ISSN 1948-5182 (online)

World Journal of *Hepatology*

World J Hepatol 2016 December 8; 8(34): 1489-1540

Published by Baishideng Publishing Group Inc

World Journal of Hepatology

A peer-reviewed, online, open-access journal of hepatology

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2014-2017

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World Journal of Hepatology

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Three issues per month Volume 8 Number 34 December 8, 2016

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World Journal of Hepatology
Volume 8 Number 34 December 8, 2016

AIM AND SCOPE

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

WJH covers topics concerning liver biology/pathology, cirrhosis and its complications, liver fibrosis, liver failure, portal hypertension, hepatitis B and C and inflammatory disorders, steatohepatitis and metabolic liver disease, hepatocellular carcinoma, biliary tract disease, autoimmune disease, cholestatic and biliary disease, transplantation, genetics, epidemiology, microbiology, molecular and cell biology, nutrition, geriatric and pediatric hepatology, diagnosis and screening, endoscopy, imaging, and advanced technology. Priority publication will be given to articles concerning diagnosis and treatment of hepatology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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INDEXING/ABSTRACTING

World Journal of Hepatology is now indexed in PubMed, PubMed Central, and Scopus.

NAME OF JOURNAL
World Journal of Hepatology

ISSN
ISSN 1948-5182 (online)

LAUNCH DATE
October 31, 2009

FREQUENCY
36 Issues/Year (8th, 18th, and 28th of each month)

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World Journal of Hepatology
Baishideng Publishing Group Inc
8226 Regency Drive, Pleasanton, CA 94588, USA
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Fax: +1-925-2238243
E-mail: editorialoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
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PUBLICATION DATE
December 8, 2016

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World Journal of Hepatology

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DOI: 10.4254/wjh.v8.i34.1489

World J Hepatol 2016 December 8; 8(34): 1489-1496
ISSN 1948-5182 (online)

Role of nitric oxide in liver transplantation: Should it be routinely used?

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Author contributions: Both authors contributed to the manuscript.

Conflict-of-interest statement: No relevant conflicts of interest were declared for each author.

Manuscript source: Invited manuscript

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Received: April 29, 2016
Peer-review started: May 4, 2016
First decision: July 4, 2016
Revised: August 6, 2016
Accepted: October 17, 2016
Article in press: October 18, 2016
Published online: December 8, 2016

Abstract

Ischemia-reperfusion injury (IRI) continues to be a major contributor to graft dysfunction, thus supporting the need for therapeutic strategies focused on minimizing organ damage especially with growing numbers of extended criteria grafts being utilized which are more vulnerable to cold and warm ischemia. Nitric oxide (NO·) is highly reactive gaseous molecule found in air and regarded as a pollutant. Not surprising, it is extremely bioactive, and has been demonstrated to play major roles in vascular homeostasis, neurotransmission, and host defense inflammatory reactions. Under conditions of ischemia, NO· has consistently been demonstrated to enhance microcirculatory vasorelaxation and mitigate pro-inflammatory responses, making it an excellent strategy for patients undergoing organ transplantation. Clinical studies designed to test this hypothesis have yielded very promising results that includes reduced hepatocellular injury and enhanced graft recovery without any identifiable complications. By what means NO· facilitates extra-pulmonary actions is up for debate and speculation. The general premise is that they are NO· containing intermediates in the circulation, that ultimately mediate either direct or indirect effects. A plethora of data exists explaining how NO·-containing intermediate molecules form in the plasma as S-nitrosothiols (*e.g.*, S-nitrosoalbumin), whereas other compelling data suggest nitrite to be a protective mediator. In this article, we discuss the use of inhaled NO· as a way to protect the donor liver graft against IRI in patients undergoing liver transplantation.

Key words: Liver; Nitric oxide; Ischemia-reperfusion; Nitrite; Transplantation

Core tip: Our manuscript assesses the basic and clinical literature of nitric oxide and liver transplantation and creates a scientific/clinical justification for its routine use.

Fukazawa K, Lang JD. Role of nitric oxide in liver trans-

plantation: Should it be routinely used? *World J Hepatol* 2016; 8(34): 1489-1496 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1489.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1489

INTRODUCTION

Liver transplantation has become a viable treatment option for recipients suffering from irreversible liver failure for more than three decades. However, the number of recipients on the waiting list continues to grow due to the major mismatch between organ supply and demand, creating tremendous pressure on for the development of techniques to expand the donor pool. There is about 7000 liver transplants performed annually with a trend that in increasing due to demand. As a result, according to national transplant registry database from the Organ Procurement and Transplant Network, about 1000 potential recipients on the waiting list die annually (Figure 1). Therefore, strategies are actively being sought to increase in donor pool. The transplant community has evaluated an option to relax the standard for donors to include donors with suboptimal quality [more damage from anoxic preservation and ischemia-reperfusion injury (IRI)], including the advanced age donor, prolonged cold and warm ischemia time, and hepatic steatosis. Currently, extended criteria donors make up 5%-10% of all donors and this number is increasing. IRI to the liver remains a significant contributor to graft dysfunction, or primary non-function, resulting in increased intensive care unit and hospital stay, increase financial burden, re-transplantation and, in a worst case scenario, death[1].

Nitric oxide (NO·) is an important endogenously produced biological mediator affecting vascular function, metabolic function and host defense mechanisms[2]. It is produced by macrophages, dendritic cells and plays a critical role in host innate and adaptive immunological processes[3,4]. Inhaled NO· has been clinically used to treat pulmonary hypertension due to its vasodilating effect in pulmonary microcirculation without causing any unfavorable systemic hemodynamic changes. More recent evidence has suggested a relative NO· deficiency due to IRI and that the use of preemptive inhaled NO· can attenuate liver IRI during liver transplantation.

ENDOGENOUS NO· AND THE LIVER DURING ISCHEMIA-REPERFUSION

Liver graft injury from ischemia-reperfusion is the principal mechanism of liver injury related to procedures involving clamping of hepatic inflow such as hepatectomy and liver transplantation. Cessation of oxygen delivery to sinusoidal microcirculation causes severