent activation function, and Softmax for the output layer. All possible combinations of hyperparameters were investigated, and the hyperparameters with the highest average validation AUROC (area under the receiver-operating characteristic curve) were considered as optimal hyperparameters, and after that, the final model was tested for performance by root-mean-square error (RMSE) and mean absolute error (MAE) calculation. The importance of variables for the model was calculated. ANN structural model was constructed according to Haykin[36].

Our primary analysis attempted to analyze the prediction ability of machine learning and LR model in terms of AUROC. Accuracy was defined as the sum of the number of cases with true positive and true negative results divided by the total number of test sets. Statistical calculations were performed using the SPSS 28.0 software for Windows.

RESULTS

During the period from September 2017 to June 2021, 145 DDLT cases were included in the present study. Of the total patients included, 88 (60.6%) presented any further stage of postoperative AKI during the 7-d follow-up, 22 (15.1%) developed stage 1 AKI, 36 (24.8%) developed stage 2, and 30 (20.6%) developed stage 3 AKI (Table 4); renal replacement therapy (RRT) was required in 12 patients (8.7%). All patients' preoperative baseline information, donors, and grafts characteristics according to the occurrence of AKI are shown in Tables 5 and 6. The intraoperative data related to IOAH, blood derivatives transfusion, and piggy-back clamping, and laboratorial tests until the seventh postoperative (PO) day are shown in Table 7.

In the LR analysis, Nagelkerke R2 statistic was 0.147. Hosmer and Lemeshow goodness of fit test was not significant at 5% (P = 0.247). The six following factors were confirmed as predictors (Table 8): Biological (not adjusted) MELD score ≥ 25 (OR = 1.999, 95%CI = 1.586-2.503, P < 0.001), pre-existing KD (OR = 1.279, 95%CI = 0.916-1.686, P < 0.001), ECD (OR = 1.191, 95%CI = 0.711-1.787, P = 0.002), IOAH (OR = 1.935, 95%CI = 1.505-2.344, P < 0.001), MBT (OR = 1.830, 95%CI = 1.428-2.241, P < 0.001), serum lactate at the end of LT (OR = 2.001, 95%CI = 1.616-2.421, P < 0.001). The relationships between the significant variables were explored by a relationship map detailed in Figure 1.

Data of the two models with regard to AUROC for predicting AKI of all stages are detailed in Figure 2. ANN had the largest test AUROC (0.81, 95%CI: 0.75-0.83) and highest accuracy (0.68) than LR analysis [AUROC (0.71, 95%CI: 0.67 to 0.76), accuracy = 0.68].

Importance plot for ANN is shown in Figure 3 (KD and MELD score ranked first and second, respectively). Multilayer perceptron ANN presented one hidden layer by hyperbolic tangent activation function with four nodes in the layer, as presented in the ANN structural model diagram (Figure 4), and the prediction RMSE was 0.47 and the prediction MAE was 0.38.

DISCUSSION

As described elsewhere[36], the findings in the present study demonstrated a high incidence of postoperative AKI, and the predictive ability of ANN and LR models for this complication. An important point in this research is that AKI prediction was focused on the identification of significant risk factors at the end of the procedure, thus enabling the adoption of preventive measures or early therapies for AKI in the postoperative period.

In the present study, the severity of chronic liver disease, pre-existing KD, marginal grafts, hemodynamic instability, MBT, and consequent inadequate tissue perfusion during LT were predictors of AKI after DDLT, and the relationship map illustrated through a visual pattern, the relationship

Table 5 Patients' preoperative baseline information according to the occurrence of acute kidney injury after deceased-donor liver transplantation (*n* = 145)

	No AKI (*n* = 57)	AKI (*n* = 88)	*P* value
Male gender, *n* (%)	29 (50.8)	49 (55.6)	0.441
Age (yr), mean (± SD)	53.2 (± 13.56)	56.2 (± 13.26)	0.352
BMI, mean (± SD)	18.2(± 4.54)	22.7 (± 4.92)	0.065
Biological MELD score, mean (± SD)	21.67 (± 2.15)	26.05 (± 3.05)	< 0.001
Previous ascites, *n* (%)	24 (42.1)	52 (59.0)	0.013
Previous encephalopathy, *n* (%)	18 (31.5)	39 (44.3)	0.025
Previous upper digestive bleeding, *n* (%)	21 (36.8)	45 (51.1)	0.018
Preexisting KD, *n* (%)	15 (26.3)	60 (68.1)	< 0.001
HCC, *n* (%)	20 (35.0)	37 (42.0)	0.069
Systemic arterial hypertension, *n* (%)	28 (49.1)	46 (52.2)	0.083
Diabetes mellitus, *n* (%)	23 (40.3)	43 (48.8)	0.254

AKI: Acute kidney injury; LT: Liver transplantation; SD: Standard deviation; KD; Kidney dysfunction; BMI: Body mass index; MELD: Model for End-stage Liver Disease; HCC: Hepatocellular carcinoma.

Table 6 Donor and graft characteristics according to the occurrence of acute kidney injury after deceased-donor liver transplantation (*n* = 145)

	No AKI (*n* = 57)	AKI (*n* = 88)	*P* value
Donor > 60 yr, *n* (%)	16 (28.0)	31 (35.2)	0.346
Donor BMI > 27-30 kg/m^2, *n* (%)	14(24.5)	28 (31.8)	0.039
Graft macrosteatosis > 30%, *n* (%)	11 (19.2)	32 (36.3)	0.024
GCIT > 8 h, *n* (%)	0	0	-
GWIT > 40-45 min	38 (66.6)	54 (61.3)	0.349
Donor ICU stay > 4 d, *n* (%)	11 (19.2)	22 (25.0)	0.088
Donor controlled sepsis, *n* (%)	05 (8.7)	11 (12.5)	0.061
History of alcoholism of donor, *n* (%)	08 (14.0)	15 (17.0)	0.255
Donor sCr > 1.2 mg/dL, *n* (%)	16 (28.0)	31 (35.2)	0.024
Donor hypotensive episodes (< 60 mmHg) > 1 h, *n* (%)	10 (17.5)	18 (20.4)	0.127
Donor serum bilirubin > 2.0 mg/dL, *n* (%)	25 (43.8)	48 (54.5)	0.087
Donor serum ALT > 170 U/L, *n* (%)	11 (19.2)	22 (25.0)	0.073
Donor serum AST > 140 U/L, *n* (%)	05 (8.7)	13 (14.7)	0.023
Use of dopamine doses > 10 microg/kg per min, *n* (%)	10 (17.5)	13 (14.7)	0.176
Donor peak serum sodium > 155 mEq/L, *n* (%)	02 (3.5)	5 (5.6)	0.219
ECD (3 or more factors above), *n* (%)	07 (12.2)	31 (35.2)	< 0.001

AKI: Acute kidney injury; LT: Liver transplantation; BMI: Body mass index; GCIT: Graft cold ischemia times; GWIT: Graft warm ischemia times; ICU: Intensive care unit: sCr: Serum creatinine; ALT: Alanine transaminase; AST: Aspartate transaminase; ECD: Extended criteria donor.

between the variables, although it is important to understand that a visual relationship does not always mean statistical causation. As demonstrated in our study, in the case of machine learning-based techniques, the importance of each variable in the dataset can be indicated by the characteristic importance measure, which can improve the transparency of the algorithm according to He *et al*[20].

Table 7 Intraoperative events in 145 deceased-donor liver transplantations according to the occurrence of postoperative acute kidney injury

	Without AKI (*n* = 57)	With AKI (*n* = 88)	*P* value
IOAH (bleeding/PRS), *n* (%)	14 (24.5)	54 (61.3)	< 0.001
MBT, *n* (%)	5 (8.7)	15 (17.0)	< 0.001
Vasoactive drugs, *n* (%)	38(66.6)	48 (54.5)	0.197
Cryoprecipitate transfusion, *n* (%)	10 (17.5)	18 (20.4)	0.169
Piggy-back clamping, *n* (%)	30 (52.6)	48 (54.5)	0.072
SL (mmol/L) at the end of LT, mean (± SD)	1.4 (± 0.3)	2.8 (± 0.7)	< 0.001
Lower serum fibrinogen (mg/dL), mean (± SD)	242 (± 34)	214 (± 24)	0.090

AKI: Acute kidney injury; IOAH: Intraoperative arterial hypotension; MBT: Massive blood transfusion; SL: Serum lactate; SD: Standard deviation.

Table 8 Logistic regression analysis of risk factors for acute kidney injury after deceased-donor liver transplantation (*n* = 145)

Logistic regression	Beta coeficient	OR	95%CI		*P* value
Biological MELD score ≥ 25	0.194	1.999	1.586	2.503	< 0.001
Pre-existing KD, *n* (%)	0.115	1.279	0.916	1.686	< 0.001
ECD (3 or more factors above)	0.911	1.191	0.711	1.787	0.002
IOAH (bleeding/PRS), *n* (%)	0.169	1.935	1.505	2.344	< 0.001
MBT, n (%)	0.125	1.830	1.428	2.241	< 0.001
SL (mmol/L) ≥ 2.0 at the end of LT	0.110	2.001	1.616	2.421	< 0.001

Hosmer and Lemeshow goodness of fit test not significant at 5% (P = 0.701); Nagelkerke R2 statistic = 0.163). LR: Logistic regression; AKI: Acute kidney injury; MELD: Model for End-stage Liver Disease; OR: Odds ratio; CI: Confidence interval; KD: Kidney dysfunction; ECD: Extended criteria donor; IOAH: Intra-operative arterial hypotension; MBT: Massive blood transfusion; SL: Serum lactate.

According to our results, ANN had larger AUROC and higher accuracy to predict AKI after DDLT than LR, which is consistent with the previous study with different machine learning tools, whereas the performance of the ANN was inferior to that of all other machine learning techniques in prediction of AKI after LT[19]. Multilayer perceptron has already been associated to a good performance in predicting in-hospital mortality, reinforcing the good performance of ANN to predict clinical outcomes, although there have been some reports that the performance of the machine learning techniques is not superior to that of LR model in predicting mortality[18].

Regarding the risk factors identified in the present research, several other authors have already described that higher MELD scores[37] were associated with AKI after LT[20,38]. Xu *et al*[21] showed that MELD score > 25 was a predictor of AKI, and in patients with MELD scores > 30, the most required RRT [11,39]. Moreover, in the cirrhosis scenario, the functional renal disorders can be added as risk factors for AKI, such as recipient HRS[11,23,40]. Donor marginal liver grafts of ECD were identified elsewhere as a strong predictor of PGD[24-26] and post-LT AKI[20]. Patients undergoing LT can experience IOAH and consequent AKI because of multiple factors, including the duration of surgery, massive bleeding[16, 40-42], the severity of the PRS[36,43,44], and the severity of the end-stage liver disease[21,45-49]. In addition, MBT may be an additional risk factor for postoperative AKI[34,49,50].

The present retrospective study has important limitations, regarding sample size and moreover, the lack of evaluation of clinical outcomes of patients according to the occurrence of post-LT AKI, either for short or long-term evolution of patients. Despite these limitations, the high incidence of AKI reported highlights the importance of this issue, and the predictors identified may provide a focus for further research. ANN methods may provide feasible tools for forecasting AKI after LT, and perhaps provide a high-performance predictive model that may ultimately improve perioperative management of these patients at risk for this serious complication.

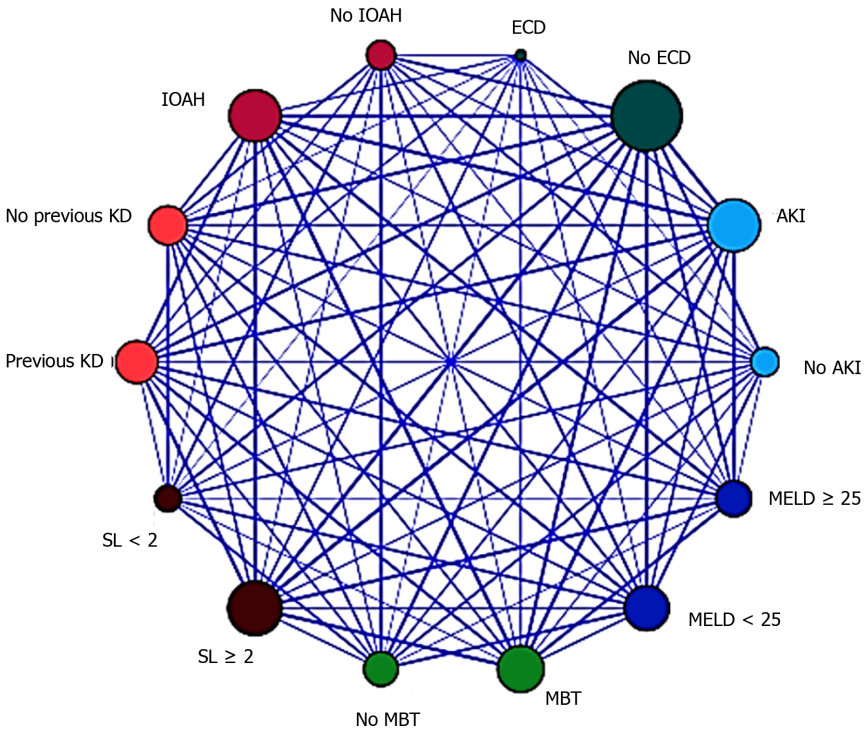

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Figure 1 Relationship map between the selected variables in the logistic regression for acute kidney injury after deceased-donor liver transplantation (*n* = 145). MELD: Model for End-stage Liver Disease; KD: Kidney dysfunction; ECD: Extended criteria donor; IOAH: Intra-operative arterial hypotension; MBT: Massive blood transfusion; SL: Serum lactate. LR: Logistic regression; AKI: Acute kidney injury.

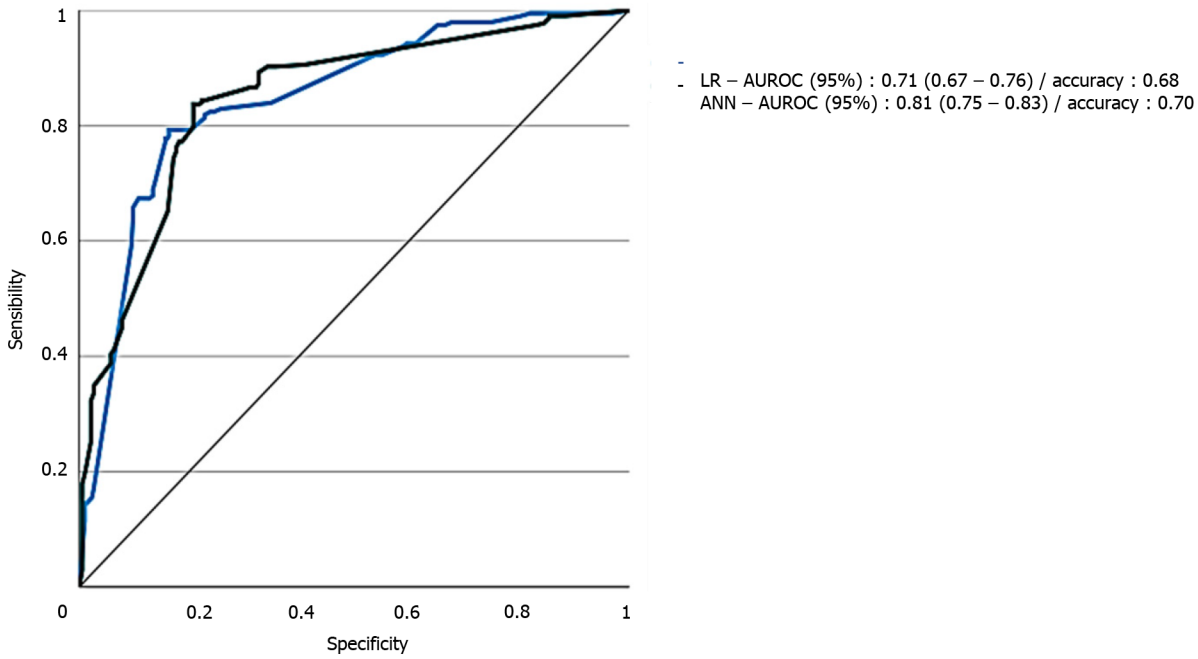

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Figure 2 Area under the receiver-operating characteristic curve of the two different models for predicting acute kidney injury (*n* = 145). LR: Logistic regression; AUROC: Area under the receiver-operating characteristic curve; ANN: Artificial neural network; AKI: Acute kidney injury.

CONCLUSION

According to our results, the severity of chronic liver disease, pre-existing KD, marginal grafts, hemodynamic instability, MBT, and inadequate tissue perfusion during LT are predictors of AKI after

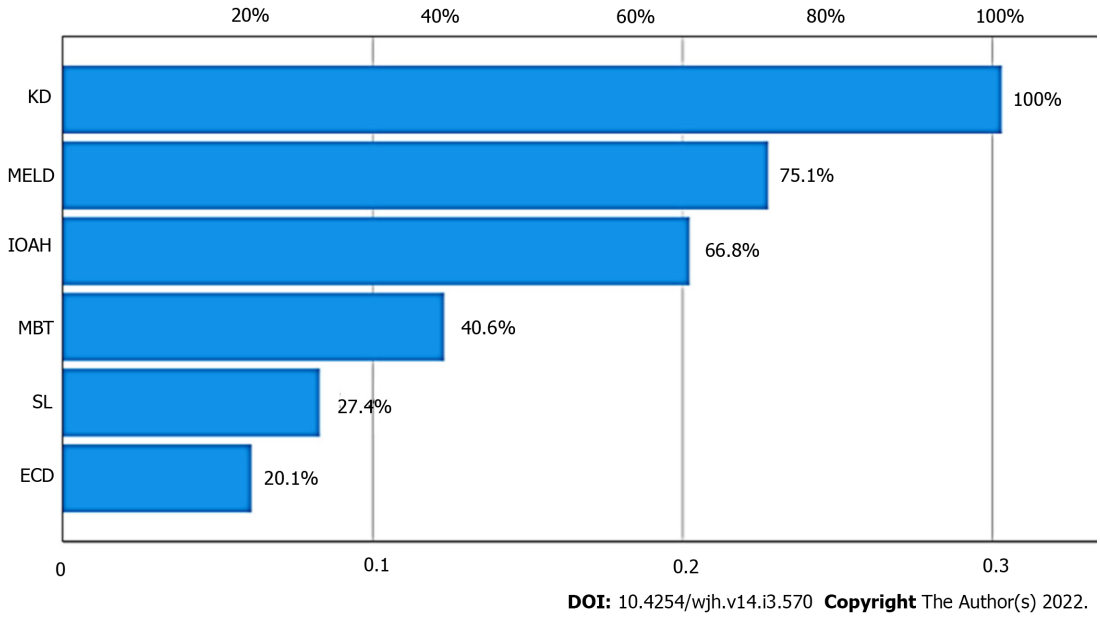

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Figure 3 Variance importance plot of predictors of acute kidney injury for artificial neural network. KD: Kidney dysfunction; MELD: Model for End-stage Liver Disease; IOAH: Intra-operative arterial hypotension; MBT: Massive blood transfusion; ECD: Extended criteria donor; AKI: Acute kidney injury; ANN: Artificial neural network.

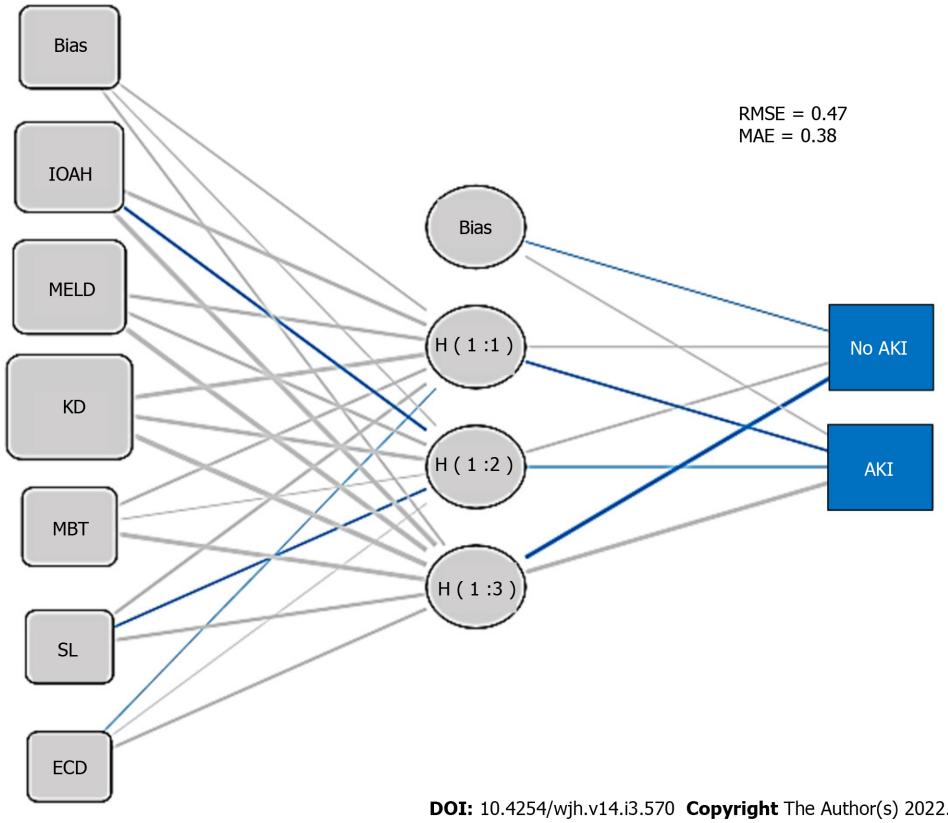

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Figure 4 Artificial neural network structural model diagram for acute kidney injury after deceased-donor liver transplantation. IOAH: Intra-operative arterial hypotension; MELD: Model for End-stage Liver Disease; KD: Kidney dysfunction; MBT: Massive blood transfusion; ECD: Extended criteria donor; AKI: Acute kidney injury; ANN: Artificial neural network; RMSE: Root-mean-square error; MAE: Mean absolute error.

DDLT, and ANN has better prediction performance than LR in this scenario.

ARTICLE HIGHLIGHTS

Research background

Acute kidney injury (AKI) post-liver transplantation (LT) is a serious complication, and its prediction with validated tools is crucial.

Research motivation

To improve the perioperative management of patient candidates for LT.

Research objectives

To identify the risk factors for AKI after deceased-donor liver transplantation (DDLT) and validate a prediction tool for this complication.

Research methods

Logistic regression (LR) analysis for predictor identification, and comparative analysis of artificial neural network (ANN) and LR prediction performance were performed.

Research results

The severity of liver disease, preexisting kidney dysfunction, marginal grafts, hemodynamic instability, massive blood transfusion, and SL were predictors of postoperative AKI, and ANN had better prediction performance than LR.

Research conclusions

ANN has better performance than the classical LR for AKI prediction after DDLT.

Research perspectives

A risk score of AKI after DDLT can be developed according to these identified predictors.

FOOTNOTES

Author contributions: Bredt LC, Peres LAB, Risso M, and Barros LCAL contributed equally to this study with regard to conception and design, literature review and analysis, manuscript drafting, critical revision, and editing, and approval of the final version.

Institutional review board statement: The study was approved by the Research Ethics Board at Assis Gurgacz University (No. 4.190.165). The study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki.

Conflict-of-interest statement: All authors that contributed equally to this manuscript declare no potential conflicts of interest and no financial support.

Data sharing statement: All authors declare that the original anonymous dataset is available on request from the corresponding author (lcbredt@gmail.com).

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Retrospective Study

Pediatric liver transplantation outcomes from a single center in Thailand

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Abstract

BACKGROUND

Liver transplantation (LT) has become an acceptable curative method for children with several liver diseases, especially irreversible acute liver failure and chronic liver diseases. King Chulalongkorn Memorial Hospital is one of Thailand's largest liver transplant centers and is responsible for many pediatric cases.

AIM

To report the experience with pediatric LT and evaluate outcomes of living-related *vs* deceased-donor grafts.

METHODS

This evaluation included children who underwent LT between August 2004 and November 2019. Data were retrospectively reviewed, including demographics,

diagnoses, laboratory values of donors and recipients, the pediatric end-stage liver disease (PELD) or model for end-stage liver disease (MELD) score, graft source, wait time, perioperative course, postoperative complications, and survival rates. Continuous data were reported using the median and interquartile range. The Mann–Whitney *U*-test was used to compare the wait time between the living-related and deceased-donor groups. The chi-square or Fisher's exact test were used to compare the frequencies of between-group complications. Survival rates were calculated using the Kaplan–Meier method.

RESULTS

Ninety-four operated pediatric liver transplant patients were identified (54% were females). The median age at transplantation was 1.2 (0.8-3.8) years. The median PELD and MELD scores were 20 (13-26.8) and 19.5 (15.8-26.3), respectively. Most grafts (81.9%) were obtained from living-related donors. The median wait time for the living donors was significantly shorter compared with the deceased donors at 1.6 (0.3-3.1) mo *vs* 11.2 (2.1-33.3) mo (P = 0.01). Most patients were diagnosed with biliary atresia (74.5%), and infection was the most common complication within 30 d post-transplantation (14.9%). Without a desensitization protocol, 9% of transplants were ABO-incompatible. Eight hepatitis B core antibodies (anti-HBc)-negative recipients received positive anti-HBc grafts without different observed complications. The overall survival rate was 93.6% and 90.3% at 1 and 5 years, respectively. No graft loss during follow-up was noted among survivors.

CONCLUSION

A significant number of pediatric LT cases were reported in Thailand. Based on relatively comparable outcomes, ABO-incompatible and HBc antibody-positive grafts may be considered in an organ shortage situation.

Key Words: Pediatric; Liver transplantation; Living-donor; Hepatitis B; ABO-incompatible; Survival

Core Tip: Pediatric liver transplantation (LT) is an acceptable life-saving operation for several chronic liver diseases and irreversible acute liver failure. This single-center data was analyzed from pediatric LTs performed between August 2004 and November 2019. This study evaluated the most extensive series of pediatric liver transplant recipients in Thailand in the past two decades. Preoperative and postoperative data, including complications and survival, were reviewed. The overall 5-year survival rate was > 90%. In addition, the satisfying outcomes of ABO-incompatible living-donor and hepatitis B core antibody-positive graft transplantation were also highlighted.

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INTRODUCTION

Liver transplantation (LT) has long been accepted as the standard treatment for end-stage liver disease due to acute fulminant hepatic failure and various chronic liver disorders. Starzl *et al*[1] were among the first group to demonstrate the possibility of this procedure in 1963 at the University of Colorado, Denver, CO, United States. This attempt faced several surgical and hemostatic challenges, requiring a very high dose of steroids and mercaptopurine due to the lack of effective immunosuppression regimens. The 1-year patient survival was about 30% at that time. However, the 1-year survival improved, close to 70%, because of the introduction of immunosuppressive drugs in the early 1980s[2]. Better organ-preservation techniques, enhanced surgical skills, and the availability of more effective immunosuppressive agents are also responsible for improved success rates. The shortage of organs from deceased donors led to a rapid expansion of living-donor programs, especially for children. The first successful living-donor LT was performed in 1989 in a pediatric patient[3]. The traditional indications for LT in children include end-stage liver disease with a predicted life expectancy of < 1 year, acute liver failure, unresectable hepatic tumors, and liver-based metabolic defects. The 5-year success rates for graft and patient survival for these life-threatening indications have been reported at a range of 85%-90%[4].

This success has led to an understandable urge to slightly contemplate more nontraditional indications for LT, including growth failure, intractable pruritus, or bone mass loss from cholestatic disorders, a neurodevelopmental abnormality from metabolic liver disease, and liver tumors in the absence of significant extrahepatic disease.

The first human LT was performed in Thailand at King Chulalongkorn Memorial Hospital, Bangkok, Thailand, on 28 November 1987 by Sriwatanawongsa *et al*[5]. Later, Nonthasoot *et al*[6] reported outcomes of LT from 1 January 2002 to 30 June 2013 for 120 adults and 24 pediatric LT cases. The data showed that the 1- and 5-year survival rates improved to 86% and 72%, respectively. As for pediatric patients, 16 and 8 had living-donor and cadaveric LT, respectively. The median age was 2 years old, and the most common primary indication was biliary atresia (83.3%). Patient survival at 1 and 5 years was 96% and 91%, respectively.

The objective of this study was to report the experience with pediatric LT performed at the center of the current study and evaluate outcomes of living-related *vs* deceased-donor grafts.

MATERIALS AND METHODS

Study population and data collection

All pediatric liver transplant recipients who underwent LT at the center of this study between August 2004 and November 2019 were included. The data of all pediatric LT cases, including demographics, diagnoses, laboratory values of donors and recipients, the pediatric end-stage liver disease (PELD) or model for end-stage liver disease (MELD) score, graft source, wait time, perioperative course, immunosuppression type, postoperative complications, causes of death, and survival times, were retrospectively reviewed. The follow-up time was the duration in months from LT to the latest date of a doctor visit. Laboratory data were collected from preoperative assessment in both donors and recipients, including the ABO blood group and viral serology [hepatitis B surface antigen, hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc), hepatitis C virus antibody, immunoglobulin G antibody to Epstein–Barr virus, and immunoglobulin G antibody to cytomegalovirus (anti-CMV IgG)]. Postoperative complications were categorized into three groups according to time to events after transplantation as early complications (within 30 d), middle complications (between 30 d and 1 year), and late complications (after 1 year). CMV viremia was defined as a detectable virus in blood quantified at ≥ 1000 DNA copies/mL using quantitative polymerase chain reaction or comparable positive antigenemia assay. Patient survival was defined as the time between LT and patient mortality. Moreover, graft survival was defined as the time between LT and graft loss, either by patient mortality or by graft failure necessitating retransplantation.

Statistical analyses

Statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc. Chicago, IL, United States). Categorical data were represented as numbers with percentages. Moreover, continuous data were reported using medians and interquartile ranges. The wait time was compared between the living-related and deceased-donor groups using the Mann-Whitney *U*-test. Frequencies of complications between groups were compared using chi-square or Fisher's exact test as appropriate. The survival rates were plotted using the Kaplan–Meier curves, and the differences in selected factors were evaluated using the log-rank test. Statistical significance was defined by P values < 0.05.

RESULTS

Overall characteristics of patients

The most common diagnosis was biliary atresia (74.5%) in all 94 pediatric transplant recipients identified during the study period. Other less common diagnoses included fulminant hepatic failure, progressive familial intrahepatic cholestasis, Alagille syndrome, and others (Figure 1). Table 1 shows the patients' characteristics. Most patients were < 2 years old (64.9%). The median age at transplantation was 1.2 (0.8–3.8) years, and significantly lower in the living-donor group [1.1 (0.8-1.9) years] compared with the deceased-donor group [9.7 (3.5-13.5) years; P < 0.001]. The median wait time for the living donors was significantly shorter than that for deceased donors at 1.6 (0.3–3.1) mo *vs* 11.2 (2.1–33.3) mo (P = 0.01). The median follow-up time was 4.0 (2.2–7.3) years.

Donor and recipient serology

Table 2 shows the serology data. Eight ABO-incompatible transplants were performed in the living-donor group in infants (< 1 year old) without a desensitization protocol. Positive donor anti-HBc grafts were transplanted to eight patients with negative anti-HBc (8.5%). Five of the 54 recipients were anti-HBc-positive, although every patient in this group received negative donor anti-HBc grafts. All recipients received at least one primary hepatitis B Virus (HBV) vaccination at birth and a booster dose

Table 1 Patient characteristics

Data set		Living donors (*n* = 77)	Deceased donors (*n* = 17)	Total (*n* = 94)
Sex	Male (*n*, %)	36 (46.8)	7 (41.2)	43 (45.7)
Age (yr)[1]	Median (IQR)	1.1 (0.8-1.9)	9.7 (3.5-13.5)	1.2 (0.8-3.8)
Diagnosis of biliary atresia (*n*, %)		59 (76.6)	11 (64.7)	70 (74.5)
PELD score (age < 12 yr, *n* = 84)	Median (IQR)	19 (12.5-26)	25 (17.5-31.3)	20 (13-26.8)
	n	74	10	84
MELD score (age ≥ 12 yr, *n* = 10)	Median (IQR)	19 (18-19)	23 (15-30)	19.5 (15.8-26.3)
	n	3	7	10
Wait time (mo)[2]	Median (IQR)	1.6 (0.3–3.1)	11.2 (2.1–33.3)	1.7 (0.4–4.0)

[1]$P < 0.001$.
[2]$P = 0.01$.
PELD: Pediatric end-stage liver disease, MELD: Model for end-stage liver disease.

Table 2 Donor and recipient laboratory findings

Data set		Living donors (*n* = 77)	Deceased donors (*n* = 17)	Total (*n* = 94)
ABO incompatibility[1]	*n*, %	8 (10.4)	0 (0)	8 (8.5)
Positive donor anti-HBc	*n*, %	4 (5.2)	4 (23.5)	8 (8.5)
Anti-CMV IgG (*n*, %)[2]	D+/R-	8 (11.0)	2 (18.2)	10 (11.9)
	D+/R+	62 (84.9)	9 (81.8)	71 (84.5)
	D-/R+	3 (4.1)	0 (0)	3 (3.6)
	D-/R-	0 (0)	0 (0)	0 (0)
Recipient positive serology (*n*/total, %)	HBsAg	0/44 (0)	0/9 (0)	0/53 (0)
	Anti-HBs	33/43 (76.7)	3/10 (30)	36/53 (67.9)
	Anti-HBc	3/44 (6.8)	2/10 (20)	5/54 (9.3)
	Anti-HCV	1/42 (2.4)	0/10 (0)	1/52 (1.9)
	Anti-EBV IgG	31/43 (72.1)	8/9 (88.9)	39/52 (75)

[1]Median age at liver transplantation (IQR) = 0.9 (0.5-1.0) years.
[2]Total 84 patients.
D: Donor; R: Recipient; HBsAg: Hepatitis B surface antigen; Anti-HBs: Hepatitis B surface antibody; Anti-HBc: Hepatitis B core antibodies; Anti-HCV: Hepatitis C virus antibodies; Anti-EBV: Epstein-Barr virus antibodies.

before LT. HBc antibody-positive graft recipients also received lamivudine prophylaxis for *de novo* HBV infection after LT. Most recipients (74/84, 88.1%) had positive anti-CMV IgG. All CMV-naïve recipients (10/84, 11.9%) received positive anti-CMV IgG grafts.

Immunosuppression use within the first 30 postoperative d

All patients postoperatively received corticosteroids in conjunction with at least one main T-cell suppression immunosuppressant. Tacrolimus (78.7%) was the preferred calcineurin inhibitor over cyclosporine (21.3%). Other additional immunosuppressive drugs used included azathioprine (30.9%), mycophenolate mofetil (26.6%), and sirolimus (3.2%). Apart from corticosteroids, more than half of the patients (58.5%) required a combination of two or more immunosuppressive agents.

Complications after LT

Infection was the most common early complication (within 30 d after LT; 14.9%). Other less common early complications included vascular complications, chylous ascites, acute cellular rejection, bile leakage, and post-transplant lymphoproliferative disorder (PTLD, Figure 2). One patient who received living-related-donor LT had hepatic artery thrombosis, which required a second emergent deceased-donor LT. PTLD occurred in 10.6% of the patients within the first year after transplantation, whereas

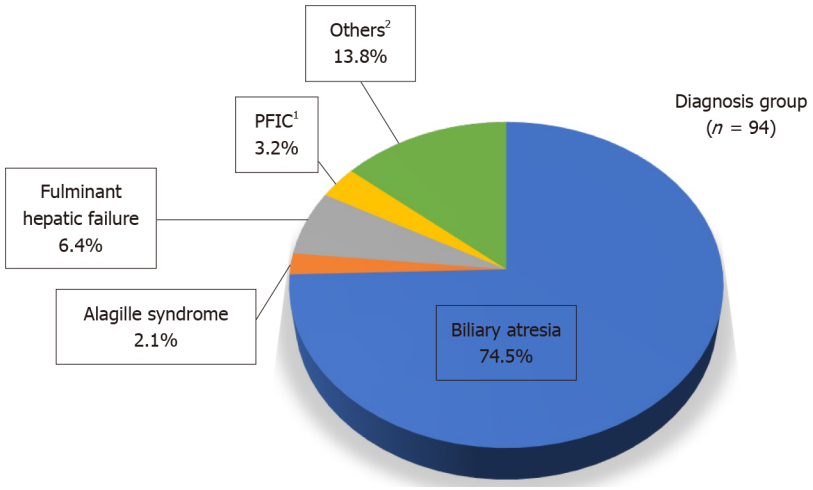

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Figure 1 Preoperative diagnoses. [1]PFIC: Progressive familial intrahepatic cholestasis; [2]Others include idiopathic neonatal hepatitis, Budd-Chiari syndrome, hepatoblastoma, hepatocellular carcinoma, bile acid synthesis disorder, autoimmune hepatitis, glycogen storage disease, Caroli disease, Abernethy malformation, hepatic artery thrombosis after prior liver transplantation, and cryptogenic cirrhosis.

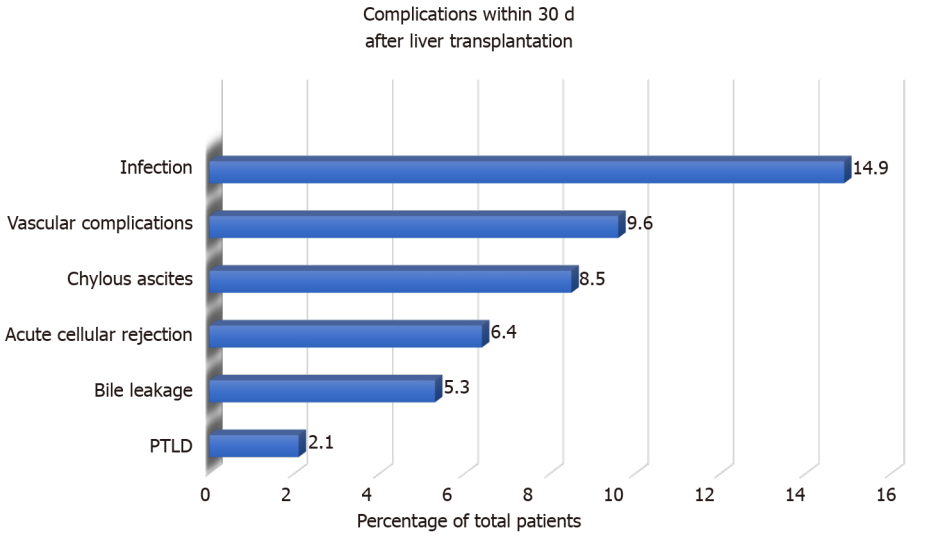

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Figure 2 Early postoperative early complications. PTLD: Post-transplant lymphoproliferative disorder.

3.2% developed PTLD after the first year. Early CMV viremia within the first year after LT was detected in 40% of CMV-naïve patients compared with 4.8% of CMV-seropositive patients (P = 0.004). Food allergy was found in 23 patients (24.5%), 65.2% of which were *de novo* food allergies, defined as the occurrence of allergic symptoms after LT. No donors with a history of allergy were identified among these *de novo* food allergy recipients. The median age at LT was 13 (10-19.8) mo, whereas the median time to the event was 148 (92-347) d. The common culprits were cow milk (66.7%), egg (46.7%), and wheat (20%).

No observed different vascular, infection, or rejection complications were noted in the ABO-incompatible and positive donor anti-HBc graft transplantation cases. Four patients among the eight ABO-incompatible LT recipients had no complications. One patient developed early sepsis, candida urinary tract infection, hepatic artery stenosis, portal vein thrombosis, and bile leakage. Two patients developed PTLD within the first year after LT. One patient expired 4 d after LT due to sepsis.

Post-transplant survival

The overall survival rate was 93.6% and 90.3% at 1 and 5 years (living-donor group = 92.2% and 88.1% at 1 and at 5 years, respectively; deceased-donor group = 100% at 1 and 5 years), respectively (Figure 3). All patients with acute liver failure (6.4%) survived after LT with normal neurodevelopmental outcomes. Six (75%) of the eight total deaths occurred within the first year after transplantation. The

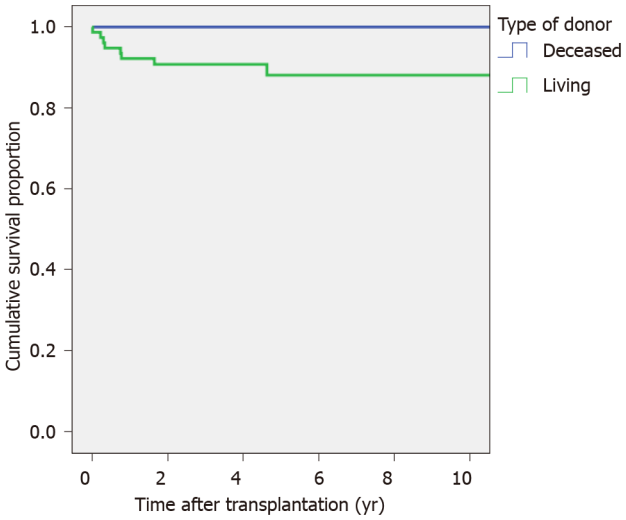

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Figure 3 Survival proportion after transplantation according to donor type.

preoperative diagnoses were biliary atresia (7/8, 87.5%) and bile acid synthesis disorder (1/8, 12.5%). The causes of mortality were sepsis (three patients), acute renal failure and shock (two patients), bacterial pneumonia (one patient), and unknown (two patients).

DISCUSSION

Pediatric LT is considered the standard management for children with several chronic liver diseases and irreversible acute liver failure. King Chulalongkorn Memorial Hospital, affiliated with the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, is the leading hospital for pediatric LT and is responsible for most procedures in Thailand. At the aforementioned hospital, 23 children underwent LT (average, 2.6 cases per year) from 2004 to 2013. The remaining 71 patients underwent LT from 2014 to November 2019 (average, 14.2 cases per year). The most common indication for pediatric LT in the current series was biliary atresia (74.5%), consistent with other series previously reported[7-9]. The liver graft allocation policy for pediatric recipients in the current study includes the use of living-donor grafts as default and deceased-donor grafts when the former is unavailable or impossible due to donor limitations. Most grafts came from living donors (81.9%); however, this rate was lower than recent reports from Japan and Turkey. Kasahara *et al*[7] reported on 414 and 22 Living- and deceased-donor transplantations up to 2016 from the Organ Transplantation Center at the National Center for Child Health and Development in Japan. In Turkey, Yankol *et al*[8] reported on 135 cases of pediatric LT, 91.4% of which involved living donors. However, the rate of living-donor LT in the current study was higher than in Western and Middle Eastern countries, including centers in the United States (6%)[9] and Iran (40.6%)[10]. Regarding other patient characteristics, the median age of patients [1.2 (0.8-3.8) years] in the current study is relatively lower compared with other studies. Moreover, the median PELD and MELD scores were 20 (13-26.8) and 19.5 (15.8-26.3), respectively, which is comparable to other studies[8, 9].

The most common early complication in the current series was an infection, consistent with other reports. Nikeghbalian *et al*[10] reported in-hospital complications encountered by 34.7% of the patients, and the most common being infections (26.8%), bleeding (23.4%), and vascular complications (18%). The United States Studies in Pediatric Liver Transplantation (SPLIT) group[11] also reported that infection was the most common complication. It occurred in nearly half of all patients (46%) and could be as severe as multisystem organ failure or cardiopulmonary failure. Infants, which accounted for most of the pediatric LT cohorts in the current study, were at the highest risk of developing an infection. Eight pediatric recipients received positive anti-HBc grafts and were started on lamivudine after LT. These patients had good graft survival and complications that were comparable to the negative anti-HBc group without HBV infection reactivation that is likely because of the antiviral prophylaxis. In addition, anti-HBs and revaccination of patients with low or undetectable anti-HBs before LT were also monitored because keeping the anti-HBs > 200 mIU/mL before LT could be sufficient to prevent *de novo* HBV infection[12]. However, one case with biliary atresia and cirrhosis diagnosis developed *de novo* HBV infection after receiving a negative anti-HBc liver graft. This patient had a high anti-HB titer (anti-HBs > 1000 IU/L) before LT but had elevated transaminases at 3.83 years after LT before the HBV infection was diagnosed. Sintusek *et al*[13] reported the unexpectedly high prevalence of HBV immunity loss after LT (46%, 57%, and 82% at 1, 2, and > 3 years following LT). Positive anti-HBc grafts may be

considered with relatively positive outcomes in the face of organ shortages for LT. Oral antivirals for HBV and careful monitoring of viral serology should be performed in endemic areas given the high prevalence of HBV immunity loss after LT.

Regarding vascular complications, hepatic artery thrombosis after pediatric LT ranges from 5.7% to 8.4% and is an important cause of graft loss[11]. Portal vein thrombosis is another vascular complication that can occur after LT. Vascular complications occurred in 9.6% of the cohorts of the current study. One patient, who underwent LT in 2010, developed hepatic artery thrombosis and required a second LT. However, the rate of vascular complications significantly declined with careful selection of donors and ultrasound monitoring. Doppler ultrasound after reperfusion was routinely performed to ensure good vascular flow, and daily monitoring continued 1 wk after LT.

Herein, the good outcome of ABO-incompatible LT was highlighted in eight < 1-year-old pediatric recipients without a desensitization protocol. No differences in terms of complications or graft and patient survival were found. This is likely because differentiation and maturity of the immune system are closely tied to children's age. Isoagglutinin titers in < 1-year-old infants are lower than adult levels [14]. In a systematic review and meta-analysis, Lee *et al*[15] reported comparable patient survival in both groups. However, the ABO-incompatible group was inferior regarding graft survival and several complications. Graft survival could be comparable in pediatric patients and those using rituximab. However, another more recent systematic review and meta-analysis by Kang *et al*[16] showed consistently lower patient and graft survival with pediatric ABO-incompatible LT than the ABO-compatible group. The authors concluded that ABO-incompatible LT is an important choice to consider for emergency LT in the absence of blood type-matching liver source although it is not an optimal treatment in terms of graft and patient survival rates.

Patient survival rates at 1 and 5 years after a pediatric LT are 97.3% and 94.2%, respectively, according to a recent SPLIT registry database from 2011 to 2018[17]. The survival rates from the largest single-center report on pediatric LT in Iran were up to 84.4% and 77.8% at 1 and 5 years, respectively [10]. Thus, the overall survival rates of the current series of 93.6% and 90.3% at 1 and 5 years, respectively, are comparable.

The limitations of the current study include the inherent nature of retrospective studies. Some detailed information may be missing from the medical records, including specific causes of mortality or mention of minor infections that could have occurred at home or other hospitals. Also, the pretransplant viral serology of donors and recipients was not available in all cases due to missing data from the records. The main strength of the current study is that it is the most extensive report on pediatric LT in Thailand covering the two decades. The experiences in the current study also highlighted a good proportion of living-donor LT in children with relatively comparable outcomes and excellent graft and patient survival compared to global data.

CONCLUSION

In conclusion, pediatric LT has been accepted as a life-saving procedure for patients with acute liver failure and chronic end-stage liver diseases. The transplant center of the current study is responsible for a large number of pediatric LT procedures in Thailand. Herein, the experiences in pediatric LT with excellent outcomes concerning survival and complications are reported. The current series was mostly comprised of living-related donor liver grafts, which had the advantage of shorter wait times compared to deceased-donor grafts. The experiences with ABO-incompatible LT in < 1-year-old patients without a desensitization protocol and HBc antibody-positive LT with relatively comparable outcomes were also highlighted, leading to the assertion that such grafts should be considered in the face of organ shortages. Overall, the results in the previous 15 years regarding pediatric LT are promising.

ARTICLE HIGHLIGHTS

Research background

Pediatric LT has been accepted as a curative method for children with several liver diseases. The success rates have improved due to better organ-preservation techniques, enhanced surgical skills, and the availability of newer immunosuppressive agents. Organ shortage has become a rising problem worldwide, especially in Eastern countries.

Research motivation

King Chulalongkorn Memorial Hospital is the leading hospital in Thailand for pediatric LT. Several reports on pediatric LT were noted in the United States, Europe, Middle East, and East Asian countries. However, data from South East Asia, especially related to ABO-incompatible LT, are scarce.

Research objectives

The current study aimed to report experiences with pediatric LT performed at the center of this study and evaluate outcomes of living-related *vs* deceased-donor grafts.

Research methods

The current retrospective study included 94 children who underwent LT and were followed up for a median time of 4 years thereafter. Data of donors and recipients, including postoperative complications and survival rates, were reviewed and analyzed.

Research results

In the current study, 94 pediatric LT performed at the center of this study were reported. The median age at transplantation was 1.2 (0.8-3.8) years. Most grafts (81.9%) were obtained from living-related donors. The median wait time for the living donors was significantly shorter than that for deceased donors at 1.6 (0.3-3.1) *vs* 11.2 (2.1-33.3) months (P = 0.01). Most patients were diagnosed with biliary atresia (74.5%), and infection was the most common complication within 30 d post-transplantation (14.9%). In addition, 9% of transplants were ABO-incompatible without a desensitization protocol. No observed different vascular, infection, or rejection complications were noted. Eight (8.5%) recipients who tested negative for HBc antibodies received positive anti-HBc grafts with no observed different infection or rejection complications. The overall survival rate was 93.6% and 90.3% at 1 and 5 years, respectively. No graft loss during follow-up was noted among the survivors.

Research conclusions

Living-donor-related LT has saved many lives with shorter wait times compared with deceased-donor surgeries. Based on relatively comparable outcomes, ABO-incompatible and HBc antibody-positive liver grafts may be considered in the face of organ shortages. The survival results in the previous 15 years are promising.

Research perspectives

The current study suggests that living-donor liver transplantation (LT) can save many lives and has a good outcome with shorter wait times in the face of organ shortage. ABO-incompatible LT can be considered in pediatric < 1-year-old recipients without a sensitization protocol. Hepatitis B core (HBc) antibody-positive liver grafts may also be used. Nonetheless, special attention should be focused on high titers of anti-hepatitis B surface before LT and lifelong postoperative antiviral prophylaxis. More studies on living-donor pediatric LT and protocols for these special donor groups are needed.

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FOOTNOTES

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Observational Study

Predictors of mortality at 28-days in infection associated acute kidney injury in cirrhosis

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Abstract

BACKGROUND

Acute kidney injury (AKI) in cirrhosis is important complication with poor outcomes. And infections are common cause for acute decompensation. Infections in cirrhosis lead to acute deterioration of hemodynamics leading to precipitation of AKI.

AIM

To study predictors of mortality in patients with infection-associated AKI in cirrhosis.

METHODS

This was a prospective, observational study conducted at tertiary care centre from January 2018 till April 2019. Total 119 participants with cirrhosis of liver presenting with AKI were included into the study. AKI was defined as per international club of Ascites-AKI criteria 2015. Patients were grouped into infection AKI and non-infection AKI. Non-infection AKI included patients with diuretic induced AKI and pre-renal AKI. Logistic regression analysis was used to determine predictors of mortality at 28-d.

RESULTS

Out of 119 patients, alcohol (n = 104) was most common etiology of cirrhosis. The infection AKI included 67 (56%) patients and non-infection AKI (n = 52) included pre-renal AKI in 36 (30%) and diuretic-induced AKI in 16 (14%) patients. Infection AKI had significantly higher bilirubin, higher international normalized ratio (INR), low serum sodium, higher total leukocyte count (TLC) and higher prevalence of hepatic encephalopathy (HE) as compared to non-infection AKI. Infection AKI had higher progression of AKI (19/67 *vs* 2/52; P = 0.01) and 28-d mortality (38/67 *vs* 4/5; $P \leq 0.01$) as compared to non-infection AKI. At 28-d, non-

survivors (n = 42) had significantly higher bilirubin, higher INR, low serum sodium, higher TLC and higher prevalence of HE as compared to survivors (n = 77). On subgroup analysis of Infection AKI group, on multivariate analysis, serum bilirubin as well as presence of HE were independent predictors of 28-d mortality. There was no significant difference of mortality at 90-d between two groups.

CONCLUSION
Infection AKI in cirrhosis has a dismal prognosis with higher 28-d mortality as compared to non-infection AKI. Serum bilirubin and presence of HE predict 28-d mortality in infection AKI.

Key Words: Infection; Acute kidney injury; Mortality; Cirrhosis; Bilirubin; Hepatic encephalopathy

Core Tip: The infections in cirrhosis are the most common cause for acute decompensation and organ failure. Acute kidney injury (AKI) in cirrhosis is itself an indicator for worsening hemodynamics. In the present study, we compared infection associated AKI and non-infection AKI. We found higher 28-d mortality in infection AKI than non-infection AKI. In addition to altered hemodynamics, pathogen associated molecular patterns and damage-associated molecular patterns produced as a result of sepsis contribute to multiorgan failure, especially renal dysfunction. Moreover, higher bilirubin and presence of hepatic encephalopathy predicted 28-d mortality in patients with infection AKI. This provides an insight that the combination of infection and AKI in cirrhosis portends a dismal prognosis and therefore, on admission, early identification of infection and aggressive management may improve outcome in these patients.

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INTRODUCTION

The onset of jaundice, ascites, hepatic encephalopathy (HE), or gastrointestinal (GI) bleed marks the decompensation of a well-compensated chronic liver disease. The occurrence of ascites is an important benchmark in history of cirrhosis as it tilts the balance[1]. It signifies the presence of clinically significant portal hypertension, liver cell dysfunction, hypoalbuminemia, alteration in hemodynamics due to imbalance of vasoconstrictors and vasodilators in the splanchnic and systemic circulation. The splanchnic pooling of blood and systemic vasodilation leads to reduced effective arterial blood volume over a period of time resulting in refractory ascites. Ascites predisposes a patient with cirrhosis to increased incidence of spontaneous bacterial peritonitis (SBP) and acute kidney injury (AKI). In 25-year inception cohort study of patients with cirrhosis by D'Amico *et al*[2], it was shown that as stages of cirrhosis progress from 1 to 6, there is decreased 5-year survival. On competing risk analysis, they showed 0.50-0.97 risk of death within 1-year of onset of infections, renal failure or acute-on-chronic liver failure (ACLF) in decompensated cirrhosis[3,4].

The unique structural organization and dual blood supply of the liver plays important role in its immune function. Cirrhosis is associated with immune dysfunction. There is associated impaired Kupffer cell function, sinusoidal capillarization with continuous basement membrane formation leading to impaired exchange of cargo between sinusoidal blood and hepatocytes[5]. The gut dysbiosis in cirrhosis leads to increased portal blood endotoxemia, increased lipopolysaccharide levels which due to portosystemic shunting bypasses the liver and reaches directly in systemic circulation. This increases the risk of acquiring bacterial infections in cirrhosis. In addition, there is reduced neutrophil count due to splenic sequestration, associated neutrophil dysfunction with reduced chemotaxis, reduced monocyte and macrophage function with and impaired phagocytosis, impaired natural killer cell function. The CD4 and CD8 T cell function is also reduced[6-8]. Liver dysfunction leads to reduced complement proteins and hypoalbuminemia. All these factors predispose patients with cirrhosis towards acquiring infection. Sepsis, on the other hand is a precursor to multiorgan dysfunction. Therefore, we aimed to compare infection associated AKI with non-infection AKI in patients with cirrhosis of liver. We also determined the predictors of mortality in patients with infection AKI.

MATERIALS AND METHODS

Study population and data collection

This was a prospective observational study which included consecutive patients with liver cirrhosis with AKI admitted in Department of Medicine at Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, India from Jan 2018 to June 2019. Liver cirrhosis was defined as per clinical, biochemical, and radiological parameters on ultrasound (nodular liver, portal vein diameter > 13 mm, splenomegaly, presence of collateral) and liver biopsy if needed. After written and informed consent, patients aged 18-70 years with cirrhosis of the liver presenting with AKI were included into the study. Patients aged < 18 years or > 70 years, pregnancy, chronic respiratory disease, chronic kidney disease, and hepatocellular carcinoma were excluded from the study. A detailed history and clinical examination were performed in all patients. AKI was defined as an increase in serum creatinine ≥ 0.3 mg/dL within 48 h; or a percentage increase in serum creatinine ≥ 50% from baseline which is known, or presumed, to have occurred within the prior 7 d. Baseline serum creatinine was defined as serum creatinine obtained in the previous 3 mo. In patients with more than one value within the previous 3 mo, the value closest to the admission time to the hospital was taken as the baseline value.

AKI staging

AKI staging was done as per international club of Ascites criteria; Stage 1 was defined as an increase in serum creatinine ≥ 0.3 mg/dL or ≥ 1.5-2 ULN from baseline, Stage 2 as an increase in serum creatinine > 2-3 ULN from baseline and Stage 3 as increase in serum creatinine > 3 ULN from baseline or serum creatinine ≥ 4.0 mg/dL with an acute increase ≥ 0.3mg/dL or initiation of renal replacement therapy. Further "Progression" of AKI was defined as progression to a higher stage and/or need of renal replacement therapy and "Regression" was defined as regression of AKI to a lower stage. The response to treatment was defined as "Full response" when serum creatinine value decreased to within 0.3 mg/dL of the baseline value, "Partial response" if reduction of serum creatinine ≥ 0.3mg/dL above baseline value and "No response" if there was no response in creatinine values. All patients were followed till 3 months to evaluate for 28-d and 90-d mortality. On admission, all patients were evaluated for the presence of infection by performing ascitic fluid analysis, blood culture, urine examination, urine culture, sputum gram stain and culture, chest X-Ray, and any other body fluid examination as indicated.

Pneumonia: Any new lung infiltrate with either symptom (cough, sputum, pleuritic pain, dyspnoea) or rales/crepitation on auscultation with components of systemic inflammatory response, *i.e.*, temperature > 38 °C or < 36 °C or TLC > 10000/mm^3 or < 4000/mm^3 or respiratory rate > 20/min or $PaCO_2$ < 32 mmHg or pulse > 90/min.

SBP: Either ascitic fluid PMNs (polymorphonuclear) > 250 cells/mm^3 with/without a positive ascitic fluid culture.

Spontaneous bacterial empyema: Either pleural fluid PMNs > 250 cells/mm^3 with positive culture or > 500 cells/mm^3 irrespective of culture positivity.

Bacteraemia: Blood culture positivity without any source of infection.

Cellulitis

Urinary tract infection (UTI): Urine microscopy showing WBC > 10/high power field with/without positive culture.

All patients with AKI were grouped into infection AKI, diuretic induced AKI and pre-renal AKI. Diuretic induced AKI was defined as patients who were on diuretics (furosemide and spironolactone) for the control of ascites with negative work up for infection or pre-renal causes. Pre-renal AKI was defined in patients with cirrhosis presenting with upper GI bleed, fluid losses due to diarrhea or vomiting *etc.* and with negative work up for infections and no history of diuretics. Diuretic induced AKI and pre-renal AKI were grouped as non-infection AKI.

Statistical analysis

All continuous variables were taken as mean ± SD (range) or median [IQR; Q1, Q3] and categorical variables as frequency and percentages. For comparison of continuous variables, Mann–Whitney U test/Student t-test and for categorical variables, χ^2 and Fisher exact tests were used. 28-and 90-d mortality was assessed using survival analysis. $P < 0.05$ was taken as significant. SPSS v21.0 (IBM, USA) was used for analysis.

RESULTS

Out of 140 patients of cirrhosis with AKI were admitted during the study period, 21 patients did not fulfil inclusion criteria (Figure 1). Finally, 119 patients of cirrhosis with AKI were included into the study.

The most common etiology of cirrhosis was alcohol (n = 98), chronic hepatitis B and C (n = 5 each), non-alcoholic steatohepatitis related cirrhosis (n = 4), both alcohol and chronic hepatitis C (n = 4), both alcohol and chronic hepatitis B (n = 2), and autoimmune cirrhosis (n = 1).

Among 119 patients, infection with AKI was present in 67 (56%). Non-infection AKI included 36 (30%) patients with pre-renal and 16 (14%) patients with diuretic induced AKI (Figure 1). Out of 67 patients of infection AKI, SBP was present in 30 (45%), pneumonia in 9 (13%), cellulitis in 7 (10%), UTI in 2 (3%), splenic abscess in 1 (1.5%) and source of infection unidentified in 18 (27%) patients.

At baseline, Infection AKI group had higher creatinine (2.6 mg/dL *vs* 2.2 mg/dL, P = 0.016) as compared to non-infection AKI. Further, infection AKI group had higher mean serum bilirubin, higher INR, lower serum albumin, lower serum sodium, higher haemoglobin, higher TLC, and higher prevalence of HE than non-infection AKI group respectively (Table 1).

Infection AKI had higher progression of AKI (19/67 *vs* 2/52; P = 0.01) and higher 28-d mortality (38/67 *vs* 4/52; P < 0.001) than non-infection AKI group respectively. In non-infection AKI group, four non-survivors belonged to prerenal AKI. At 90-d, there was no significant difference of mortality among infection AKI and non-infection AKI group (49/67 *vs* 13/52; P = 0.2) respectively (Table 2).

Overall, out of 119 patients, at 28-d, there were 77 survivors and 42 non-survivors. On univariate analysis, survivors had lower serum bilirubin, lower INR, lower TLC, and lower prevalence of HE compared to non-survivors. The multivariate analysis revealed higher bilirubin and presence of HE to predict 28-d mortality (Table 3).

In subgroup analysis of Infection AKI group, non-survivors (n = 38) had higher TLC, higher bilirubin, higher INR and higher prevalence of HE as compared to survivors (n = 29). On multivariate analysis, serum bilirubin and presence of HE were independent predictors of 28-d mortality (Table 4).

As per CANONIC grading of ACLF, there were 35 patients in no ACLF, 23 in ACLF grade-1, 27 in ACLF grade-2, 34 in ACLF grade-3. At 28-d, there was mortality of one patient with no ACLF, three in ACLF grade-1, 13 in ACLF grade-2, and 25 in ACLF grade-3. At 90-d, there was a mortality of five patients in no ACLF, five in ACLF grade-1, 18 in ACLF grade-2, and 34 in ACLF grade-3 (Figure 2). In infection AKI group (n = 67), 55 patients had ACLF and 12 had no ACLF.

DISCUSSION

The study gives three important findings in relation to infection AKI in cirrhosis of liver: (1) prevalence of infection AKI in cirrhosis; (2) One-and three-mo mortality; and (3) predictors of mortality at 28-d in infection AKI group. There was a 56% prevalence of infection AKI in patients with cirrhosis presenting with AKI. Remaining patients had AKI due to pre-renal and diuretic-related causes. This study had SBP as the cause of infection in 45% followed by pneumonia (13%) and cellulitis (10%) in acute decompensation of cirrhosis. The CANONIC series had SBP in 25% of all infections and source of infection was undefined in 13%[9]. The International Club of Ascites Global Study Group also showed higher prevalence of SBP (35% *vs* 27%) and pneumonia (28% *vs* 19%) in Asia compared to Europe respectively[2]. The Global study group showed higher rates of ACLF in Asia compared to global data (46% *vs* 35%; P < 0.01) in patients with cirrhosis with infection respectively[10]. Our study had ACLF in 82% of patients in infection AKI group. We had selectively included patients of cirrhosis with AKI and as renal dysfunction is a late manifestation in the course of cirrhosis, this may be the reason behind the higher rates of ACLF in our study population as compared to previous studies which included all patients with acute decompensation of cirrhosis.

The previous data from India suggest higher rates of acute viral hepatitis A and E as a cause for acute insult in acute decompensation of cirrhosis and ACLF[11]. However, recent studies show a trend towards increasing rates of infection with multidrug resistant (MDR) and extremely drug resistant (XDR) bacteria in Asia[12]. The Global study showed higher prevalence of MDR (76% *vs* 16%) and XDR bacteria (33% *vs* 1%-16%) in Indian centers, as compared to Western centers respectively[10].

Presence of infections in cirrhosis activates systemic inflammation and results in multi-organ dysfunction[13]. The pathogen associated molecular patterns (PAMPs) arising from the gut and damage associated molecular patterns (DAMPs) released from necrotic hepatocytes stimulate toll like receptors (TLRs) on hepatocytes and cause release of Interleukin (IL)-1, IL-6, tumor necrosis factor (TNF)-alpha, *etc.* The cytokine storm, PAMPs and DAMPs in the circulation increase the expression of TLR4 receptors in the kidneys leading to increased permeability, proteinuria, and alteration in vascular tone. Through various molecular pathways, oxidative stress and apoptosis in tubular epithelial cells increases and results in sepsis induced AKI[14,15]. In animal studies, pharmacological interventions targeting TLR receptors in the kidney have shown reduced injury in sepsis induced AKI[16]. Shah *et al*[17] showed that there is already increased expression of TNF-alpha, TLR4, *etc.* in kidneys in cirrhosis, making them

Table 1 Baseline characteristics of patients in Infection and non-infection acute kidney injury groups

Variables	Infection AKI (*n* = 67)	Non-infection AKI (*n* = 52)	*P* value
Age (yr, mean ± SD)	42 ± 23	41 ± 21	0.23
Males, *n* (%)	58 (86%)	47 (90%)	0.31
Hb (gm/dL)	8.5 (3.6-14.7)	8.1 (3-14)	0.037
TLC (× 10^3/mm^3)	17 (2-40)	7.8 (2.5-18)	0.001
Platelet count (× 10^9/L)	110 (60-200)	130 (80-220)	0.335
Bilirubin (mg/dL)	11.3 (0.8-46.6)	4.4 (0.8-27.9)	0.003
INR	2.1 (0.9-3.9)	1.9 (0.9-3.6)	0.045
Albumin (gm/dL)	2.3 (1.6-3.9)	2.5 (1-3.7)	0.04
Creatinine (mg/dL)	2.6 (1.4-6)	2.2 (1.2-5.4)	0.016
Sodium (mEq/L)	132.5 (116-164)	135.9 (120-151)	0.04
HE, *n*	47	22	0.03
CTP	12 (6-15)	11 (6-14)	0.73
MELD	27 (11-38)	24 (10-35)	0.95

Data expressed as median (range) otherwise expressed. Hb: Hemoglobin; INR: International normalized ratio; CTP: Child-Turcotte Pugh score; MELD: Model for end stage liver disease.

Table 2 Mortality data among infection and non-infection acute kidney injury groups

Mortality	Infection AKI (*n* = 67)	Non-infection AKI (*n* = 52)		*P* Value
		Diuretic-induced (*n* = 16)	Pre-renal (*n* = 36)	
28-d (*n* = 42)	38	0	4	< 0.0001
90-d (*n* = 20)	11	3	6	0.206

Data expressed as frequency. AKI: Acute kidney injury.

susceptible to inflammatory insult and gut decontamination with norfloxacin prevents renal dysfunction after LPS stimulation. The present study revealed that 1-mo mortality in the infection AKI group was significantly higher than non-infection AKI (38 *vs* 4; $P < 0.0001$) respectively. It is likely that the greatest impact of infection as an acute insult is on short term mortality, and if aggressive and appropriate management is given timely, it may improve renal function also.

Various studies on histopathology of renal tissues in cirrhosis have shown direct renal damage due to high bilirubin levels. There is formation of bile casts in the tubular lumen and accumulation of conjugated bilirubin in tubular epithelial cells, which leads to mitochondrial damage with defective oxidative phosphorylation. All above changes predispose patients of cirrhosis with jaundice to cholemic nephropathy[18]. Nazar *et al*[19] evaluated response of terlipressin in treatment of hepatorenal syndrome type-1 and found serum bilirubin level > 10 mg/dL as a predictor of poor response to therapy. The response rate in patients with bilirubin > 10 mg/dL was 13% as compared to 67% in bilirubin values < 10 mg/dL ($P = 0.001$). We reported higher mean bilirubin values in the infection AKI group than non-infection AKI group (11.3 *vs* 4.4 mg/dL) respectively. Possibly with increasing severity of chronic liver disease as assessed by MELD score, the immune function worsens and there is propensity to get infection in these patients. Our study also revealed significantly higher serum bilirubin values (15.7 *vs* 4.2 mg/dL) in non-survivors than survivors at 28-d, respectively.

In ACLF, HE is multifactorial. Sepsis, metabolic disturbances like hypokalemia, hyponatremia secondary to diuretic use or volume loss, liver dysfunction with hyperammonemia can precipitate HE. We have shown previously that there is increasing cerebral edema in patients with increasing grades of ACLF[20]. Therefore, HE also marks a poor prognosis in patients with infection AKI.

Our study has some limitations. The sample size could be higher, due to which subgroup analysis could not be done as the number of patients were small in individual groups. Second, being a tertiary care institute, most of the patients were referred from primary and secondary care centers after receiving antibiotics, therefore, culture reports were not available in all the patients. Also, the data on

Table 3 Univariate and multivariate analysis of survivors and non-survivors at 28-d

Variables	Survivors (*n* = 77)	Non-survivors (*n* = 42)	Univariate	Multivariate
Age (yr, mean ± SD)	41 ± 21	40 ± 22	0.73	-
Males, *n* (%)	69 (86.9%)	36 (85.7%)	0.41	-
Hb (gm/dL)	8.3 (4-14)	8.4 (3.4-14)	0.838	-
TLC (× 10^3/mm^3)	11 (2.5-37)	17.4 (2-39)	0.001	-
Platelet count (× 10^9/L)	114.6 (100-200)	130 (60-220)	0.520	-
Bilirubin (mg/dL)	4.2 (0.5-30)	15.7 (0.2-46)	0.001	< 0.001
INR	1.8 (1-3.7)	2.1 (1.2-3.8)	0.006	-
Albumin (gm/dL)	2.4 (1-3.7)	2.4 (1.8-3.9)	0.689	-
Sodium (mEq/L)	135 (116-164)	131 (120-146)	0.336	-
HE, *n*	32	37	< 0.001	< 0.01

Data expressed as median (range) otherwise expressed. INR: International normalized ratio; Hb: Hemoglobin; TLC: Total leukocyte count; HE: Hepatic encephalopathy.

Table 4 Univariate and multivariate analysis of survivors and non-survivors in infection acute kidney injury group (*n* = 67) at 28-d

Variables	Survivors (*n* = 29)	Non-survivors (*n* = 38)	Univariate	Multivariate
Age (yr, mean ± SD)	40 ± 21	40 ± 22	0.81	-
Males, *n* (%)	23 (79%)	35 (92%)	0.9	-
Hb (gm/dL)	8.1 (4-14)	7.5 (3.4-14)	0.06	-
TLC (× 10^3/mm^3)	10 (2.5-36)	18.3 (2-39)	0.001	-
Platelet count (× 10^9/L)	112 (65-203)	125 (60-220)	0.520	-
Bilirubin (mg/dL)	4.6 (1.2-30)	16.3 (1.5-46)	0.004	0.01
INR	1.9 (1.3-3.7)	2.1 (1.1-3.8)	0.005	-
Albumin (gm/dL)	2.3 (1-3.6)	2.4 (1.5-3.9)	0.73	-
Sodium (mEq/L)	135 (116-154)	132 (119-148)	0.45	-
HE, *n*	10	37	< 0.001	< 0.01

Data expressed as median (range) otherwise expressed. INR: International normalized ratio; Hb: Hemoglobin; TLC: Total leukocyte count; HE: Hepatic encephalopathy.

beta blockers was not available at baseline for all the patients and could not be analyzed.

In ACLF, renal dysfunction is multifactorial with the presence of sepsis, circulatory dysfunction either due to volume loss or sepsis and higher bilirubin levels. We showed that pre-renal, upper GI bleed and diuretic-induced AKI is less severe with favorable outcomes after successful management with very low rate of recurrence. On the other hand, in patients with infections, it is not only the control of infection, but also the number of organ failures which is crucial to determine the final outcome of these patients. Finally, higher grades of ACLF in patients with infection, AKI having liver dysfunction and cerebral failure has worst prognosis with high 28-d mortality.

CONCLUSION

Infections lead to worsening hemodynamics in cirrhosis which results in organ failures. Renal dysfunction in these patients further complicates the clinical scenario. We noted that higher bilirubin levels and Hepatic encephalopathy in patients with infection associated AKI portends a dismal prognosis. The present study emphasizes the worse prognosis with infection and need of early identification and aggressive management on admission to improve short-term mortality.

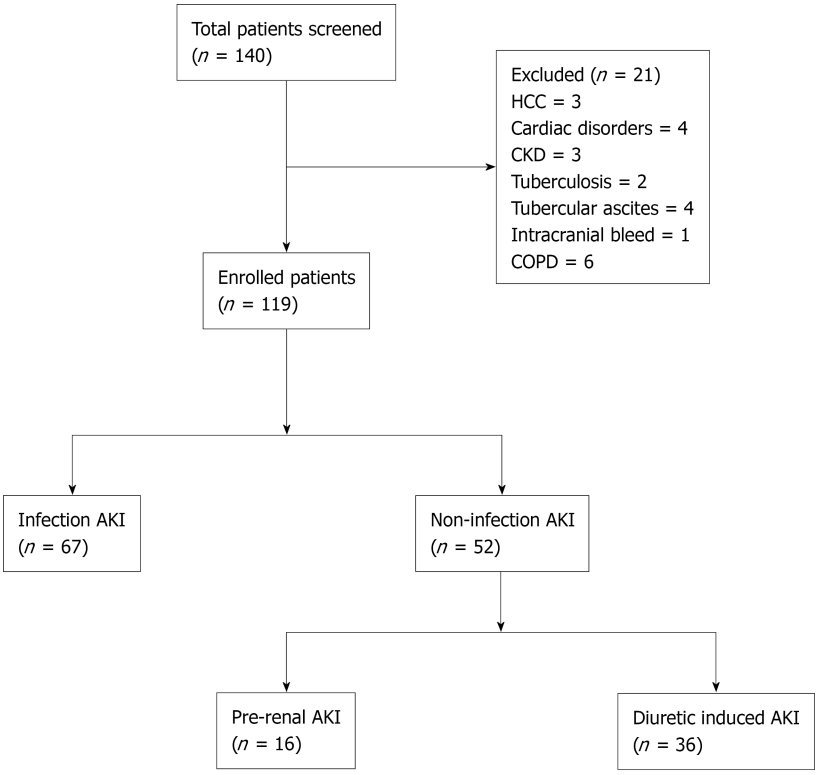

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Figure 1 Flow of patients into the study. AKI: Acute kidney injury; HCC: Hepatocellular carcinoma; CKD: Chronic kidney disease.

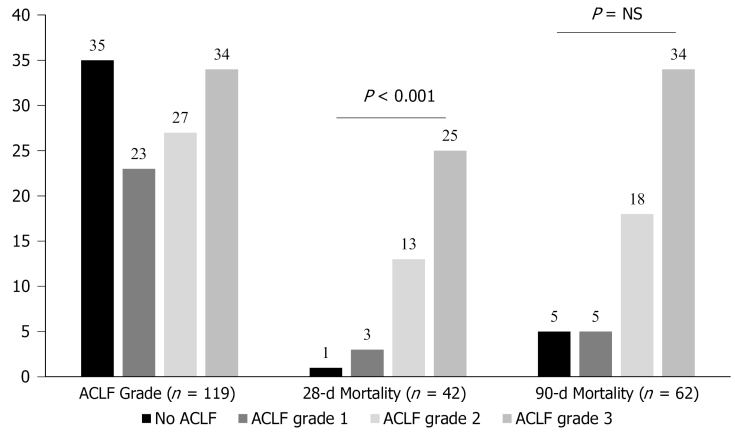

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Figure 2 Distribution of acute-on-chronic liver failure grades and 28- and 90-d mortality as per acute-on-chronic liver failure grades among patients of all groups. ACLF: Acute-on-chronic liver failure.

ARTICLE HIGHLIGHTS

Research background

Acute kidney injury (AKI) in cirrhosis has dismal outcomes. Recent data suggests infections being most common insult for acute decompensation of cirrhosis. Infections lead to acute deterioration of already compromised hemodynamics in cirrhosis.

Research motivation

Infections in cirrhosis is a precursor towards multi-organ dysfunction. Kidney failure is one of the early manifestation in cirrhosis which has a potential for reversibility. Identifying high risk of mortality in patients with AKI in cirrhosis may warrant early institution of treatment, especially in presence of infection. This may help to develop new protocols to salvage kidney in presence of infections in

cirrhosis.

Research objectives

To compare infection and non-infection AKI in cirrhosis, and to determine predictors of mortality at 28-d in patients with infection associated AKI.

Research methods

It was a prospective, observational study conducted at a tertiary care hospital for a period of 1 year. After written, informed consent total 119 patients with AKI in cirrhosis were included into the study. AKI was defined as per International Club of Ascites-AKI 2015 criteria. Patients were divided into infection and non-infection AKI groups. Non-infection AKI included patients with pre-renal and diuretic induced AKI. Infection and non-infection AKI groups were compared for clinical and laboratory data. In infection AKI group logistic regression analysis was performed to determine 28-d predictors of mortality.

Research results

There were 119 patients of cirrhosis with AKI. Alcohol (n = 104) was most common etiology of cirrhosis. The infection AKI group had 67 (56%) patients and non-infection AKI had 52 (44%) patients which included pre-renal AKI in 36 (30%) and diuretic-induced AKI in 16 (14%). Infection AKI patients had higher progression of AKI (19/67 *vs* 2/52; P = 0.01) and 28-d mortality (38/67 *vs* 4/5; $P \leq 0.01$) as compared to non-infection AKI patients. On subgroup analysis of Infection AKI group, on multivariate analysis, serum bilirubin as well as presence of HE were independent predictors of 28-d mortality. There was no significant difference of mortality at 90-d between two groups.

Research conclusions

This study says that AKI in cirrhosis with infection has high short term mortality. High bilirubin and presence of hepatic encephalopathy predicts high 28-d mortality in infection associated AKI. Probably AKI in patients with cirrhosis is multifactorial with sepsis, volume depletion, bilirubin as important factors.

Research perspectives

High bilirubin levels can contribute to nephropathy as well as encephalopathy. Still, we do not have effective therapies for high bilirubin values. Future research should focus on drugs to lower bilirubin levels. And probably more data is needed on infections in cirrhosis.

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FOOTNOTES

Author contributions: Gupta T was the guarantor and designed the study; Ranga N was involved in acquisition of data and drafted the initial manuscript; Goyal SK performed statistical analysis and interpretation of data; Gupta T revised the manuscript critically for important intellectual content.

Institutional review board statement: The study was reviewed and approved by the institutional ethics committee at Pt. BD Sharma Institute of Medical Sciences, Rohtak (India).

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ORIGINAL ARTICLE

Observational Study

Benign course of residual inflammation at end of treatment of liver transplant recipients after sofosbuvir based therapy

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Abstract

BACKGROUND

Persistent inflammation on histology after successful hepatitis C (HCV) treatment has been reported. However, data regarding the long-term impact in liver transplant recipients is limited, particularly after using direct-acting antiviral (DAA) therapies.

AIM

To evaluate the impact of successful treatment with DAAs on histological changes and occult HCV and to describe the clinical course of residual inflammation in liver transplant recipients.

METHODS

We conducted a case series of 13 chronic HCV infected liver transplant recipients successfully treated with DAAs between December 2013 and May 2014. All patients were treated for 24 wk and had non-detectable serum HCV RNA by the time of biopsy. Only patients with at least one liver biopsy at or after treatment were included. We examined liver biopsies for evidence of residual inflammation and the presence of intrahepatic HCV RNA.

RESULTS

Persistent inflammation was seen in 12/13 patients on end of treatment biopsy. Inflammation was still seen in the available five follow-up biopsies (range 38-48 wk after the end of treatment). Intrahepatic HCV RNA was undetectable in all biopsies. All patients had preserved graft function for a mean follow-up of 2.5 years, except one that developed chronic rejection.

CONCLUSION

After successful HCV treatment with DAAs, liver transplant recipients may have persistent inflammation on biopsy without evidence of intracellular RNA. The clinical outcome remained favorable in most patients. Further studies with a larger number and longer follow-up are needed to establish the implication of this finding on long-term graft function.

Key Words: Immunosuppression; Liver transplantation; Recurrent hepatitis C; Sustained virologic response; Interferon

Core Tip: Unexplained residual inflammation can be seen in a subset of liver transplant recipients successfully treated with direct-acting antiviral therapies; however, it does not seem to affect graft function. An extensive clinical and histopathologic workup should still be performed to exclude other potentially treatable conditions.

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INTRODUCTION

Until the emergence of direct-acting antivirals (DAAs), hepatitis C virus (HCV) related liver cirrhosis was the most common indication for liver transplant in adults[1,2]. Unfortunately, HCV recurrence after transplant is universal, with immediate exposure after graft reperfusion, leading to accelerated fibrosis, eventually cirrhosis, and graft failure if untreated[3]. Graft survival in HCV infection has been inferior to transplant for other disease etiologies[4], and HCV remains a common indication for re-transplantation[5], leading to a high burden on transplant resources. Eradication of HCV in the immediate pre-or early post-transplant setting can preserve graft function, but historically a difficult goal to achieve. The availability of DAAs has made significant improvement in the efficacy and tolerability in the post-transplant population[6,7], compared to the interferon (IFN) based regimens. The latter had a lower rate of sustained virologic response (SVR) in this unique patient population (as low as 30%), with a higher discontinuation rate due to adverse events, including graft rejection[8].

Although SVR is the critical clinical endpoint, there are mixed results concerning the post-SVR histologic benefit and detection of intra hepatocyte HCV (occult HCV). Prior reports on HCV recurrence following liver transplantation showed histological improvement after achieving SVR with IFN based regimens[9], whereas other studies reported ongoing inflammation in a subset of patients[10,11]. One study included 36 Liver transplant (LT) recipients treated with IFN based regimens showed persistent inflammation in 69% of the post SVR biopsies and identified occult hepatitis C in only one out of 32 biopsies that were tested[11].

Data on inflammation following SVR after DAA are scarce. A study that included nine LT recipients treated with DAAs identified residual inflammation after SVR in four patients and HCV RNA in the tissue sample in four patients[12]. Another study included LT recipients with recurrent HCV and advanced fibrosis (F3-F4) showed improved liver function in the majority of patients; however, regression of fibrosis by elastography (48 wk after treatment) was only seen in 39/77 subjects (51%). Although details on liver histology for these patients were not reported, this remains concerning for residual inflammation in some patients that could have progressed over time[13].

We believe that the finding of persistent inflammation in LT recipients after SVR using DAAs requires further research work as it is of clinical importance to the transplant team. At the same time, many of the available series included a small sample and focused mainly on histology without providing long-term clinical data.

The study aimed to evaluate the impact of successful treatment with DAAs on histologic changes and the occurrence of occult HCV in liver transplant recipients with chronic HCV. The secondary aim was to describe the long-term clinical course of residual inflammation if present.

MATERIALS AND METHODS

Patients population and data collection

We reviewed all chronic HCV infected liver transplant recipients treated with DAA regimens between December 2013 and May 2014. We excluded patients who did not achieve SVR or did not have at least one liver biopsy at or after the end of treatment. The study protocol was approved by the Institutional Review Board of the Cleveland Clinic Foundation in Florida. Data collected at baseline and during patient follow-up included; age, gender, race, date of transplant, baseline and end-of-treatment liver enzymes and viral load, HCV treatment regimen, other serologic autoimmune and virological markers, immunosuppression treatment regimen, and liver histology at end-of-treatment. When available, before-treatment and follow-up liver histology were also reported.

Outcome definitions

SVR: Defined as the absence of HCV RNA by polymerase chain reaction12 and 24 weeks after completion of treatment. The Linear Range of the used assay was 15 IU/mL to 100000000 IU/mL.

End of treatment liver biopsy: for the purpose of our study, this was defined as liver biopsy performed within 12 weeks after HCV RNA becomes undetectable.

Post-treatment liver biopsy: biopsy was done at least 6 mo after the end of treatment.

Biopsy method: All biopsies were performed percutaneously using an 18-gauge coaxial needle *via* ultrasound guidance. Two core tissue samples were obtained and placed in a formalin container. All samples reviewed by the pathologist contained at least 20 portal tracts to be considered adequate. Tissue sections were processed and stained with hematoxylin-eosin and trichrome stains. For the purpose of this study, the liver biopsies were evaluated by an expert liver pathologist with over 20 years of experience reading liver biopsies in an academic transplant center. The pathologist was blinded to the patients' clinical data, diagnoses, and previous biopsy interpretation. Evaluation of fibrosis and inflammation was described using Batts-Ludwig grading and staging[14]. Biopsies showing inflammation were carefully examined by the pathologist for the presence of rejection, de novo autoimmune hepatitis, and evidence of hepatotropic and non-hepatotropic viral hepatitis, including cytomegalovirus, Epstein-Barr virus, and Herpes simplex virus.

Method for HCV quantification on liver samples: Total RNA was isolated from five to six 10 μm cuts (curls) from formalin-fixed, paraffin-embedded (FFPE) biopsies in a Maxwell 16/LEV instrument using the Maxwell®16 LEV RNA FFPE Purification Kit (Promega). The concentration of purified RNA was quantified in NanoDrop 2000. 50 μL of each RNA was diluted in 950 μL of SPEX buffer and ran in a COBAS® AmpliPrep/COBAS® TaqMan® System using the HCV test (Roche Molecular Systems Inc.). The viral load, when detected, was expressed as IU/100 ng of RNA.

RESULTS

Out of 46 patients treated for HCV following liver transplant during the study time, 13 patients met the inclusion criteria. Their baseline and demographic characteristics are summarized in Table 1.

Treatment regimens

One patient started treatment before transplant (125 days before transplant), while 12/13 patients started after transplant (mean 5 years from transplant, range 32 days – 18 years). Eight patients (62%) were treated with Sofosbuvir plus Ribavirin, three patients (23%) were treated with Sofosbuvir plus Simeprevir, and two patients (15%) were started on Sofosbuvir plus Ribavirin then switched to Sofosbuvir plus Simeprevir because of worsening anemia. The total treatment duration in all patients was 24 wk.

Virologic response

Serum HCV RNA was undetectable in all patients at end-of-treatment and remained undetectable for another 12 and 24 wk post-treatment, consistent with SVR 12 and 24 (Figure 1).

End of treatment biopsy

Biopsies at the end of treatment were reviewed in all included patients (mean time from treatment start to biopsy was 25 wk, range 20-33 wk). The biopsies were performed to evaluate abnormal liver function tests or assess the resolution of inflammation after HCV eradication.

Table 1 Patients baseline and end of treatment charactarestics

	Gender	Age attreatment start	HCV genotype	Immune suppression	Baseline ALT U/L	Baseline RNA × 10^6	Time transplant to treatment (m)	Treatment regimen	End of treatment ALT U/L	End of treatment biopsy			
										Treatment to biopsy (wk)	Inflammation grade	Fibrosis stage	Other findings
1	F	59	1a	MMF, tacrolimus	57	1.3	22 mo	SOF/SIM	18	25	2	1	
2	M	57	1a	Tacrolimus	12	0.3	32 d	SOF/RBV	17	22	0	0	Mild centrilobular dilatation with focal hemorrhage
3	M	59	1a	MMF, tacrolimus	85	4.0	13 mo	SOF/RBV > SOF/SIM	11	32	2	0	
4	M	61	1a or 1b	Tacrolimus	20	0.1	5 mo	SOF/RBV	16	22	3	0	
5	M	68	1a or 1b	Tacrolimus	44	1.2	8.5 yr	SOF/RBV	18	25	1	3	
6	F	76	1a	Tacrolimus	20	10.6	5.5 mo	SOF/SIM	48	25	2	0	Histocytes granuloma
7	F	80	1a	Tacrolimus	12	5.2	18 yr	SOF/SIM	15	24	3	2	
8	M	62	1a	MMF, tacrolimus	166	1.7	43 d	SOF/RBV	9	30	1	0	Steatosis (< 5%)
9	M	60	3a	Tacrolimus, prednisone	23	0.9	4 mo	SOF/RBV	82	33	1	0	Rare councilmen bodies
10	M	58	1a	Tacrolimus	25	1.7	20 mo	SOF/RBV	30	25	4	3	Mild TCMR cannot be ruled out
11	M	53	3a	Cyclosporine	78	5.2	4.5 yr	SOF/RBV	37	23	4	2	Mild TCMR cannot be ruled out. Two portal tracts show non-necrotizing granulomas
12	F	61	2b	Tacrolimus	43	7.6	6 mo	SOF/RBV	34	20	2	1	
13	M	65	1a	Tacrolimus	94	2.0	7.5 yr	SOF/RBV > SOF/SIM	14	29	2	2	Mild absence of bile ducts

ALT: Alanine aminotransferase; MMF: Mycophenolate mofetil; RBV: Ribavirin; SIM: Simeprevir; SOF: Sofosbuvir.

Although all patients had undetectable RNA by the time of biopsy, active inflammation was present in 12/13 patients. Eight patients (62%) had grade 1-2 inflammation, and four (31%) had grades 3-4, Table 1.

The inflammation observed was consistent with chronic HCV with the presence of chronic portal inflammation, lymphoid aggregates, lobular inflammation, and acidophil bodies (Figure 2). There was a histologic suggestion of mild T cell-mediated rejection on biopsy in 2 patients, but clinically deemed not to have rejection, as one had normal aminotransferases, and the other one had spontaneous normalization of aminotransferases without adjusting their immune suppression regimen. One patient had a

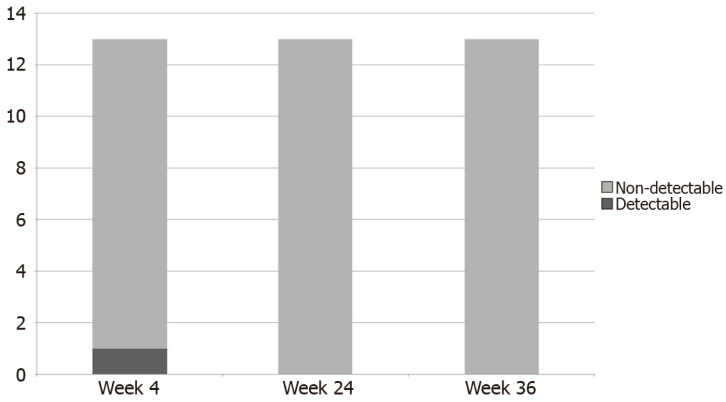

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Figure 1 Hepatitis C RNA during and after treatment.

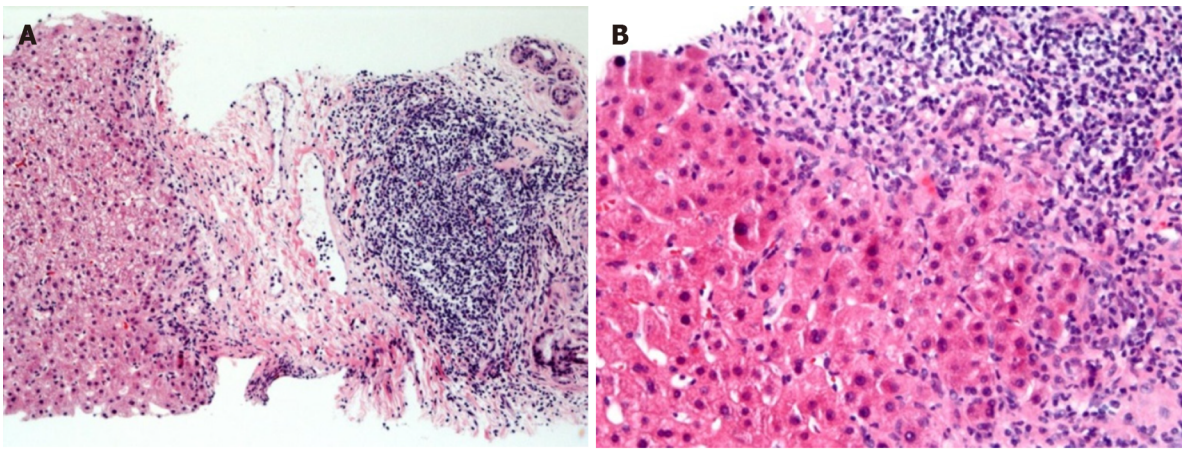

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Figure 2 End of treatment liver biopsy. A: Portal tract showing fibrosis and a robust lymphoid aggregate reminiscent of a germinal center in the biopsy tissue from patient 12 who completed treatment for hepatitis C (Hematoxylin and eosin, X50); B: There is interface hepatitis as the portal lymphocytic infiltrates spill into the surrounding liver parenchyma in the biopsy tissue from patient 9 (Hematoxylin and eosin, 100X).

mild absence of bile ducts concerning for early chronic rejection. Two patients had occasional non-necrotizing granulomas in portal tracts without an identifiable cause. Steatosis was present in only one patient and was mild < 5%.

We compared pre- and post-therapy histological grades of inflammation in five patients (biopsies were performed at a mean of 17 wk before treatment start, range 10-26 wk). The mean time between pre and end of treatment biopsies was 43 wk, range 35-55 wk. Inflammation increased in three patients (by one point), decreased in one patient (by one point), and remained the same in one, Table 2.

Post-treatment liver biopsy

Post-treatment follow-up biopsies were available in five patients (range 38-48 wk after the end of treatment). Compared to end-of-treatment biopsies, inflammation decreased in 4 out of 5 patients (by one point) and increased in one patient (by one point). Fibrosis increased by one point in two patients and remained the same in two. No other causes of inflammation were identified clinically or histologically, Table 3.

HCV RNA was undetected on all available end-of-treatment (13) and post-treatment liver biopsies (5).

Clinical follow up

The clinical course for all patients was tracked for a mean of 2.5 years after the end of treatment. None of the patients had HCV relapse or worsening liver function. All had preserved graft function, normal aminotransferase, and alkaline phosphatase levels except one that had chronically elevated alkaline phosphatase and was later diagnosed with chronic ductopenic rejection (the patient that had a mild absence of bile ducts on the end of treatment biopsy).

Table 2 End of treatment biopsy compared to prior to treatment biopsy

Patient number	Weeks from biopsy to treatment start	Prior to treatment inflammation grade	End of treatment	
			Inflammation	
			Grade	Change
1	10	1	2	↑
5	14	2	1	↓
10	22	3	4	↑
11	18	3	4	↑
13	26	2	2	↔

↑: Increased; ↔ : Unchanged; ↓: Improved.

Table 3 End of treatment biopsy compared to follow up biopsy

Patient number	End of treatment biopsy		Weeks between both biopsies	ALT	Follow up biopsy	
	Inflammation	Fibrosis			Inflammation	Fibrosis
3	2	0	38	20	1 ↓	0 ↔
4	3	0	42	15	2 ↓	1 ↑
5	1	3	43	20	2 ↑	3 ↔
10	4	3	48	22	2 ↓	3↔
11	4	2	44	71	3 ↓	3 ↑

↑: Increased; ↔ : Unchanged; ↓: Improved.
ALT: Alanine aminotransferase.

DISCUSSION

Even after the wide use of DAAs, HCV-related cirrhosis remains one of the leading indications for liver transplant and re-transplant in adults[5]. Due to their safety profile, DAAs allow treating more patients with decompensated cirrhosis prior to transplant; however, treatment is commonly deferred to the post-transplant period to avoid reducing the transplant priority or allowing receipt of a hepatitis C positive organ[15]. DAAs show high SVR rates in transplant recipients; however, data regarding the histological impact of these drugs in terms of inflammatory changes is limited. Throughout our early DAA experience, we evaluated end of treatment and follow-up biopsies after achieving undetectable RNA. We noted that biopsies still showed persistent inflammation at the end of treatment in 12/13 patients, with no improvement from pre-treatment in 4/5 patients.

Additionally, all the available follow-up biopsies (up to 48 wk from the end of treatment) still showed persistent inflammation. We thoroughly evaluated different possibilities that could explain this persistent inflammation. Although no clinically significant drug interactions are reported between immune suppressive regimens and the included DAAs in our study[16], a cure of HCV can potentially influence immune suppression drug levels, and rejection may be a concern. Prior studies have shown an impact of ongoing HCV infection on CYP3A4[17] and eventually on cyclosporine and tacrolimus levels. In one report, lower doses of these immunosuppressants were needed to reach the same therapeutic level compared to non HCV infected patients[18], raising the possibility that resolution of HCV infection can lead to lower immunosuppressant level with subsequent rejection. This possibility was carefully examined in our patients; findings suggestive of mild T cell-mediated rejection were present only in two patients on end of treatment biopsies. The histological changes present were minimal and, when correlated, clinically deemed insignificant as the patients did well clinically without additional interventions or adjustment of immune suppression regimen. Persistent unexplained hepatitis in the liver allograft has been previously reported as idiopathic post-transplant hepatitis (IPTH), chronic hepatitis of unknown etiology, with a variable prevalence ranging from 10%-50% in the adult population[19]. The implication of this diagnosis in a chronic HCV setting is unclear as most of the studies excluded this patient population[20,21].

Furthermore, in our study, inflammation was present in higher frequency (in 12/13 end of treatment biopsies and all 5 post-treatment biopsies), suggesting the presence of another etiology. Prior studies also described a characteristic pattern of plasma cell hepatitis[10,22,23] in patients who achieved SVR after receiving IFN based therapy. This unique pattern was not seen in our patients. One possible explanation is that plasma cell hepatitis is seen more in IFN treated patients, given the immune stimulant effect of IFN leading to exposure of new antigens on hepatocytes[24]. All our patients treated with IFN free regimens can possibly explain the absence of this histologic pattern.

Prior studies reported occult HCV infection, a "controversial" term, indicating persistence of HCV RNA within hepatocytes and/or peripheral blood mononuclear cells despite successfully achieving SVR [25]. Although the active liver disease has not been reported with this finding, it has been shown that this persistent low-level HCV replication promotes persistence of both humoral and T-cellular HCV specific markers, that inversely correlated with time from SVR but can persist for up to 9 years[26]. Our patients, in theory, are at high risk for developing occult HCV for multiple reasons. First, it has been proposed that following treatment with DAA, there is a higher potential for developing occult HCV when compared to IFN based treatment due to the lack of induced immunologic response of the interferon effect[27]. Second, the risk of occult HCV in the immune-compromised patients is likely increased because of the limited ability of the immune system for complete viral clearance, similar to end-stage renal disease patients on dialysis[28]. However, the occurrence of occult infection remains questionable[29], and data following DAA have been inconsistent. One study reported detectable HCV RNA in hepatocytes or peripheral blood mononuclear cells in five out of nine post-transplant patients who were treated with DAA and had elevated liver enzymes despite achieving SVR[12].

In contrast, another study did not find evidence of intracellular RNA in 4 patients with persistent liver enzyme elevation after DAA[30]. The discrepancy in results could be related to the lack of method standardization used among studies to detect HCV RNA in tissue; different sensitivities have been reported depending on the used method and tissue processing before analysis[27,31]. Due to the retrospective nature of our study, we used FFPE specimens. We found no HCV RNA particles on the available end of treatment and post-treatment biopsies indicating that occult infection is not the underlying etiology of residual inflammation in our cohort.

The way viral infections induce liver inflammation is complex; one of the identified triggers of this immune response is the activation of transmembrane and cytosolic receptors that sense both the viral nucleic acid and certain host nucleic acid segments, particularly DNA derived from mitochondrial damage. It has been presumed that this plays a role in some non-viral liver injury models as acetaminophen hepatotoxicity and ischemic injury[32]. The persistent inflammation seen in our subjects could be triggered by the host rather than the remaining viral RNA. However, this is not certain as we did not immunologically characterize the inflammatory cells on liver biopsies for HCV-specific T-cell responses, and this can be an area for future research.

Another likely explanation for the persistent inflammation in our series can be the lag of the histological improvement behind viral clearance and biochemical improvement. Our study did not show complete resolution of inflammation on the end of treatment biopsies nor on post-treatment biopsies. However, most of our patients had post-treatment liver biopsy within 6 mo after completion of treatment, which might not be enough time for inflammation to resolve. Moreover, it should be noted that the change in inflammation grades and fibrosis stages between both biopsies is subtle; hence, we cannot exclude the possibility of this being secondary to sampling variation rather than a true change.

Our study limitations include the small number of patients, mostly genotype 1 treated with some early sofosbuvir-based regimens. However, we believe that our sample is relatively larger than similar studies that evaluated histologic changes post-transplant in this setting and that the findings are likely generalizable to other DAA regimens. Moreover, we did not check for HCV RNA in peripheral blood mononuclear cells due to the retrospective nature of our study, so there is a possibility that we may have missed occult infection in the mononuclear cells. However, in the setting of residual inflammation on liver biopsy that is consistent with HCV activity, we believe it is more important to examine the liver tissue, which was negative for HCV RNA particles, making occult HCV infection a less likely explanation for this persistent inflammation.

CONCLUSION

Our case series is among the few available that report the histologic findings and clinical outcomes in transplant recipients after achieving SVR using DAAs. We were also able to rule out occult HCV, and we followed the patients clinically for 2.5 years showing a benign course of the residual inflammation in most subjects. Based on our findings, the residual inflammation appears to have a favorable outcome, but it is crucial to exclude other causes of inflammation thoroughly. Moreover, based on our results and prior studies, we believe that checking occult HCV is not routinely necessary from a clinical standpoint. Liver transplant recipients often require liver biopsy for various reasons; recognizing the natural history of this residual inflammation is important to the transplant team. Further studies with a larger and more diverse patient population and longer follow-up will help better characterize the long-term outcome of

this persistent inflammation following SVR.

ARTICLE HIGHLIGHTS

Research background

Liver transplant recipients may undergo liver biopsy for different indications, and persistent inflammation in patients who receive DAAs can be seen despite achieving sustained virologic response (SVR).

Research motivation

Data on the significance of persistent inflammation on histology after successful treatment of hepatitis C infection with Direct-acting antiviral (DAA) therapies is scarce.

Research objectives

We aimed to examine the impact of successful treatment with DAAs on histological changes and to describe the clinical course of residual inflammation in liver transplant recipients.

Research methods

A case series of chronic hepatitis C liver transplant recipients received DAA post-liver transplant and achieved sustained virologic response. Only patients with at least one liver biopsy were included.

Research results

Thirteen patients were included in this case series; all achieved SVR. Twelve patients were found to have persistent inflammation at the end of treatment biopsy. Five patients had follow-up biopsies, all of which had persistent inflammation. However, all patients had preserved graft function up to 2.5 years, except one who had chronic rejection.

Research conclusions

Persistent inflammation can be seen in liver transplant recipients treated with DAAs; however, it did not appear to affect the outcome.

Research perspectives

The findings of our case series shed light on the significance of persistent inflammation in liver transplant recipients post successful DAAs treatment. Further studies are needed to include a more diverse patient population.

FOOTNOTES

Author contributions: Zervos XB and Tzakis A designed the research; Ismail B, Sears D performed the research; Ismail B, Bejarano P, Ruiz P analyzed the data; Sears D, Benrajab KM, Ismail B, and Zervos XB wrote the paper; all authors contributed to critical revision of the manuscript, and saw and approved the final version.

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Informed consent statement: All study participants, or their legal guardian, provided informed written consent prior to study enrollment.

Conflict-of-interest statement: Authors have no relevant relationships or conflict of interest to disclose.

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ORIGINAL ARTICLE

Observational Study

Interrelationship between physical activity and depression in nonalcoholic fatty liver disease

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Abstract

BACKGROUND

Nonalcoholic fatty liver disease (NAFLD) is associated with a sedentary lifestyle and depressive symptoms. It is also well established that physical inactivity and depressive symptoms are related. However, an investigation of the interaction between all of these factors in NAFLD has not been previously conducted.

AIM

To investigate the interrelationship between physical inactivity and depressive symptoms in individuals with NAFLD.

METHODS

Data from the Rancho Bernardo Study of Healthy Aging were utilized. 589 individuals were included in the analyses (43.1% male; 95.8% non-Hispanic white; aged 60.0 ± 7.0 years). NAFLD was defined by using the hepatic steatosis index, depression using the Beck Depression Inventory, and physical activity by self-report of number of times per week of strenuous activity. Multivariable generalized linear regression models with Gamma distribution were performed to investigate the proposed relationship.

RESULTS

About 40% of the sample had evidence of NAFLD, 9.3% had evidence of depression, and 29% were physically inactive. Individuals with NAFLD and depression were more likely to be physically inactive (60.7%) compared to individuals with neither NAFLD nor depression (22.9%), individuals with depression without NAFLD (37.0%), and individuals with NAFLD without depression (33.3%). After accounting for various comorbidities (*i.e.*, age, sex, diabetes, hypertension, obesity), individuals with NAFLD and higher levels of physical activity were at a decreased odds of having depressive symptoms [16.1% reduction (95% confidence interval: -25.6 to -5.4%), $P = 0.004$], which was not observed in those without NAFLD.

CONCLUSION

Individuals with NAFLD have high levels of physical inactivity, particularly those with depressive symptoms. Because this group is at high risk for poor outcomes, practitioners should screen for the coexistence of depressive symptoms and NAFLD. This group should receive appropriate interventions aimed at increasing both participation and levels of intensity of physical activity.

Key Words: Liver disease; Outcomes research; Psychiatric disorders; Exercise

Core Tip: Physical inactivity and depressive symptoms are common in individuals with nonalcoholic fatty liver disease (NAFLD). Individuals with both NAFLD and depression are more likely to be sedentary than individuals without NAFLD or in individuals with NAFLD without depressive symptoms. Because this group is at high risk for poor outcomes, practitioners should screen for the coexistence of depressive symptoms and NAFLD. This group should receive appropriate interventions aimed at increasing both participation and levels of intensity of physical activity. It is therefore desirable that individuals with NAFLD should be screened for the presence of depressive symptoms to help determine appropriate interventions.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver condition in the United States and globally[1]. NAFLD is a multisystem disease which can affect many organ systems and increase the risk of cardiovascular disease and type 2 diabetes mellitus, as well as numerous other conditions[2,3]. NAFLD, along with these other conditions, has been associated with a variety of behavioral factors, including a lack of physical activity, poor nutritional status, and substance consumption[4].

Specifically, a sedentary lifestyle has been related to the onset of NAFLD[5,6]. One potential pathway explaining this relationship is that a lack of physical activity is associated with obesity, which in turn is a major risk factor for NAFLD[5,7]. Increases in physical activity and exercise can lead to mobilization of fat from the liver and is suggested as a treatment for NAFLD[8-10]. Therefore, physical activity is an important behavior to understand in the context of NAFLD, both as a risk factor and as a treatment. Another factor that is highly related to physical inactivity is elevated depressive symptoms (both severity and frequency)[11]. In fact, there is a bi-directional relationship between a sedentary lifestyle and depressive symptoms such that physical inactivity is a risk factor for depressive symptoms and depressive symptoms are a risk factor for physical inactivity[5,12].

NAFLD has also been associated with depressive symptoms[13]. Individuals with NAFLD that also have a major depressive disorder are at an increased risk of developing other conditions such as cardiovascular diseases and stroke[14]. In general, individuals with elevated depressive symptoms have worse health outcomes, including increased morbidity and mortality [14]. Since one potential intervention for NAFLD is increasing levels of physical activity, it is important to consider the potential impact of depressive symptoms on the likelihood of participating in physical activity. It has been well established that individuals with depressive symptoms are less adherent to treatment for chronic illness, particularly treatments that involve behavioral changes[15].

Previous research has demonstrated the relationship between NAFLD and physical inactivity, between NAFLD and depressive symptoms, and between physical inactivity and depressive symptoms; however, we were not able to identify previous literature that explored the interaction of NAFLD, physical inactivity, and depressive symptoms together. The current investigation assesses the presence of these three factors in a community sample in order to explore the potential interrelationships.

MATERIALS AND METHODS

Data source and study population

The Rancho Bernardo Study (RBS) of Healthy Aging has been previously described in detail[16]. Briefly, between 1972 and 1974, 6339 (82%) adults from the predominantly white and middle to upper middle class southern California community of Rancho Bernardo were enrolled in a longitudinal study focusing on healthy aging. In addition, RBS focused on determining risk factors for cardiovascular disease, diabetes, cognitive function, and bone disease. Participants were followed *via* 12 subsequent clinic visits occurring approximately every four years as well as annual mailers to follow-up on health status and vital status through July 2019.

Our study utilized data from 1781 participants who completed clinic visit 7 (1992-1996). Clinic visit 7 was chosen because it assessed the factors necessary to establish presence or absence of NAFLD. Of these, we excluded 17 participants for missing the hepatic steatosis index (HSI), 55 participants for missing Beck Depression Inventory (BDI), and 221 participants who had a history of hepatitis, iron overload (iron ≥ 198 mcg/dL in men and ≥ 170 mcg/dL in women), or excessive alcohol consumption. As depression can manifest differently in older adults[17] and physical activity levels are different in older adults, we further excluded 899 participants aged 70 and over, leaving 589 participants in the final analytical sample (Figure 1). All participants provided written informed consent prior to participation at each visit.

Measurements

Demographic factors, lifestyle factors, laboratory measures, and medical history data were collected at clinic visit 7 (1992-1996). Lifestyle information was obtained through standard questionnaires and included smoking status [non-smoker, former smoker (quit ≥ 2 years); active smoker], sedentary lifestyle (reported physical activity < 3 times per week) and excessive alcohol consumption (≥ 2 drinks/day in men and ≥ 1 drinks in women). Metabolic components were calculated by the following definitions: (1) Obesity pattern was categorized into lean (BMI: 18.5-25 kg/m^2); overweight (25-29.9 kg/m^2) and obese (≥ 30 kg/m^2); (2) Hypertension was defined as having a systolic blood pressure of > 140 mmHg or diastolic blood pressure of > 90 mmHg from an average of three measurements and/or use of antihypertensive medications; (3) Hyperlipidemia was defined as a serum cholesterol level of ≥ 200 mg/dL, LDL of ≥ 130 mg/dL, and HDL ≤ 40 mg/dL in men or ≤ 50 mg/dL in women; (4) Diabetes mellitus was defined by a fasting glucose level ≥ 126 mg/dL, post-challenge plasma glucose level of at least 200 mg/dL, and history of physician-diagnosed diabetes or use of diabetes medication; (5) Insulin resistance was defined by the homeostasis model assessment of insulin resistance[18]; and (6) Metabolic syndrome was defined as having at least three of the following: waist circumference > 102 cm in men or > 88 cm in women, fasting plasma glucose > 110 mg/dL, blood pressure > 130/85 mmHg, elevated triglycerides > 150 mg/dL, and HDL ≤ 40 mg/dL in men or ≤ 50 mg/dL in women[19].

Definition of depression and physical activity

We categorized the presence of depression as a BDI score of ≥ 10[20]. Individuals that scored less than 10 were considered to not have depression. We categorized physical activity into 3 groups: (1) "physical inactivity" if participants didn't engage in any level of physical activity at least three times per week; (2) "ideal physical activity" if participants regularly (≥ 3/week) engaged in strenuous activity; and (3) "moderate physical activity" that encompassed everyone else.

Definition of nonalcoholic fatty liver disease

NAFLD was defined by using the HSI, validated previously and used in epidemiologic studies[13,21,22] in the absence of secondary causes of liver disease. HSI was calculated by the following equation: 8 × (alanine aminotransferase/aspartate aminotransferase ratio) + BMI (+2 for diabetes; +2 for female). The published cut-off score of 36 was utilized to define the presence of NAFLD. Participants with a HSI of < 36 and no secondary causes of liver disease were presumed to not have the presence of NAFLD (non-NAFLD).

Statistical analysis

We compared demographic, lifestyle factors, clinical factors and medical history of the study cohort by the presence of NAFLD, depression and level of physical activity using a non-parametric Kruskal-Wallis test for continuous variables and chi-square test for categorical analysis. Multivariable generalized

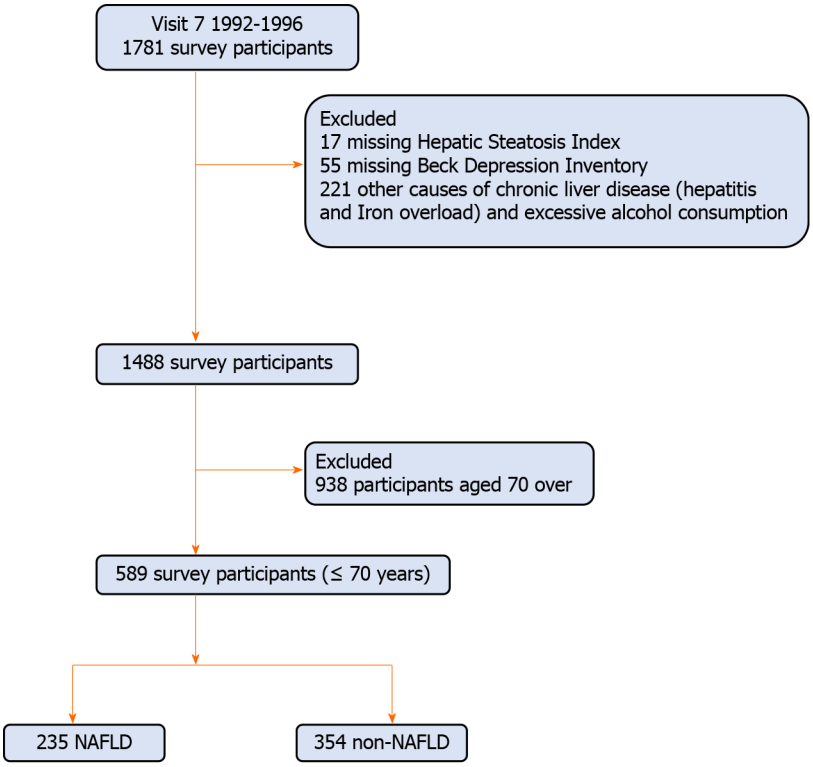

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Figure 1 Flow chart of study cohort selection. NAFLD: Nonalcoholic fatty liver disease.

linear regression model (GLM) with Gamma distribution was performed on BDI score to evaluate the effect of physical activity and NAFLD after adjusting for age, sex, current smoker, diabetes, hypertension, hyperlipidemia, obesity, history of cardiovascular disease and cancer. The adjusted relationship between factors and BDI scores was estimated using coefficients from GLM models, which were exponentiated to yield a percentage change in the outcome associated with each factor. Independent predictors of depression were studied using multivariable logistic regression. All differences reported here are statistically significant otherwise mentioned at the 0.05 Level. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Of 589 study subjects (43.1% male; 95.8% non-Hispanic white; mean (SD) age 60.0 (7.0) years), 235 (39.9%) subjects had evidence of NAFLD and 55 (9.3%) had evidence of depression. Furthermore, 12.6% had diabetes, 75.7% had hyperlipidemia, 20.0% had hypertension, 26.7% had insulin resistance, 29.0% were physically inactive and 11.9% were active smokers.

Compared to individuals without NAFLD, individuals with NAFLD were statistically significantly more commonly male (52.3% *vs* 37.0%), more likely to be overweight/obese (93.6% *vs* 28.0%) more likely to have insulin resistance (46.4% *vs* 13.6%), hyperlipidemia (85.5% *vs* 69.2%), diabetes (23.4% *vs* 5.4%), and metabolic syndrome (43.4% *vs* 6.5%) (all $P < 0.02$). Among individuals with NAFLD, 36.6% fell into the physical inactivity category and 24.7% were in the ideal physical activity category; whereas among individuals without NAFLD, 24.0% fell into the physical inactivity category and 36.7% were in the ideal physical activity category ($P < 0.002$) (Table 1). Individuals with NAFLD had a statistically significantly higher mean BDI score than those without NAFLD (4.49 *vs* 3.67, $P = 0.004$), although the mean scores were relatively low in each group.

Of the entire cohort, 4.8% had both NAFLD and depression, 4.6% had depression without NAFLD, 35.1% had NAFLD without depression and 55.5% had neither depression nor NAFLD. Demographic, lifestyle and general health comorbidities of participants according to the presence of NAFLD and depression status are presented in Table 2. Compared to individuals with NAFLD but no depression, individuals with both NAFLD and depression were more likely to have a history of arthritis (17.6% *vs* 10.5%). Compared to individuals with depression but no NAFLD, individuals with both NAFLD and depression were less likely to be lean (7.1% *vs* 85.2%) and have a higher rate of insulin resistance (42.9% *vs* 3.7%) and metabolic syndrome (39.3% *vs* 7.4%).

Table 1 Demographic, lifestyle and general health comorbidities of participants according to the presence of nonalcoholic fatty liver disease

	All (*n* = 589)	Non-NAFLD (*n* = 354)	NAFLD (*n* = 235)	*P* value
Age, mean ± SD	59.97 ± 6.97	59.99 ± 7.40	59.94 ± 6.29	0.3959
Male, %	254 (43.12%)	131 (37.01%)	123 (52.34%)	0.0002
White, %	564 (95.76%)	340 (96.05%)	224 (95.32%)	0.6686
Smoking status, %				
Current	70 (11.90%)	47 (13.31%)	23 (9.79%)	0.1958
Former	239 (40.65%)	141 (39.94%)	98 (41.70%)	0.6706
Non-smoker	279 (47.45%)	165 (46.74%)	114 (48.51%)	0.674
Regular exercise, %				
Physically Inactive	171 (29.03%)	85 (24.01%)	86 (36.60%)	0.001
Moderate physical activity	230 (39.05%)	139 (39.27%)	91 (38.72%)	0.8949
Ideal physical activity	188 (31.92%)	130 (36.72%)	58 (24.68%)	0.0021
Obesity, % BMI				
Lean	270 (45.84%)	255 (72.03%)	15 (6.38%)	< 0.0001
Overweight	234 (39.73%)	99 (27.97%)	135 (57.45%)	< 0.0001
Obese	85 (14.43%)	0 (0.00%)	85 (36.17%)	< 0.0001
History of CVD, %	40 (6.79%)	23 (6.50%)	17 (7.23%)	0.7278
History of arthritis, %	68 (11.54%)	39 (11.02%)	29 (12.34%)	0.6226
History of cancer (any), %	97 (16.47%)	48 (13.56%)	49 (20.85%)	0.0195
Insulin resistance, %	157 (26.66%)	48 (13.56%)	109 (46.38%)	< 0.0001
Hypertension, %	117 (19.86%)	66 (18.64%)	51 (21.70%)	0.3623
Hyperlipidemia, %	446 (75.72%)	245 (69.21%)	201 (85.53%)	< 0.0001
Diabetes, %	74 (12.56%)	19 (5.37%)	55 (23.40%)	< 0.0001
Metabolic syndrome[1], %	125 (21.26%)	23 (6.52%)	102 (43.40%)	< 0.0001
BDI, mean ± SD	4.00 ± 3.71	3.67 ± 3.62	4.49 ± 3.80	0.0041

CVD: Cardiovascular disease; NAFLD: Nonalcoholic fatty liver disease; SD: Standard deviation. NCEP ATP III (2005 revision).

[1]*P* value by nonparametric Kruskal-Wallis Test for continuous variables, chi-square test for categorical variable, Data are presented as the mean ± SD for numerical variables and count (%) for categorical variables.

For the individuals that had NAFLD and depression, 60.7% fell within the physical inactivity category which is statistically significantly greater than all of the other groups [individuals with neither NAFLD nor depression (22.9%), individuals with depression without NAFLD (37.0%), and individuals with NAFLD without depression (33.3%)] (Figure 2). Characteristics of individuals according to the presence of NAFLD and physical activity are presented in Supplementary Table 1.

In stratified analyses across the presence of NAFLD, accounting for age, sex, current smoker, diabetes, hypertension, hyperlipidemia, obesity, history of cardiovascular disease and any cancer in GLMs, individuals with NAFLD and higher levels of physical activity experienced greater odds of having a lower BDI score [16.1% reduction (95% confidence interval: -25.6 to -5.4%), $P = 0.004$]. This association between level of activity and BDI scores was not observed in those without NAFLD (Table 3).

To assess the association of physical activity and NAFLD on BDI scores, GLMs were performed (Table 4). In the unadjusted model, compared with non-NAFLD individuals with an ideal level of physical activity, NAFLD individuals with physical inactivity had an increased BDI score [46.8% increase (19.3 to 80.8%), $P < 0.001$]. Even in the fully adjusted model, this result was consistently observed [36.3% increase (9.1% to 70.2%) $P < 0.001$]. Non-NAFLD Individuals with physical inactivity did not statistically significantly differ from non-NAFLD individuals with an ideal level of physical activity ($P = 0.465$).

Table 2 Demographic, lifestyle and general health comorbidities of participants according to the presence of depression and nonalcoholic fatty liver disease

	Individuals with NAFLD			Individuals without NAFLD		
	No depression (*n* = 207)	Depression (*n* = 28)	*P* value	No depression (*n* = 327)	Depression (*n* = 27)	*P* value
Age, mean ± SD	59.91 ± 6.34	60.15 ± 6.00	0.9433	60.02 ± 7.32	59.61 ± 8.44	0.9813
Male, %	115 (55.56%)	8 (28.57%)	0.0073	125 (38.23%)	6 (22.22%)	0.0978
White, %	197 (95.17%)	27 (96.43%)	0.7671	316 (96.64%)	24 (88.89%)	0.0471
Smoking status, %						
Current	19 (9.18%)	4 (14.29%)	0.3934	41 (12.58%)	6 (22.22%)	0.1563
Former	86 (41.55%)	12 (42.86%)	0.8949	133 (40.80%)	8 (29.63%)	0.2549
Non-smoker	102 (49.28%)	12 (42.86%)	0.5236	152 (46.63%)	13 (48.15%)	0.8789
Regular exercise, %						
Physically inactive	69 (33.33%)	17 (60.71%)	0.0048	75 (22.94%)	10 (37.04%)	0.0992
Moderate physical activity	86 (41.55%)	5 (17.86%)	0.0157	129 (39.45%)	10 (37.04%)	0.8051
Ideal physical activity	52 (25.12%)	6 (21.43%)	0.6706	123 (37.61%)	7 (25.93%)	0.2259
Obesity, % BMI						
Lean	13 (6.28%)	2 (7.14%)	0.8609	232 (70.95%)	23 (85.19%)	0.1132
Overweight	118 (57.00%)	17 (60.71%)	0.7094	95 (29.05%)	4 (14.81%)	0.1132
Obese	76 (36.71%)	9 (32.14%)	0.6365	0 (0.00%)	0 (0.00%)	-------
History of CVD, %	14 (6.76%)	3 (10.71%)	0.4488	21 (6.42%)	2 (7.41%)	0.8417
History of arthritis, %	21 (10.14%)	8 (28.57%)	0.0054	37 (11.31%)	2 (7.41%)	0.5331
History of any cancer, %	46 (22.22%)	3 (10.71%)	0.1595	47 (14.37%)	1 (3.70%)	0.1196
Insulin resistance, %	97 (46.86%)	12 (42.86%)	0.6902	47 (14.37%)	1 (3.70%)	0.1196
Hypertension, %	44 (21.26%)	7 (25.00%)	0.6519	61 (18.65%)	5 (18.52%)	0.9861
Hyperlipidemia, %	177 (85.51%)	24 (85.71%)	0.9767	226 (69.11%)	19 (70.37%)	0.8918
Diabetes, %	50 (24.15%)	5 (17.86%)	0.4601	16 (4.89%)	3 (11.11%)	0.1682
Metabolic syndrome[1], %	91 (43.96%)	11 (39.29%)	0.6394	21 (6.44%)	2 (7.41%)	0.8451

CVD: Cardiovascular disease; SD: Standard deviation; NAFLD: Nonalcoholic fatty liver disease, NCEP ATP III (2005 revision).

[1]*P* value by nonparametric Kruskal-Wallis Test for continuous variables, chi-square test for categorical variable. Data are presented as the mean ± SD for numerical variables and count (%) for categorical variables.

In multivariable logistic regression, we included in the model: NAFLD, diabetes, age, sex, smoking status, hypertension, hyperlipidemia, cardiovascular disease, and cancer. The statistically significant risk factors of depression were NAFLD Odds Ratio (OR 2.01 1.08-3.72), $P = 0.028$), being male [OR 0.37 (0.19-0.72), $P = 0.003$] and physical inactivity [OR 1.68 (0.78-3.65), $P = 0.005$] (Supplementary Table 2).

DISCUSSION

This study investigated the interrelationships between NAFLD, depressive symptoms and physical activity. Our results demonstrate a strong likelihood of physical inactivity in individuals with NAFLD and depression, which was at a higher rate than was seen in individuals without NAFLD or in individuals with NAFLD without depressive symptoms.

Similar findings have been found in individuals with type 2 diabetes[23]. Various symptoms of depression (lack of motivation, low self-esteem, feelings of helplessness, anhedonia) might explain why individuals with depressive symptoms are more often physically inactive[24], but having only depression in this cohort did not explain the inactivity level. The co-existence between NAFLD and depression is likely to associate with physical inactivity.

Table 3 Univariable and multivariable changes in beck depression inventory according to physical activity, stratified by the presence of nonalcoholic fatty liver disease

	NAFLD		Non-NAFLD	
	% change (95%CI)	*P*	% change (95%CI)	*P*
Unadjusted	-16.91 (-26.28 - -6.35)	0.0024	-8.96 (-18.25 - 1.38)	0.0871
Age-sex adjusted	-14.74 (-24.42 - -3.83)	0.0095	-6.79 (-16.35 - 3.87)	0.203
Model 1	-14.6 (-24.34 - -3.61)	0.01	-3.43 (-13.64 - 7.98)	0.54
Model 2	-16.12 (-25.6 - -5.44)	0.004	-3.27 (-13.49 - 8.15)	0.5592

Generalized linear regression with a gamma error distribution and a log-link function. Level of Physical activity is defined as a continuous variable (1 = inactive, 2 = moderate, 3 = Ideal); Model 1 adjusted for Physical activity, age, sex, current smoker, DM, hypertension, hyperlipidemia and obesity; Model 2 adjusted for all variables in model 1 + history of CVD and history of cancer. NAFLD: Nonalcoholic fatty liver disease.

Table 4 Univariable and multivariable changes in beck depression inventory score according to the presence of nonalcoholic fatty liver disease and level of physical activity

	Unadjusted		Age-sex adjusted		Model 1		Model 2	
Group	% change (95%CI)	*P*	% change (95%CI)	*P*	% change (95%CI)	*P*	% change (95%CI)	*P*
Non-NAFLD with physical ideal	Reference		Reference		Reference		Reference	
Non-NAFLD with physical moderate	16.67 (-3.33 - 40.82)	0.108	11.5 (-7.68 - 34.67)	0.2584	7.77 (-11.05 - 30.58)	0.4447	6.99 (-11.65 - 29.56)	0.4891
Non-NAFLD with physical inactivity	19.85 (-2.87 - 47.89)	0.0913	14.15 (-7.5 - 40.87)	0.2174	8.25 (-12.74 - 34.29)	0.4708	8.34 (-12.58 - 34.26)	0.4645
NAFLD with physical ideal	1.23 (-20.04 - 28.15)	0.919	3.31 (-18.23 - 30.53)	0.7849	-2.43 (-24.49 - 26.08)	0.851	-2.19 (-24.33 - 26.45)	0.866
NAFLD with physical moderate	23.08 (-0.09 - 51.63)	0.051	23.99 (0.79 - 52.53)	0.042	15.68 (-7.7 - 44.98)	0.206	16.24 (-7.23 - 45.65)	0.1911
NAFLD with physical inactivity	46.84 (19.28 - 80.75)	0.0003	43.06 (16.39 - 75.84)	0.0007	35.02 (8.03 - 68.74)	0.0083	36.25 (9.1 - 70.16)	0.0064

Generalized linear regression with a gamma error distribution and a log-link function. Level of Physical activity is defined as a continuous variable (1 = Poor, 2 = moderate, 3 = Ideal); Model 1 adjusted for Physical activity, age, sex, current smoker, DM, hypertension, hyperlipidemia and obesity; Model 2 adjusted for all variables in model 1 + history of CVD and history of cancer. NAFLD: Nonalcoholic fatty liver disease.

Depression and NAFLD occur together more often than would be predicted by chance[25]. There are many potential factors that may help to explain this overlap, including the presence of diabetes and obesity, both risk factors for NAFLD and depression[25]. Another area of overlap is the increase in circulating inflammatory cytokines in both depression and NAFLD[26]. In addition, physical inactivity is a risk factor for both depression and NAFLD[5,11]. However, further investigation is needed to clarify this bi-directional relationship between depression and NAFLD.

The findings of the current study show that both physical inactivity and depressive symptoms are common in individuals with NAFLD. In addition, individuals with NAFLD and depressive symptoms are much more likely to be physically inactive than people with depression without NAFLD and those without either. NAFLD is a risk factor for all-cause mortality and exercise is an antidote to this. The combination of depression and NAFLD is significantly associated with low level of physical activity, which in itself is a risk for all-cause mortality. It is therefore desirable that individuals with NAFLD should be screened for the presence of depressive symptoms. Depressive symptoms are likely to contribute to a low level of physical activity, and if treated, may increase participation in more vigorous activity for greater durations. Additionally, increased physical activity has been shown to help mobilize fat from the liver[8,9], and increased physical activity has been shown to have anti-depressive effects [27]. Therefore, it may be important to screen for the combined presence of NAFLD and depression, treat each appropriately, and aim to maximize participation in physical activity. Longitudinal studies

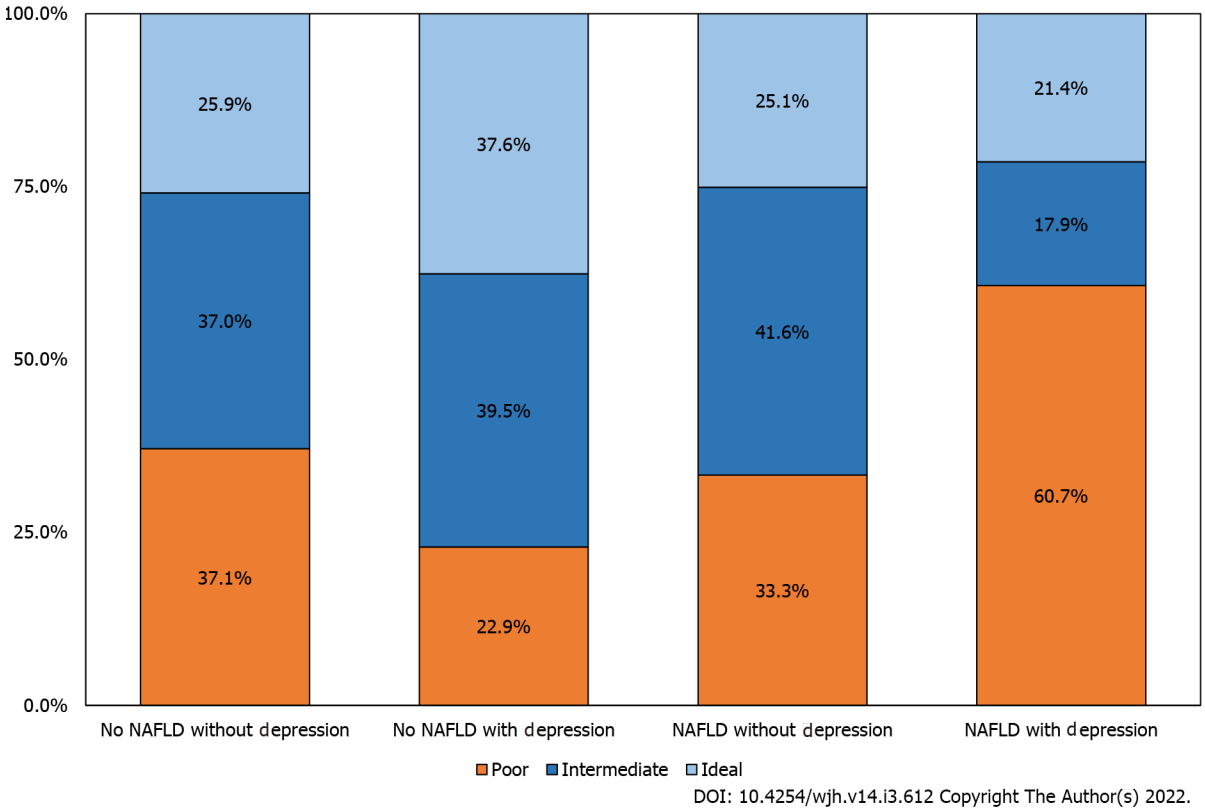

Figure 2 Percentage distribution of physical activity, by the presence of nonalcoholic fatty liver disease and depressive symptoms.
NAFLD: Nonalcoholic fatty liver disease.

investigating these interrelationships are needed to determine if physical inactivity is one of the factors that may link depressive symptoms to subsequent poor health outcomes in NAFLD patients.

Some limitations should be noted. Due to the cross-sectional nature of the current investigation, no causal relationships nor directionality can be inferred between physical inactivity, depressive symptoms, and NAFLD. Another limitation is that we used a noninvasive test (HSI) to identify NAFLD rather than a liver biopsy or other sensitive radiologic tests since these were not available. An objective method of physical activity assessment (*i.e.*, an activity monitor) was not available. In addition, these data were collected in 1992-1996, therefore an older version of the BDI was used and the diagnosis of viral hepatitis was relatively new at the time. We also acknowledge that our findings are not generalizable to the general population, as all participants were well educated, medically insured, predominantly white, and middle to upper-middle-class. Lastly, participants have a relatively low prevalence of obesity, diabetes, and metabolic syndrome compared to the National Health and Nutrition Examination Survey III[28] which may have influenced the results.

CONCLUSION

Individuals with NAFLD have high levels of physical inactivity, particularly those with depressive symptoms. Because this group is at high risk for poor outcomes, practitioners should screen for the coexistence of depressive symptoms and NAFLD. This group should receive appropriate interventions aimed at increasing both participation and levels of intensity of physical activity.

ARTICLE HIGHLIGHTS

Research background

Since one potential intervention for nonalcoholic fatty liver disease (NAFLD) is increasing levels of physical activity, it is important to consider the potential impact of depressive symptoms on the likelihood of participating in physical activity. It has been well established that individuals with depressive symptoms are less adherent to treatment for chronic illness, particularly treatments that involve behavioral changes.

Research motivation

Previous research has demonstrated the relationship between NAFLD and physical inactivity, between NAFLD and depressive symptoms, and between physical inactivity and depressive symptoms; however, we were not able to identify previous literature that explored the interaction of NAFLD, physical inactivity, and depressive symptoms together.

Research objectives

The current investigation assesses the presence of NAFLD, physical inactivity, and depressive symptoms in a community sample in order to explore the potential interrelationships.

Research methods

Data from the Rancho Bernardo Study were used. 589 individuals were included in the analyses (43.1% male; 95.8% non-Hispanic white; aged 60.0 ± 7.0 years). NAFLD was defined by using the hepatic steatosis index, depression using the Beck Depression Inventory, and physical activity by self-report of number of times per week of strenuous activity. Multivariable generalized linear regression models with Gamma distribution were performed to investigate the proposed relationship.

Research results

About 40% of the sample had evidence of NAFLD, 9.3% had evidence of depression, and 29% were physically inactive. Individuals with NAFLD and depression were more likely to be physically inactive (60.7%) compared to individuals with neither NAFLD nor depression (22.9%), individuals with depression without NAFLD (37.0%), and individuals with NAFLD without depression (33.3%). After accounting for various comorbidities (*i.e.*, age, sex, diabetes, hypertension, obesity), individuals with NAFLD and higher levels of physical activity were at a decreased odds of having depressive symptoms [16.1% reduction (95% confidence interval: -25.6 to -5.4%), $P = 0.004$], which was not observed in those without NAFLD.

Research conclusions

Individuals with NAFLD have high levels of physical inactivity, particularly those with depressive symptoms. Because this group is at high risk for poor outcomes, practitioners should screen for the coexistence of depressive symptoms and NAFLD. This group should receive appropriate interventions aimed at increasing both participation and levels of intensity of physical activity.

Research perspectives

Further investigation is needed to clarify this bi-directional relationship between depression and NAFLD. Future work should explore screening for the combined presence of NAFLD and depression to determine if treatment with appropriate physical activity interventions can enhance outcomes.

FOOTNOTES

Author contributions: Weinstein AA, de Avila, L, Golabi P, Gerber LH, and Younossi ZM designed the research study; Paik JM analyzed the data; and Weinstein AA, Kannan S, de Avila L, and Paik JM wrote the manuscript; all authors have read and approve the final manuscript.

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ORIGINAL ARTICLE

Observational Study

Assessment of fibroblast growth factor 19 as a non-invasive serum marker for hepatocellular carcinoma

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Abstract

BACKGROUND

Fibroblast growth factor 19 (FGF-19) is one of the founding members of the endocrine FGF subfamily. Recently, it has been the subject of much interest owing to its role in various physiological processes affecting glucose and lipid metabolism and the regulation of bile acid secretion as well as cell proliferation, differentiation, and motility. Additionally, FGF-19 secretion in an autocrine style has reportedly contributed to cancer progression in various types of malignancies including hepatocellular carcinoma (HCC).

AIM

To estimate the serum FGF-19 concentrations in HCC cases and assess its diagnostic performance for the detection of HCC.

METHODS

We recruited 90 adult participants and divided them into three equal groups: Healthy controls, cirrhosis patients, and HCC patients. Serum FGF-19 concentrations were measured using the Human FGF-19 ELISA kit.

RESULTS

We detected a high statistically significant difference in serum FGF-19 levels among the three groups. The highest level was observed in the HCC group, followed by the cirrhosis and control groups (236.44 ± 40.94 *vs* 125.63 ± 31.54 *vs* 69.60 ± 20.90 pg/mL, respectively, $P \leq 0.001$). FGF-19 was positively correlated with alpha fetoprotein (AFP; $r = 0.383$, $P = 0.003$) and international normalised ratio ($r = 0.357$, $P = 0.005$), while it was negatively correlated with albumin ($r = -0.500$, $P \leq 0.001$). For the detection of HCC, receiver operating characteristic curve analysis showed that the best cut-off point of AFP was > 8.2 ng/mL with an area

under the curve (AUC) of 0.78, sensitivity of 63.33%, specificity of 83.33%, positive predictive value (PPV) of 79.2%, negative predictive value (NPV) of 69.4%, and total accuracy of 78%. However, FGF-19 at a cut-off point > 180 pg/mL had an AUC of 0.98, sensitivity of 100%, specificity of 90.0%, PPV of 90.0%, NPV of 100%, and total accuracy of 98%.

CONCLUSION

FGF-19 represents a possible novel non-invasive marker for HCC. It may improve the prognosis of HCC patients due to its utility in several aspects of HCC detection and management.

Key Words: Fibroblast growth factor 19; FGF-19; Fibroblast growth factors; Tumour biomarkers; Hepatocellular carcinoma; Detection; Cirrhosis

Core Tip: We recruited 90 adult participants and divided them into three equal groups: Healthy controls, cirrhosis patients, and hepatocellular carcinoma (HCC) patients. We detected a high statistically significant difference in fibroblast growth factor 19 (FGF-19) levels among the three groups, with the highest level occurring in the HCC group, followed by the cirrhosis and control groups (236.44 ± 40.94 *vs* 125.63 ± 31.54 *vs* 69.60 ± 20.90 pg/mL, respectively, $P \leq 0.001$). For the detection of HCC, receiver operating characteristic curve analysis showed that FGF-19 demonstrated a better diagnostic performance than alpha fetoprotein (area under the curve = 0.98 *vs* 0.78). Consequently, we can conclude that FGF-19 represents a possible novel non-invasive marker for HCC.

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INTRODUCTION

Fibroblast growth factor 19 (FGF-19) is one of the founding members of the endocrine FGF subfamily [1]. Recently, it has been the subject of much interest owing to its role in various physiological processes affecting glucose and lipid metabolism and bile acid secretion as well as cell proliferation, differentiation, and motility[2-4]. Additionally, FGF-19 secretion in an autocrine style has reportedly contributed to cancer progression in various types of malignancies including hepatocellular carcinoma (HCC)[5-9].

FGF-19 has a restricted pattern of expression. It is mostly expressed in the terminal ileum in response to the bile-acid-stimulated intestinal Farnesoid X receptor (FXR)[10], and then, through the portal circulation to the liver, it attaches to its receptor, fibroblast growth factor receptor 4 (FGFR4), and a co-factor known as β-klotho. This action initiates the transcription of various genes that negatively regulate bile acid synthesis through the downregulation of CYP7A1[11].

Although FGF-19 is formed principally in the ileum and FGF-19 expression is almost absent in the human liver under normal conditions, current studies propose that FGF-19 may be autocrined by human hepatocytes under cholestatic conditions, peritumoral tissue cirrhosis, and HCC. The secretion of FGF-19 in these conditions demonstrates the protective negative feedback of FGF-19 in order to guard hepatocytes from the cytotoxicity of bile acids[12-14] and the promotion of the development and progression of HCC by bile acids through mTOR dependent mechanisms[15]. This beneficial effect of the FGF-19 pathway has also been proposed in other studies in *FXR*-/-knock out mice that developed hepatic malignancies, which were inhibited by the expression of an *FXR* transgene in the intestine[16]. This effect indicates the protective aspect of Fgf15 (the mouse homolog of human FGF-19) in relation to hepatic malignancies. Additionally, Fgf15/FGF19 mediated hepatic regeneration in mice in other studies[17,18].

However, the higher expression of FGF-19 in HCC patients has been found to promote tumour cell survival and has antiapoptotic impacts that are applied through the FGFR4-glycogen synthase kinase (GSK)3β-Nrf2 signalling pathway[19]. Moreover, Kang *et al*[20] showed that a distinctive molecular subtype of FGF-19 is correlated with a poor prognosis in HCC patients. In addition, Cui *et al*[21] and Zhao *et al*[22] reported that Fgf15 and FGF-19, respectively, promoted the progression of HCC by stimulating epithelial-mesenchymal transition and Wnt/β-catenin cascade, which is linked to tumour aggression and mortality. Furthermore, previous data has pointed to FGF-19 as a promoter of liver stem cells in HCC patients, as noted in the robust association between FGF-19 and EpCAM, which is a

moderator of cell adhesion and signalling and a special biomarker for liver cancer stem cells[23,24]. Additionally, confirmation of the role of FGF-19 signalling in HCC progression arises from the tumour-preventing effect of the selective FGFR4 inhibitor BLU9931 in a mouse HCC model with implanted FGF-19-producing, FGFR4-expressing hepatic cells[25]. These results suggest that FGF-19 may be implicated in tumour development in HCC cases.

Since FGF-19 is a serum protein secreted by HCC cells in an autocrine loop style, and systemic concentrations of FGF-19 have been found to reflect its portal concentrations[14,26], we aimed to estimate the serum FGF-19 concentrations in HCC cases and assess the diagnostic performance of FGF-19 for the detection of HCC.

MATERIALS AND METHODS

This observational study was conducted at Ain Shams University Hospitals in Cairo, Egypt from March 2021 to September 2021. This study was performed in accordance with the ethics principles of the Declaration of Helsinki and was authorised by the ethics board of the Faculty of Medicine, Ain Shams University (No. FMASU MS 66/2021). Written informed approval was obtained from all the participants before they were enrolled in the study.

We consecutively recruited 90 adult participants and divided them into three equal groups: Healthy controls, cirrhosis patients, and HCC patients. Patients with any malignant disease other than HCC were excluded. None of the HCC cases had either neoadjuvant chemotherapy or radiotherapy.

Diagnosis of cirrhosis and HCC

Cirrhosis was diagnosed according to laboratory parameters, clinical manifestations, and/or histological criteria[27]. HCC was identified through contrast-enhanced imaging studies and/or histological criteria as per the practice guidelines[28].

Measurement of serum FGF-19 concentrations

The serum FGF-19 concentrations were measured using the Human FGF-19 ELISA kit (SunRed Biological Technology Co. Ltd., Shanghai, China, Catalogue # 201-12-2199) with a sensitivity of 2.032 pg/mL, assay range of 2.5-700 pg/mL, intra-assay coefficient of variability (CV) < 10%, and inter-assay CV < 12%.

Statistical methods

Data were analysed using the Statistical Package for Social Science (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). The qualitative variables are shown as numbers and percentages, while the quantitative variables are shown as the mean, standard deviation, or median and interquartile range, as appropriate. The differences among the groups were calculated using the Chi-square test, Fisher exact test, independent *t*-test, one-way ANOVA test, or Kruskal-Wallis test, as appropriate. A receiver operating characteristic (ROC) curve analysis was applied to assess the diagnostic performance of FGF-19 and alpha fetoprotein (AFP) for HCC detection. A P value of less than 0.05 was considered statistically significant.

RESULTS

This study included 90 participants divided into control, cirrhosis, and HCC groups. The HCC group was comprised of 19 males (63.3%) and 11 females (36.7%), with a mean age of 57.37 years. In the cirrhotic group, there were 20 males (66.7%) and 10 (33.3%) females, with a mean age of 53.57 years. The control group included 18 males (60%) and 12 females (40%), with a mean age of 51.07 years (Table 1). According to the Child-Pugh class, 14 of the HCC cases (46.7%) belonged to Class C, while 18 (60%) of the cirrhotic cases belonged to Class A (P = 0.002, Table 1). There were statistically significant differences among the three groups concerning AFP, haemoglobin, platelets, alanine aminotransferase, aspartate aminotransferase (AST), albumin, international normalised ratio (INR), fasting blood glucose, and bilirubin (Table 1).

We detected a high statistically significant difference in the FGF-19 levels of the three groups. The highest level occurred in the HCC group, followed by the cirrhosis and control groups (236.44 ± 40.94 *vs* 125.63 ± 31.54 *vs* 69.60 ± 20.90 pg/mL, respectively, $P \leq 0.001$; Table 1, Figure 1). There were seven HCC patients with negative AFP; however, they had elevated FGF-19 levels (> 180 pg/mL). Serum FGF-19 levels were not significantly different according to the Child–Pugh class in the cirrhosis and HCC groups (Table 2).

The tumour characteristics of the HCC cases are shown in Table 3. Serum FGF-19 levels were higher in relation to the size of the tumour, the presence of portal vein thrombosis, jaundice, lower limb oedema, and weight loss; however, these differences did not reach statistical significance (Table 4). FGF-

Table 1 Characteristics of all participants

		Control (*n* = 30)	Cirrhosis (*n* = 30)	HCC (*n* = 30)	*P* value	*Post-hoc* analysis
Age (yr)		51.07 ± 12.38	53.57 ± 10.48	57.37 ± 10.25	0.091	
Sex	Female	12 (40%)	10 (33.3%)	11 (36.7%)	0.866	
	Male	18 (60%)	20 (66.7%)	19 (63.3%)		
Aetiology of hepatic disease			HCV (*n* = 18, 60%)	HCV (*n* = 25, 83.33%)	0.691	
			HBV (*n* = 7, 23.3%)	HBV (*n* = 3, 10%)		
			Others (*n* = 5, 16.6%)	Others (*n* = 2, 6.66%)		
Child-Pugh Class	Class A		18 (60%)	5 (16.7%)	0.002	
	Class B		6 (20%)	11 (36.7%)		
	Class C		6 (20%)	14 (46.7%)		
Fibroblast growth factor 19 (pg/mL)		69.60 ± 20.90	125.63 ± 31.54	236.44 ± 40.94	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 ≤ 0.001
Alpha fetoprotein (ng/mL)		3.35 (2.5 – 4.5)	6.4 (4 – 6.9)	513.5 (5.6 – 1500)	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 ≤ 0.001
Haemoglobin (g/dL)		13.16 ± 1.24	10.68 ± 1.11	10.49 ± 1.59	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 = 0.588
White blood cells (10^9/L)		7.09 ± 2.01	6.37 ± 2.27	5.86 ± 2.43	0.109	
Platelets (10^9/L)		288.10 ± 92.79	144.17 ± 48.27	136.13 ± 43.78	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 = 0.636
Alanine aminotransferase (U/L)		20.67 ± 7.02	65.47 ± 33.00	52.97 ± 23.25	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 = 0.044
Aspartate aminotransferase (U/L)		23.23 ± 12.69	49.87 ± 24.78	45.93 ± 20.02	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 = 0.444
Creatinine (mg/dL)		0.90 ± 0.22	0.99 ± 0.36	1.11 ± 0.51	0.112	
Urea (mg/dL)		21.70 ± 7.37	30.10 ± 18.82	32.97 ± 25.17	0.057	
Albumin (g/dL)		3.96 ± 0.34	3.33 ± 0.53	2.65 ± 0.43	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 ≤ 0.001
INR		1.09 ± 0.11	1.54 ± 0.24	1.85 ± 0.36	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 ≤ 0.001
Bilirubin (mg/dL)		0.75 ± 0.26	1.80 ± 0.74	1.97 ± 0.42	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001
						P3 = 0.211
Fasting blood glucose (μmoI/L)		5.19 ± 0.19	4.46 ± 0.28	4.46 ± 0.28	≤ 0.001	P1 ≤ 0.001
						P2 ≤ 0.001

			P3 = 1.000

HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; INR: International normalised ratio.

Table 2 Serum fibroblast growth factor 19 levels in the cirrhosis and hepatocellular carcinoma groups according to Child-Pugh score

	Child-Pugh Class	Cirrhosis (*n* = 30)	HCC (*n* = 30)
Fibroblast growth factor 19 (pg/mL)	Class A	129.311 (± 38.01)	223.320 (± 37.39)
	Class B	123.383 (± 21.51)	230.209 (± 30.96)
	Class C	116.833 (± 15.69)	246.029 (± 48.71)
	P value	0.7046	0.479

HCC: Hepatocellular carcinoma.

Table 3 Tumour characteristics of hepatocellular carcinoma cases

		HCC (*n* = 30)
Size	< 2 cm	3 (10%)
	2-3 cm	17 (56.7%)
	> 5 cm	10 (33.3%)
Number of tumour foci	Single	10 (33.3%)
	2-3	9 (30%)
	Multiple	11 (36.7%)
Portal vein thrombosis	No	21 (70%)
	Yes	9 (30%)
Metastasis	No	27
	Yes	3

HCC: Hepatocellular carcinoma.

19 was positively correlated with AFP (r = 0.383, P = 0.003) and INR (r = 0.357, P = 0.005), while it was negatively correlated with albumin (r = -0.500, $P \leq 0.001$; Table 5, Figure 2).

For the detection of HCC, the ROC curve analysis showed that the best cut-off point of AFP was > 8.2 ng/mL with an area under the curve (AUC) of 0.78, sensitivity of 63.33%, specificity of 83.33%, positive predictive value (PPV) of 79.2%, negative predictive value (NPV) of 69.4%, and total accuracy of 78%. However, FGF-19 at a cut-off point > 180 pg/mL had an AUC of 0.98, sensitivity of 100%, specificity of 90.0%, PPV of 90.0%, NPV of 100%, and total accuracy of 98% (Table 6, Figure 3).

DISCUSSION

HCC is the third highest cause of tumour death globally, with a 5-year survival rate of approximately 20% despite the developments in imaging technologies and therapeutic methodologies[29]. Unfortunately, the majority of HCC patients are diagnosed at an advanced stage of disease; therefore, early recognition of the disease is crucial to improving the prognosis and overall survival of patients[24].

Tumour markers have commonly been utilised for numerous objectives, such as diagnosis, follow-up care after treatment, optimisation of therapeutic effectiveness, and prediction of prognosis. Earlier studies have identified various serum markers for HCC which can be applied as diagnostic and prognostic markers for HCC. Although the assessment of these biomarkers is not essential for establishing a conclusive diagnosis of HCC as per the guidelines, these biomarkers play a key role in HCC diagnosis and monitoring[28,30,31]. However, it has been found that AFP, which is the most studied marker, may remain in the normal range not only in the early stages, but also in the advanced stages of HCC[32]. Moreover, an increase of AFP is occasionally detected in cirrhotic patients.

Table 4 Serum fibroblast growth factor 19 levels according to variables in the hepatocellular carcinoma group

		FGF-19 pg/mL (mean ± SD)	*P* value
Size	< 2 cm	219.9 ± 51.79	0.254
	2-3 cm	229.2 ± 36.06	
	> 5 cm	253.72 ± 44.39	
Number	Single	234.17 ± 36.38	0.885
	2 - 3	242.28 ± 45.69	
	Multiple	233.74 ± 44.22	
Portal vein thrombosis	No	230.55 ± 39.13	0.235
	Yes	250.2 ± 44.08	
Right upper quadrant pain	No	237.171 ± 41.026	0.885
	Yes	234.744 ± 43.163	
Weight loss	No	229.132 ± 34.285	0.106
	Yes	256.550 ± 52.793	
Pruritus	No	239.518 ± 39.170	0.505
	Yes	227.988 ± 47.214	
Jaundice	No	226.182 ± 29.468	0.118
	Yes	249.86 ± 50.48	
Fever	No	237.668 ± 40.531	0.834
	Yes	234.33 ± 43.54	
Oedema	No	228.945 ± 37.054	0.16
	Yes	251.44 ± 46.12	

FGF-19: Fibroblast growth factor 19.

Considering these two facts, alternative serum markers with high levels of sensitivity and specificity are needed.

It has previously been reported that FGF-19 may be associated with the pathogenesis and clinical characteristics of HCC[12,24]. Thus, we aimed to investigate the diagnostic utility of FGF-19 in HCC cases. We observed significantly higher serum FGF-19 levels in the HCC group compared to the control and cirrhosis groups. Serum FGF-19 levels were also higher in relation to the size of the tumour and presence of portal vein thrombosis; however, these differences did not reach statistical significance owing to the small sample size.

In accordance with our results, Maeda *et al*[12] detected higher serum levels of FGF-19 in their HCC group (214.5 pg/mL) compared to the cirrhosis group (100.1 pg/mL, $P < 0.001$) and the control group (78.8 pg/mL, $P = 0.002$). However, no statistically significant difference was detected between the cirrhotic cases and controls in their study.

Similar to the current results, Li *et al*[24] detected significantly higher serum FGF-19 levels in the HCC group compared to the control group (145.57 ± 118.72 *vs* 90.18 ± 13.88 pg/mL, $P = 0.044$). They also reported that FGF-19 levels were significantly raised in the HCC tissues (57.80 ± 4.39 pg/10 mg total protein) in comparison to both healthy control tissues (33.29 ± 1.53 pg/10 mg total protein, $P < 0.001$) and paired peritumoral tissues (46.33 ± 2.53 pg/10 mg total protein, $P = 0.032$). Additionally, *FGF-19* mRNA expression was significantly raised in the HCC tissues in comparison to paired peritumoral tissues (3.30 ± 1.82 *vs* 2.25 ± 0.82, respectively, $P = 0.025$). Moreover, FGF-19 expression increased significantly with a strong positive correlation ($r = 0.968$) consistent with the histological severity of hepatic disease, showing a trend in samples with steatosis (224.13 ± 115.68, $P = 0.087$), steatohepatitis (413.99 ± 159.55, $P = 0.002$), cirrhosis (613.35 ± 157.29, $P < 0.001$), and HCC (2507.28 ± 831.10, $P = 0.001$) in comparison to the paired peritumoral tissues (142.96 ± 41.32).

Our results are also consistent with those of Sun *et al*[33], who detected higher FGF-19 levels in the HCC and diabetes-HCC groups than in the control and diabetes groups (220.5, 185.1, 115.8, and 70.4 pg/mL, respectively, $P < 0.001$). All these results indicate that FGF-19 may have a role in the pathogenesis of HCC.

Table 5 Correlation between fibroblast growth factor 19 and alpha fetoprotein with patients' laboratory data

	AFP		FGF-19	
	r	*P* value	*r*	*P* value
AFP	–	–	0.383	0.003
FGF-19	0.383	0.003	–	–
Age	0.062	0.640	0.125	0.343
Haemoglobin	-0.196	0.133	-0.060	0.651
White blood cells	-0.064	0.627	-0.144	0.272
Platelets	0.018	0.893	-0.151	0.248
Alanine aminotransferase	0.036	0.786	-0.151	0.249
Aspartate aminotransferase	0.040	0.764	-0.024	0.855
Creatinine	-0.164	0.211	0.093	0.480
Urea	-0.022	0.867	0.012	0.929
Albumin	-0.213	0.102	-0.500	0.000
INR	-0.001	0.993	0.357	0.005
Bilirubin	-0.093	0.479	0.008	0.952
Fasting blood glucose	-0.135	0.477	0.056	0.767

AFP: Alpha fetoprotein; FGF-19: Fibroblast growth factor 19; INR: International normalised ratio.

Table 6 Diagnostic performance of fibroblast growth factor 19 and alpha fetoprotein for differentiation of hepatocellular carcinoma cases

	Cut-off point	AUC	Sensitivity	Specificity	PPV	NPV
FGF-19	> 180 pg/mL	0.98	100%	90%	90%	100%
AFP	> 8.2 ng/mL	0.78	63.33%	83.33%	79.2%	69.4%

AFP: Alpha fetoprotein; AUC: Area under the curve; FGF-19: Fibroblast growth factor 19; HCC: Hepatocellular carcinoma; NPV: Negative predictive value; PPV: Positive predictive value.

In line with the results of the current research, Sun *et al*[33] detected a positive association between FGF-19 and AFP in HCC patients ($P < 0.05$). However, Maeda *et al*[12] found no significant association between serum FGF-19 concentrations and AFP. Moreover, in partial agreement with the present study, Wunsch *et al*[34] observed that serum and hepatic concentrations of FGF-19 were associated with the severity of hepatic disease, as measured by laboratory parameters including albumin ($r = -0.408$, $P = 0.007$), haemoglobin ($r = -0.394$, $P = 0.01$), AST ($r = 0.328$, $P = 0.03$), and total bilirubin ($r = 0.577$, $P < 0.001$).

For HCC detection, in the study by Maeda *et al*[12], the ROC curve analysis determined a cut-off point of FGF-19 of 200 pg/mL, which had an AUC of 0.795, sensitivity of 53.2%, specificity of 95.1%, PPV of 95.9%, and NPV of 48.7%. This result was comparable to those of AFP (AUC = 0.827). However, in the current study, FGF-19 had a better diagnostic performance at a cut-off > 180 pg/mL with an AUC of 0.98, sensitivity of 100%, specificity of 90%, PPV of 90%, and NPV of 100%.

The current study was limited by a small sample size and a high ratio of patients with advanced HCC. Further studies are needed to investigate the clinical applications of the current results. FGF-19 could serve as a predictor of prognosis and a marker for follow-up after HCC treatment. Additionally, the FGF-19 pathway has received increased interest as a possible therapeutic target in chronic liver diseases[5,35-37]. In fact, anti-FGF-19 antibody therapy has been described as inhibiting HCC evolution in FGF-19 transgenic mice[38].

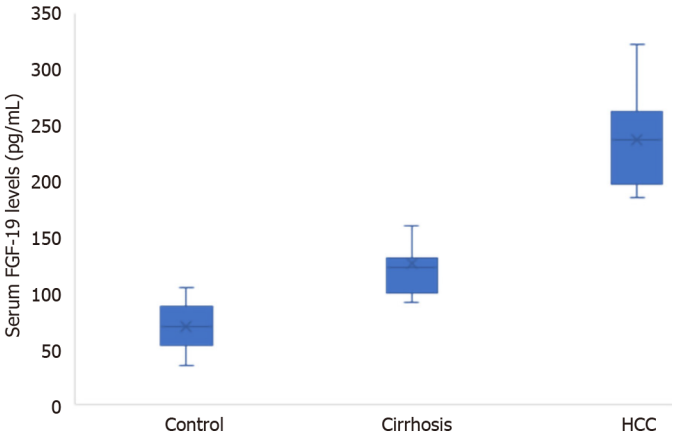

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Figure 1 Serum fibroblast growth factor 19 levels in the control, cirrhosis, and hepatocellular carcinoma groups. FGF-19: Fibroblast growth factor 19; HCC: Hepatocellular carcinoma.

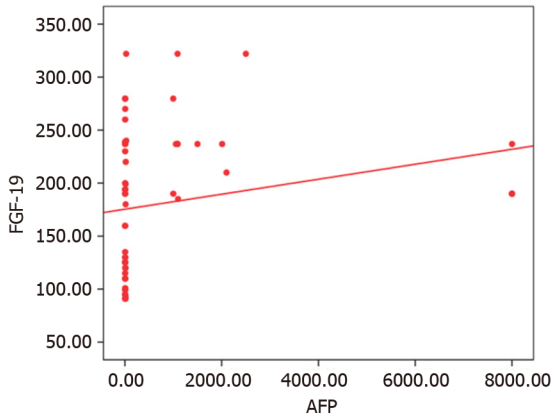

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Figure 2 Correlation between serum fibroblast growth factor 19 and alpha fetoprotein. AFP: Alpha fetoprotein; FGF-19: Fibroblast growth factor 19.

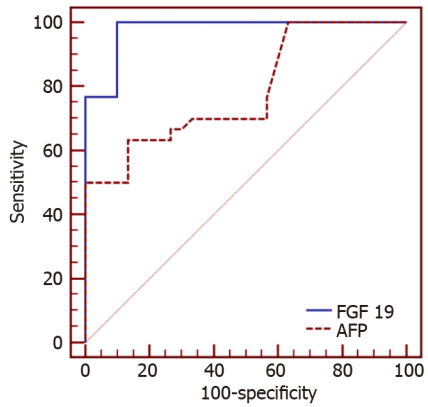

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Figure 3 Receiver operating characteristic curves for assessing the diagnostic performance of FGF-19 and alpha fetoprotein for the differentiation of HCC cases. AFP: Alpha fetoprotein; FGF-19: Fibroblast growth factor 19.

CONCLUSION

FGF-19 could be a possible novel non-invasive marker for HCC. It may improve the prognosis of HCC patients due to its utility in several aspects of HCC detection and management.

ARTICLE HIGHLIGHTS

Research background

Fibroblast growth factor 19 (FGF-19) is one of the founding members of the endocrine FGF subfamily. Recently, it has been the subject of much interest owing to its role in various physiological processes affecting glucose and lipid metabolism and bile acid secretion as well as cell proliferation, differentiation, and motility. Additionally, FGF-19 secretion in an autocrine style has reportedly contributed to cancer progression in various types of malignancies including hepatocellular carcinoma (HCC).

Research motivation

Tumour markers for HCC with a high sensitivity and specificity are necessary.

Research objectives

We aimed to estimate the serum FGF-19 concentrations in HCC cases and assess the diagnostic performance of FGF-19 for the detection of HCC.

Research methods

We recruited 90 adult participants and divided them into three equal groups: Healthy controls, cirrhosis patients, and HCC patients. Serum FGF-19 concentrations were measured using the Human FGF-19 ELISA kit.

Research results

We detected a high statistically significant difference in the FGF-19 levels between the three groups, with the highest level occurring in the HCC group, followed by the cirrhosis and control groups (236.44 ± 40.94 *vs* 125.63 ± 31.54 *vs* 69.60 ± 20.90 pg/mL, respectively, $P \leq 0.001$). For the detection of HCC, ROC curve analysis showed that FGF-19 produced a better diagnostic performance than alpha fetoprotein with an AUC of 0.98 *vs* 0.78.

Research conclusions

FGF-19 may be a possible novel non-invasive marker for HCC.

Research perspectives

FGF-19 could serve as a predictor of prognosis and a marker for follow-up after HCC treatment. Furthermore, the FGF-19 pathway may be a therapeutic target for the management of HCC.

FOOTNOTES

Author contributions: Mohamed GA, Nashaat EH, and ElGhandour AM designed the study; Fawzy HM participated in the acquisition of the data; Mohamed GA, Nashaat EH, Fawzy HM, and ElGhandour AM participated in the analysis and interpretation of the data; Mohamed GA, Nashaat EH, Fawzy HM, and ElGhandour AM revised the article critically for important intellectual content; Mohamed GA wrote the manuscript.

Institutional review board statement: The study was reviewed and approved by the institutional review board of Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Informed consent statement: Informed consent was obtained from every participant before the enrollment into the study.

Conflict-of-interest statement: All authors have nothing to disclose.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Effect of a specific *Escherichia coli Nissle 1917* strain on minimal/mild hepatic encephalopathy treatment

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Abstract

BACKGROUND

Hepatic encephalopathy (HE) can be considered a result of dysregulated gut-liver-brain axis function, where cognitive impairment can be reversed or prevented by the beneficial effects induced by "gut-centric" therapies, such as the administration of nonabsorbable disaccharides, nonabsorbable antibiotics, probiotics and prebiotics.

AIM

To assess the short-term efficacy and safety of the probiotic *Escherichia coli Nissle* (EcN) 1917 strain compared to lactulose and rifaximin in patients with minimal/mild HE.

METHODS

From January 2017 to March 2020, a total of 45 patients with HE were enrolled in this prospective, single-centre, open-label, randomized study. Participants were randomly assigned at a ratio of 1:1:1 to one of the treatment groups: The EcN group (n = 15), lactulose group (n = 15) or rifaximin group (n = 15) for a 1 mo

intervention period. The main primary outcomes of the study were changes in serum ammonia and Stroop test score. The secondary outcomes were markers of a chronic systemic inflammatory response (IL-6, IL-8, and IFN-γ) and bacteriology of the stool flora evaluated by specialized nonculture techniques after a 1 mo intervention period.

RESULTS

Patients who were given rifaximin or EcN showed a more significant reduction in serum ammonia and normalization of *Bifidobacteria* and *Lactobacilli* abundance compared to the lactulose group. However, the most pronounced restoration of the symbiotic microflora was associated with EcN administration and characterized by the absence of *E. coli* with altered properties and pathogenic enterobacteria in patient faeces. In the primary outcome analysis, improvements in the Stroop test parameters in all intervention groups were observed. Moreover, EcN-treated patients performed 15% faster on the Stroop test than the lactulose group patients (P = 0.017). Both EcN and rifaximin produced similar significant reductions in the proinflammatory cytokines INF-γ, IL-6 and IL-8. EcN was more efficient than lactulose in reducing proinflammatory cytokine levels.

CONCLUSION

The use of the probiotic EcN strain was safe and quite efficient for HE treatment. The probiotic reduced the ammonia content and the level of serum proinflammatory cytokines, normalized the gut microbiota composition and improved the cognitive function of patients with HE. The application of the EcN strain was more effective than lactulose treatment.

Key Words: Hepatic encephalopathy; Chronic liver disease; cirrhosis; Gut microbiota; *E. coli Nissle 1917*; Cognitive functions; Stroop test; Rifaximin; Lactulose

Core Tip: In a prospective, single-centre, open-label, randomized study, the short-term efficacy and safety of *Escherichia coli Nissle* (EcN) 1917 compared to that of lactulose and rifaximin in patients with hepatic encephalopathy were evaluated. The probiotic reduced the ammonia content and the level of serum proinflammatory cytokines, normalized the gut microbiota composition and improved the cognitive functions of patients with hepatic encephalopathy. The application of the EcN strain was more effective than lactulose treatment.

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INTRODUCTION

Non-alcoholic steatohepatitis is a major cause of liver cirrhosis and hepatocellular carcinoma; both primary indications for liver transplantation[1,2]. End-stage liver cirrhosis can lead to recurrent hepatic encephalopathy (HE). HE is a brain disorder caused by hepatocellular insufficiency and/or portosystemic shunting that manifests itself in a wide range of neurological or psychiatric disorders ranging from subclinical changes to coma[3]. HE, a challenging complication of advanced liver disease, occurs in approximately 30%-45% of patients with cirrhosis[4]. HE is classified using the West Haven criteria: Minimal (MHE), covert HE (grade I) or overt HE (OHE, grades II-IV)[5]. Numerous pathogenic factors contribute to the development of this disease[6].

Ammonia and mercaptans play a dominant role in the development of HE. Ammonia is formed from the nitrogen of nutrients in the intestine, primarily by the destruction of urea by urease, which is present in the colonial microflora[7]. Under normal conditions, ammonia is metabolized by the liver to urea, but under conditions of liver damage, urea can enter the systemic bloodstream and provoke nitrooxidative stress in the brain[8]. This process is accompanied by neurotransmission and cognitive function decline. Ammonia enhances the permeability of the blood-brain barrier by increasing the concentration of aromatic amino acids in brain tissues, in particular tryptophan, which leads to the synthesis of false neurotransmitters that replace real neurotransmitters (dopamine and norepinephrine) and thus interfere with normal neurotransmitters[9]. Decreased synthesis of physiological dopamine and norepinephrine

leads to inadequate neurotransmission and HE development[10]. False neurotransmitters not only can be synthesized in the central nervous system (CNS) the intestinal microflora is also a source[11]. When liver function is impaired or if there are portosystemic shunts, neurotransmitters enter the CNS, causing HE. Subsequent studies have provided some convincing evidence of the association of HE with intestinal dysbiosis. Thus, intraperitoneal administration of liposaccharides (LPS) in a mouse model of cirrhosis was associated with induction of precoma and worsening of cytotoxic cerebral oedema[12]. Moreover, small intestinal bacterial overgrowth (SIBO) is a common and increasingly recognized disorder in cirrhosis (30% to 73%)[13,14]. One of the most important predisposing factors of SIBO is small bowel dysmotility[15]. Multiple studies have shown that the presence of SIBO is strongly linked to the pathogenesis of HE[16,17]. Therefore, HE can be considered a result of dysregulated gut-liver-brain axis function, where cognitive impairment can be reversed or prevented by the beneficial effects induced by "gut-centric" therapies such as nonabsorbable disaccharides, nonabsorbable antibiotics, probiotics, prebiotics, and faecal microbiota transplantation (FMT)[18].

The treatment of choice is nonabsorbable disaccharides, such as lactulose and lactitol, which presumably acidify the stool and eradicate toxic metabolites[19]. However, treatment with lactulose is associated with nonserious (mainly gastrointestinal) adverse events such as diarrhoea[20], and one-third of these patients with HE do not respond to this standard treatment and have refractory HE[21]. Hence, newer drugs with effective improvement in HE and better side effect profiles are still being tested.

Regarding this aspect, probiotics modulating gut microbiota, and specifically those increasing urease-free strains to target ammonia production and absorption, may be considered important therapeutic options for HE patients, particularly in scenarios of noncompliance or intolerance to lactulose[22]. Probiotics are defined as live microorganisms promoted with claims that they provide health benefits when consumed in adequate amounts[23-25]. They are considered generally safe and may bring the health benefits claimed for them[26,27]. An early meta-analysis of the effects of pre-, pro-, or synbiotics that modulate the gut microbiota showed a significant improvement in MHE[22]. However, most of the assessed probiotics were limited to *Lactobacillus* or *Bifidobacterium* strains. The probiotic strain *Escherichia coli Nissle* 1917 (EcN), in contrast to a number of *Lactobacillus* or *Bifidobacterium* strains, stimulates the production of the anti-inflammatory cytokine interleukin (IL)-10[28]. Given certain metabolic processes of normal microflora and the features of the EcN strain, including short-chain fatty acid (SCFA) generation, bile acid metabolism, an increase in anti-inflammatory cytokines and a decrease in proinflammatory cytokines[29], their use may be effective for the treatment of HE in cirrhotic patients.

The aim of the present study was to assess the short-term efficacy and safety of probiotic EcN strains compared to lactulose and rifaximin in patients with mild (Stage 1-2) or MHE.

MATERIALS AND METHODS

Patient selection

This study was conducted at Bogomolets National Medical University between January 2017 and March 2020. A total of 45 patients with HE were enrolled in this prospective, single-centre, open-label, randomized study. The inclusion criteria were as follows: adult patients (age: 18-65 years) with cirrhosis diagnosed on the basis of liver biopsy, liver stiffness measurement or radiological study and the presence of minimal or mild (Grade 1-2) HE as defined by West Haven criteria; two or more documented episodes of HE in the last 6 mo, in addition to at least one episode in the last 3 mo; and a signed informed consent form. Patients were excluded if they had received L-ornithine-L-aspartate, zinc, metronidazole, neomycin, antibiotics, probiotics and yogurt consumption in the previous six weeks or if they had a history of allergy or intolerance to lactulose and/or rifaximin. The other exclusion criteria were neurologic diseases such as Alzheimer's disease, Parkinson's disease or nonhepatic metabolic encephalopathies, severe current disease (hepatic, renal, respiratory, or cardiovascular), pregnancy, any condition thought to be associated with poor compliance (*e.g.*, alcoholism or drug addiction) or any condition or circumstance that would, in the opinion of the investigator, prevent completion of the study or interfere with analysis of study results.

Study design

This prospective, open-label, single-centre, randomized clinical study compared probiotic EcN strains with lactulose and rifaximin treatment for 1 mo in patients with mild (Stage 1-2) or MHE. The 45 participants were randomly assigned at a ratio of 1:1:1 to one of the treatment groups using a computer-generated numeric sequence. The EcN group (n = 15) received probiotics (2,5-25·10^9 colony forming units - CFU/g) according to the scheme for the first 4 days, 1 capsule (QD), and then twice daily (BID) for 1 mo. Participants in the lactulose group (n = 15) received 30-60 mL in 2 or 3 divided doses so that the patient passed 2-3 semisoft stools per day for 1 month of the intervention period. The third group (rifaximin group, n = 15) was prescribed oral rifaximin 500 mg two times per day.

Patient compliance was evaluated by remnant pill counting and direct questions from an investigator after completion of the treatment. Compliance was defined as good when less than 15% of the pills were unconsumed at remnant pill counting. If it was found that a participant had missed > 15% of the

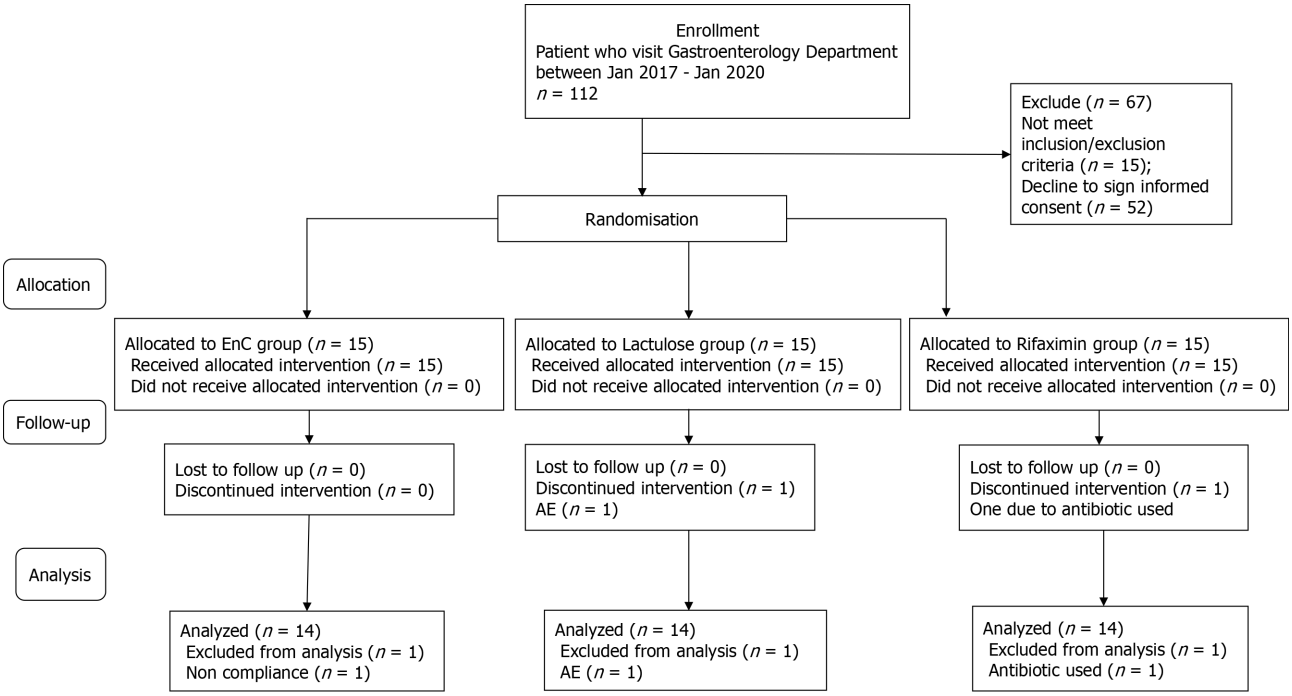

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Figure 1 Consolidated standards of reporting trials flow chart - trial protocol. AE: Adverse events.

suggested doses, the subject data were excluded from the final results. At the same time, all of the patients were asked about adverse events (AEs). In case of minor AEs, the participants had an opportunity either to continue or to cease taking the medication but nevertheless were asked to complete further visits. Patients who reported serious AEs caused by the intervention, such as diarrhoea, nausea/vomiting or sepsis; who underwent changes in previous therapy; or who had taken antibiotics other than rifaximin were not included in the final analysis.

The study protocol was approved by the Ethics Committee at Bogomolets National Medical University (protocol number: 106/2017) and was registered in the Clinical Trial.gov database under entry number NCT04787276.

Outcome measures and study procedures

After informed consent was signed, the patients provided samples of their blood serum in a fasting state, which were immediately frozen at -20 °C. Corresponding clinical and demographic data were gathered for each patient.

The main primary outcomes of the study were changes in serum ammonia and the Stroop test after a 1 mo intervention period. Cognitive functions were determined by the Stroop test[30] using the mobile application EncephalAppStroop. Each patient took the test on a smartphone twice (before and after treatment), and all results were recorded. The test consisted of two stages: without the Stroop-off effect and with the Stroop-on effect. At each stage, patients were presented with stimuli coloured red, blue, or green, and they were required to accurately label the colour. It was necessary to identify 10 stimuli in each stage of the test, and there were 5 total iterations in each stage. Before each stage, the program issued 2 training iterations. If the patient made a mistake, (i.e., pressed the wrong colour), the iteration was stopped and rebooted from the beginning, and the patient had to complete 5 iterations without error. In the Stroop-off stage, patients saw a neutral stimulus "###" on the screen in one of three colours and had to set the colour correctly. At the Stroop stage, patients saw the text stimuli, "RED", "BLUE", and "GREEN" on the screen, and each inscription could be in three possible colours (red, blue, or green), producing a total of 9 possible combinations. The patient had to evaluate the colour of the text without errors despite the written name of the colour. The stage with the Stroop effect is more complicated because there are more errors, and more time is needed to respond when the colour is not indicated by its name (for example, the word "red" is printed in blue-coloured font instead of red-coloured font). At the end of the test, the total time(s) required to complete the Stroop-off and Stroop-on stages was estimated.

The secondary outcomes of the study that were considered for investigating the efficiency of the intervention were markers of a chronic systemic inflammatory response (IL-6, IL-8, and IFN-γ) and bacteriology measured in the stool flora by specialized nonculture techniques.

All patients underwent bacteriological examination of faeces for dysbiosis. The percentage of patients in each group characterized by a decrease below the normal content of symbiotic bacteria *Bifidobacterium* (less than 10^7 CFU/g), *Lactobacilli* (less than 10^7 CFU/d), *E. coli* with normal properties (less than 10^6 CFU/d) and increase in the content of *E. coli* with altered properties (more than 10^6 CFU/g), pathogenic enterobacteria (not normally detected) and *Candida* (more than 10^4 CFU/d) was determined. Given that some patients were characterized by changes in one component of the microflora and others were within normal limits, we also determined the percentage of patients characterized by changes in the content of at least one of the representatives of microbiocenosis.

The serum levels of ammonia and cytokines were determined following a 12-h fasting period by the hospital clinical laboratory. Cytokine levels were determined (IL-6, IL-8, and IFNγ) using ELISA kits from Vector Best (Novosibirsk, Russia). The concentration of cytokines was calculated according to the calibration schedule and expressed in pg/mL.

Statistical analyses

Statistical analysis was performed using the standard software SPSS version 20.0 (SPSS, Inc., Chicago, Illinois) and GraphPad Prism, version 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Quantitative changes are presented as the mean and standard error (M ± SE), and qualitative changes are presented as percentages. To prove the normal distribution hypothesis, the Kolmogorov-Smirnov one-sample test was used. Data distribution was analysed using the Kolmogorov-Smirnov normality test. Variables with a parametric distribution were then analysed using one-way analysis of variance (ANOVA), and if the results were significant, a Tukey post hoc test was performed. Data with a nonparametric distribution were analysed using the Kruskal-Wallis test. To compare the data in the same patients before and after treatment, Student's t-test for dependent samples was employed. The χ-square test was used to assess differences between categorical data. Differences between groups were considered significant at a value of $P < 0.05$.

RESULTS

Patient characteristics

Recruitment started in January 2017 and continued until January 2020. For enrolment, the patient database of the Gastroenterology Department was used. For primary analysis, 112 patients were selected. After careful consideration for compliance with the inclusion/exclusion criteria, 15 patients were not eligible. The main reasons were the previous use of agents that can impact gut microbiota composition and overt HE (grades III-IV) as defined by West Haven criteria. A face-to-face conversation was held with all other potential participants explaining the main study criteria, purpose and methodology. After consideration of the proposal, 52 patients refused to give their informed consent. At the end of the enrolment period, with possible bias adjustment, 45 patients with HE were chosen to be included in the study. All patients were equally distributed in a random order to take the intervention for 1 mo. A CONSORT flow chart with a general protocol schedule is shown in Figure 1.

Of the 45 patients, 43 (95.5%) completed their allocated regimens. The remaining 2 patients (4.5%) were excluded from the study analysis. One patient from the lactulose group permanently discontinued participation because of diarrhoea. After AE onset, the lactulose dosage was lowered to 10 mL following 5 mL two times a day, but the event did not resolve and led to the patient's discontinuation. Another patient from the rifaximin group had been treated with antibiotics. One patient from the EcN group was excluded from the analysis due to noncompliance, as this participant received less than 85% of the prescribed intervention. Therefore, the data from 42 (93.3%) study participants were included in the final per-protocol analysis (Figure 1).

The average patient age was 48.95 ± 6.51 years, and the HE duration ranged from 5 to 12 years. Of these patients, 33.3% of patients exhibited grade I, 26.2% exhibited grade II and 40.5% exhibited MHE according to the West Haven criteria. The baseline demographic and clinical characteristics of the enrolled patients did not significantly differ between groups (Table 1).

Primary outcome analysis

After treatment with lactulose, the concentration of ammonia decreased by 11.8% ($P < 0.05$) (Figure 1). Patients who were given rifaximin or the EcN probiotic strain showed a more significant reduction in ammonia than after lactulose. In the rifaximin group, the ammonia content decreased by 35.9% ($P < 0.05$) after treatment and by 21.5% ($P < 0.05$) compared to the level of ammonia in patients receiving lactulose (Figure 2). The rate of ammonia reduction in the EcN group was 38.5% ($P < 0.05$). Moreover, the obtained data indicate that the therapeutic use of EcN was almost 30% more effective than lactulose (Figure 2).

Cognitive impairment in terms of primary outcome analysis was assessed separately in patients with HE according to the Stroop test, which was divided into two stages. In the first and simpler stage (Stroop off), the mobile application was presented to patients with a text stimulus "###" in one of three possible colours (red, blue, or green), and the patient had to accurately assess the colour. The total time

Table 1 Baseline clinical parameters in examined patients (mean ± SE or %)

	Lactulose group	Rifaximin group	EcN group	*P* value[1]
Age, yr	48.92 ± 1.64	49.07 ± 1.76	48.85 ± 1.93	0.996
Male, % (*n*)	78.6 (11)	78.6 (11)	71.4 (10)	0.877
Etiology of cirrhosis				
HCV, % (*n*)	57.1 (8)	42.9 (6)	50.0 (7)	0.940
Alcoholism, % (*n*)	21.4 (3)	35.7 (5)	28.6 (4)	
Mixed, % (*n*)	21.4 (3)	21.4 (3)	21.4 (3)	
Cirrhosis duration, years	8.14 ± 0.61	8.00 ± 0.61	8.07 ± 0.60	0.986
Time to progression from hepatitis to cirrhosis, years	4.00 ± 0.41	3.42 ± 0.38	3.56 ± 0.32	0.468
Child-pugh score				
A, % (*n*)	35.7 (5)	42.9 (6)	28.6 (4)	0.733
B, % (*n*)	64.3 (9)	57.1 (8)	71.4 (10)	
HE grade				
MHE, % (*n*)	42.9 (6)	35.7 (5)	42.9 (6)	0.979
Grade 1, % (*n*)	35.7 (5)	35.7 (5)	28.6 (4)	
Grade 2, % (*n*)	21.4 (3)	28.6 (4)	28.6 (4)	

[1]The difference between all study groups calculated using one-way ANOVA or χ^2 test for categorical data. HE: Hepatic encephalopathy; MHE: Minimal HE; EcN: *Escherichia coli Nissle*.

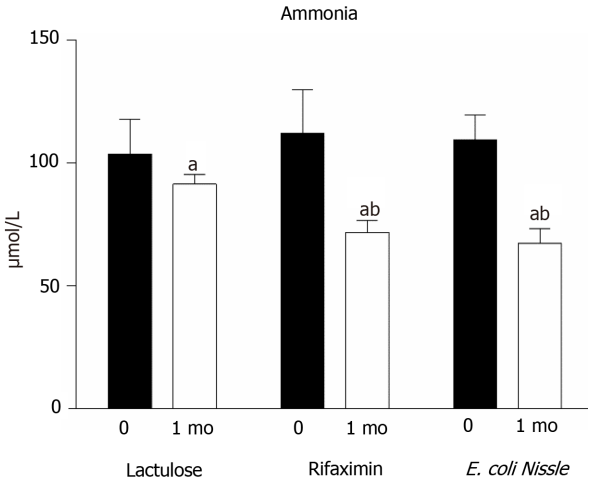

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Figure 2 Serum ammonia concentration in patients with hepatic encephalopathy receiving treatment with lactulose, rifaximin and probiotics *E.coli Nissle 1917* (*n* = 14 in each group). [a]*P* < 0.05 as compared to pre-treatment levels; [b]*P* < 0.05 as compared to the lactulose treatment. *E. coli*: *Escherichia coli*.

of correct determination of 10 presented stimuli was recorded over five iterations (i.e., the total number of responses was 50). It has been shown that the test time for patients with HE exceeded the test time of healthy people by almost 2 times, so if a healthy person correctly determined the colour of 10 text characters in an average of less than 20 s, most patients with HE needed more than 20-30 s to pass the test (the time for 5 test solutions was 160 ± 10 s, respectively).

HE treatment significantly improved patients' cognitive abilities. Under the conditions of lactulose administration, the time required to resolve the Stroop-off test was reduced by 14.9% (P = 0.028), after treatment with rifaximin by 19.0% (P = 0.001), and in EcN by 28.7% (P < 0.001). The efficiency of probiotics in restoring mental performance was higher than that of lactulose (Figure 3A).

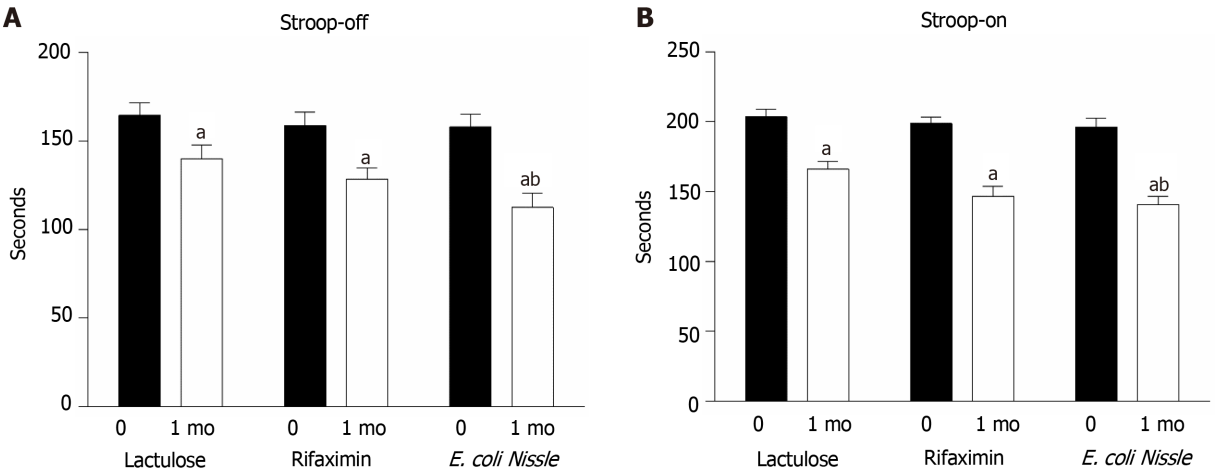

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Figure 3 The total time of evaluation of stimuli in Stroop test in patients with hepatic encephalopathy receiving treatment with lactulose, rifaximin and probiotics *E.coli Nissle 1917* (*n* = 14 in each group). A: Stroop-off; B: Stroop-on. [a]*P* < 0.05 as compared to pre-treatment levels; [b]*P* < 0.05 as compared to the lactulose treatment. *E. coli: Escherichia coli*.

In the second stage (Stroop on, with the Stroop effect), the program presented one of the three possible text stimuli "RED", "BLUE", "GREEN" in three possible colours (red, blue, or green), i.e., there were a total of 9 possible combinations, and the patient had to accurately assess the colour of the text regardless of its signage. The difficulty of this stage lies in the need to match the colour correctly while ignoring the name of the colour, so the total time to pass this test was slightly longer than that of the Stroop-off stage.

Patients with HE had a correct response rate 2 times lower than that of healthy people. The rate of Stroop's test was increased in all intervention groups: For lactulose from 203.71 ± 5.33 to 166.07 ± 5.39 (P < 0.001), for rifaximin from 198.93 ± 4.43 to 146.86 ± 7.09% (P < 0.001) and for EcN from 196.43 ± 6.25 to 140.71 ± 6.07% (P < 0.001) seconds after treatment (Figure 3B). However, complete recovery of cognitive function was not recorded for all patients. It should be noted that the efficacy of the probiotic compared to lactulose was noted according to the results of the second stage. Patients who were prescribed EcN completed the test 15% faster (P = 0.017) than the lactulose group (Figure 3B).

Secondary outcome analysis

Along with liver dysfunction, patients were diagnosed with gut dysbiotic disorders. More than 85% of patients in all groups were characterized by changes in at least one group of normoflora (Table 1). The content of *Bifidobacteria* and *Lactobacilli* was less than 10^7 CFU/g in more than 70% and 57% of patients, respectively (Table 1). Approximately 30% of patients had a reduced content of *Escherichia coli* with normal properties and an increased content of bacteria with altered properties. Pathogenic enterobacteria were detected in 35.7% of each group of patients with HE, and *Candida* were found in almost half of the patients (Table 2).

With lactulose application, the percentage of patients with dysbiotic disorders of *Bifidobacteria* and *Lactobacilli* significantly decreased. Significant improvement of other microflora indicators in this group was not registered. In the rifaximin group, normalization of *Bifidobacteria* and *Lactobacilli* was observed in 28.6% (P < 0.05) and 21.4% (P < 0.05), respectively. There was also a decrease in the number of patients with increased levels of *Escherichia coli*, pathogenic enterobacteria and *Candida*. The most pronounced restoration of the symbiotic microflora was found in the EcN group. Normalization of *Bifidobacteria* abundance was registered in 57.1% (P < 0.05) of patients, and *Lactobacilli* was registered in 35.7% (P < 0.05). After EcN treatment, *E. coli* with altered properties or pathogenic enterobacteria was not detected in any of the patients, and only one patient exhibited an increase in the content of yeast-like fungi (Table 2).

Along with liver damage, the intensification of inflammatory processes was recorded for all patients. This indicator was confirmed by an increase in the concentration of proinflammatory cytokines in the blood: IL-6, IL-8 and INF-γ were observed at frequencies 2-10 times higher than normal. For the group of patients treated with lactulose, the contents of proinflammatory INF-γ and IL-8 did not change significantly after treatment, but there was a decrease in the level of IL-6 from 9.63 ± 1.12 to 7.02 ± 1.09 (P = 0.019) pg/mL compared to the baseline level (Figure 4).

The use of rifaximin led to a significant reduction in the concentrations of serum INF-γ (11.74 ± 1.68 *vs* 8.86 ± 0.71 pg/mL; p=0.049), IL-6 (8.9 ± 0.98 *vs* 4.28 ± 0.59 pg/mL; P < 0.001) and IL-8 (82.95 ± 7.6 *vs* 63.02 ± 5.03 pg/mL; p=0.026) after treatment (Figure 4). For patients treated with probiotics, the reduction in inflammatory processes did not differ significantly from the effects of rifaximin. Thus,

Table 2 The percentage of patients with hepatic encephalopathy and concomitant changes in the microflora under treatment with lactulose, rifaximin and EcN (*n* = 14 in each group)

Group of microflora	Percentage of patients with dysbiotic disorders, %					
	Lactulose group		Rifaximin group		EcN group	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Bifidobacteria	78.6	57.1[a]	71.4	42.9[a]	85.7	28.6[a,b]
Lactobacilli	64.3	42.9[a]	57.1	35.7[a]	57.1	21.4[a,b]
E.coli with normal properties	28.6	28.6	35.7	28.6	35.7	7.1[a,b]
E.coli with altered properties	28.6	21.4	28.6	14.3[a]	28.6	0.0[a,b]
Pathogenic enterobacteria	35.7	28.6	35.7	21.4[a]	35.7	0.0[a,b]
Candida	42.9	35.7	50.0	28.6[a]	50.0	7.1[a,b]
A change in at least one group of microorganisms was revealed	85.7	71.4[a]	92.9	64.3[a]	92.9	28.6[a,b]

[a]$P < 0.05$ as compared to pre-treatment levels.
[b]$P < 0.05$ as compared to the lactulose treatment.

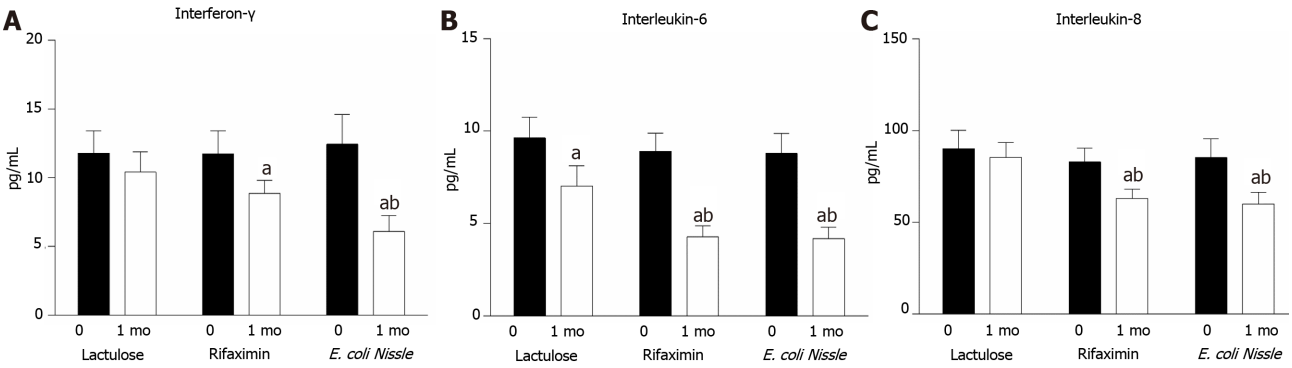

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Figure 4 Serum cytokine levels in patients with HE receiving treatment with lactulose, rifaximin and probiotics *E.coli Nissle 1917* (*n* = 14 in each group). A: INF-γ; B: IL-6; C: IL-8. [a]$P < 0.05$ as compared to pre-treatment levels; [b]$P < 0.05$ as compared to the lactulose treatment. *E. coli: Escherichia coli.*

along with EcN use, the level of INF-γ decreased by 51.0% ($P = 0.005$), IL-6 decreased by 52.3% ($P = 0.001$) and IL-8 decreased by 29.6% ($P = 0.007$) compared to the baseline value (Figure 4). By comparing the efficacy of EcN and lactulose in the treatment of HE, one can affirm the stronger anti-inflammatory properties of the probiotic, which is 20% more efficient compared to lactulose in reducing the level of the studied proinflammatory cytokines.

Adverse events

There were no reported adverse events or side effects derived from the intervention across patient included to final per protocol analysis, this was evidenced by the fact that there were no changes in biochemical tests at the end of the intervention in either group.

DISCUSSION

Microbiota dysbiosis and chronic systemic inflammation are among the risk factors for the onset and progression of pathologies such as obesity, nonalcoholic fatty liver disease and liver cirrhosis[31], but changes in the intestinal microflora and inflammation in patients with HE have not been adequately studied[32]. As a result, there is an evident need to determine the impact of chronic inflammation and microflora on the epithelium of the intestinal wall, which can also affect the development of HE in people with liver disease[33]. The reduced detoxification function of the microbiota in intestinal dysbiosis increases the load on the enzymatic systems of the liver, which aggravates its metabolic and

structural changes[34]. In addition, patients with liver cirrhosis showed a positive correlation between *Porphyromonadaceae* and *Alcaligenaceae* as well as low expressiveness of cognitive tests. These observations serve as additional confirmation that the increase in ammonia concentration is associated not only with liver dysfunction. Bajaj *et al* showed an increase in the content of ammonia-producing bacteria *Alcaligenaceae* in the intestine under HE conditions[35]. Successful recovery of the microflora can significantly reduce the activity of bacterial urease, absorption of ammonia in the intestine and the intensity of inflammatory processes and endotoxaemia, which is due to reduced absorption of toxins, including indoles, oxindoles, phenols and mercaptans[34]. Therefore, current strategies of HE treatment must also affect the intestinal microbiota.

Lactulose (4-O-β-galactopyranosyl-D-fructose) is widely used in the treatment of HE. It reduces pH levels in the intestine as a result of SCFA formation, creating conditions for the growth of acid-resistant *Lactobacteria* and *Bifidobacteria* that do not express the enzyme urease[7,36]. The literature regarding the effects of lactulose on the composition of microflora is quite contradictory. In contrast to reports on the restoration of indigenous microflora (*Lactobacillaceae*) under the influence of lactulose, Bajaj *et al* (2014) demonstrated intestinal dysbiosis and a decrease in the ratio between autochthonous and non-autochthonous bacteria with a high content of gram-positive bacteria *Enterobacteriaceae* and *Bacteroidaceae* despite treatment with lactulose[37].

Rifaximin is an antibiotic that is not absorbed in the gut and causes a mild change in the intestinal microflora, increasing the presence of beneficial species but without affecting the overall ratio of bacteria [38]. This modulating effect on the composition of the intestinal flora partly explains the clinical efficacy of rifaximin in reducing endotoxaemia and inflammatory markers that contribute to HE progression [39].

The data obtained in the current study show that the efficacy of lactulose as a gut microbiota recovery agent is not high enough, which is consistent with other works in which the efficacy of lactulose was not detected[40,41]. In contrast, treatment with rifaximin or EcN led to normalization of *Bifidobacteria* and *Lactobacilli* abundance. However, the most pronounced restoration of the symbiotic microflora was associated with EcN administration and characterized by the absence of *E. coli* with altered properties and pathogenic enterobacteria in patient features.

To our knowledge, the current study represents the first comparative analysis of the short-term efficacy of probiotic EcN strains to lactulose and rifaximin in patients with HE. One of the early RCTs failed to improve several combination tests, which showed extended reaction times in patients with MHE after treatment with EcN compared to placebo[42]. However, EcN treatment significantly improved intestinal colonization ($P < 0.001$) and tended to reduce endotoxin levels significantly on day 42 ($P = 0.07$)[42]. In contrast, our study showed the improvement of Stroop test parameters in all intervention groups after treatment. However, complete recovery of cognitive function was not recorded for all patients. Moreover, parallel with the positive shift in gut microbiota composition, patients who were prescribed EcN compared to the lactulose group completed the Stroop test 15% faster ($P = 0.017$).

Systemic inflammation also plays an important role in the pathogenesis of HE. Today, accumulated data suggest that the level of cytokines is not only an indicator of inflammation in chronic liver disease and PE but is a separate aetiological factor of this pathology. Systemic inflammation and neuroinflammation are communicated by peripheral tissues, which transmit signals to the brain through the activation of afferent fibres of the vagus and vascular endothelium. The blood-brain barrier (BBB) transmits signals to the brain through the formation of secondary mediators (NO and prostanoids) in response to cytokine stimulation. Cytokines increase the permeability of the BBB and directly penetrate the brain in areas of BBB disorders, where they cause the activation of microglia and the expression of proinflammatory mediator genes[43,44].

Probiotics increase anti-inflammatory cytokines and decrease proinflammatory cytokines in the blood [45]. Bacterial products have a significant effect on the intestinal-liver-brain axis as well as local and systemic immunity. Immunomodulatory activity is also indicated for SCFAs formed by the bacterial fermentation of polysaccharides. Most lactic acid bacteria in the human body are members of the genera *Lactobacillus, Bifidobacterium, Propionobacterium, Streptococcus*, and obligate or facultative anaerobes. These types of bacteria process carbohydrates in the intestinal lumen with the formation of SCFAs: acetic, propionic, dairy, oily, γ-oxy-oily and valerian. SCFAs play a leading role in the physiology of the large intestine, representing the main pool of anions in its lumen. SCFAs activate nerve cells by interacting with receptors associated with the G-proteins GPR41 and GPR43[46]. As recently demonstrated, SCFAs regulate the synthesis of serotonin, which is formed by enterochromaffin cells of the intestine and constitutes 95% of the body's serotonin[47]. Today, a reliable link between serotonin of intestinal origin and brain function, in particular in HE[48], may be another mechanism of communication between modulation of the microflora and disease progression.

Strain *E. coli Nissle 1917*, with the help of special adhesive organelles (type F-1A, F-1C and shaped fimbriae), has the ability to join the mucous membrane of the large intestine and organize microcolonies, forming biofilms[49]. They are also mobile because of the presence of flagella, which gives them the advantage of colonizing the colon. Thus, these bacteria have also been shown to enhance the mucosal barrier by interacting with immunomodulatory and anti-inflammatory mechanisms[49]. *E. coli* Nissle inhibits the growth of gram-negative anaerobic bacteria by secreting antimicrobial substances

(microcins) and siderophores, which capture iron and thus prevent the growth of a certain pathological bacterial strain[28].

The parameters of chronic systemic inflammation in the current study were assessed in secondary outcome analysis. Both EcN and rifaximin showed similar significant reductions in the proinflammatory cytokines INF-γ, IL-6 and IL-8 compared to baseline levels. By comparing the efficacy of EcN and lactulose in the treatment of HE, one can affirm the stronger anti-inflammatory properties of the probiotic, which is 20% more efficacious than lactulose in reducing proinflammatory cytokine levels.

New research on the beneficial effects of gut microbiota modulation and related mechanisms of their interaction with liver disease should be conducted to target better a wide variety of probiotic strains. Moreover, one of the possible gut microbiota-based interventions that may be claimed in the nearest future is FMT. Preliminary data on the possible beneficial effect of FMT find support in both animal[50] and small clinical case series[51,52]. Additionally, several randomized clinical trials are actively recruiting (NCT02862249, NCT03796598, and NCT03439982) patients with HE and cirrhosis to test the efficacy of FMT.

CONCLUSION

To summarize the described results, it can be argued that the use of the probiotic EcN strain was safe and quite efficacious for HE treatment. The probiotic reduced the ammonia content and the level of serum proinflammatory cytokines, normalized the gut microbiota composition and improved the cognitive function of patients with HE. The application of the EcN strain was more efficacious than lactulose treatment.

ARTICLE HIGHLIGHTS

Research background

Hepatic encephalopathy (HE) can be considered a result of dysregulated gut-liver-brain axis function, where cognitive impairment can be reversed or prevented by the beneficial effects induced by "gut-centric" therapies, such as the administration of nonabsorbable disaccharides, nonabsorbable antibiotics, probiotics and prebiotics.

Research motivation

The HE treatment of choice is non-absorbable disaccharides, such as lactulose and lactitol. Non-absorbable disaccharides like lactulose are associated with non-serious (mainly gastrointestinal) adverse events like diarrhea and bloating, hence, due to the side effect profile, newer drugs continue to be tested for treatment of HE. Rifaximin is an antibiotic which modulating effect on the composition of the intestinal flora partly explains the clinical efficacy in reducing endotoxaemia and inflammatory markers that contribute to HE progression. Probiotics are effective in the treatment of minimal hepatic encephalopathy. Various studies have shown some improvement in either the prevalence of minimal hepatic encephalopathy or results in neuropsychological tests with the use of probiotics.

Research objectives

To assess the short-term efficacy and safety of the probiotic *Escherichia coli Nissle* 1917 (EcN) strain compared to lactulose and rifaximin in patients with minimal/mild HE.

Research methods

In total, 45 patients with HE were enrolled in this prospective, single-centre, open-label, randomized study. Participants were randomly assigned at a ratio of 1:1:1 to one of the treatment groups: the EcN group (n = 15), lactulose group (n = 15) or rifaximin group (n = 15) for a 1 mo intervention period. The main primary outcomes of the study were changes in serum ammonia and Stroop test score. The secondary outcomes were markers of a chronic systemic inflammatory response (IL-6, IL-8, and IFN-γ) and bacteriology of the stool flora evaluated by specialized nonculture techniques after a 1 mo intervention period.

Research results

Rifaximin or EcN showed a more significant reduction in serum ammonia and normalization of *Bifidobacteria* and *Lactobacilli* abundance compared to the lactulose group. In the primary outcome analysis, improvements in the Stroop test parameters in all intervention groups were observed. Moreover, EcN-treated patients performed 15% faster on the Stroop test than the lactulose group patients (P = 0.017). Both EcN and rifaximin produced similar significant reductions in the proinflammatory cytokines INF-γ, IL-6 and IL-8.

Research conclusions

Probiotic EcN strain was safe and quite efficient for HE treatment. The probiotic reduced the ammonia content and the level of serum proinflammatory cytokines, normalized the gut microbiota composition and improved the cognitive function of patients with HE. The application of the EcN strain was more effective than lactulose treatment.

Research perspectives

New research on the beneficial effects of gut microbiota modulation and related mechanisms of their interaction with liver disease should be conducted to target better a wide variety of probiotic strains. Moreover, one of the possible gut microbiota-based interventions that may be claimed in the nearest future is fecal microbiota transplantation.

FOOTNOTES

Author contributions: Manzhalii E, Moyseyenko V and Kondratiuk V were responsible for the study conception and design, data analysis and interpretation, and manuscript drafting; Molochek N, Falalyeyeva T and Kobyliak N critically revised the article for important intellectual content; all the authors reviewed and approved the final version to be published.

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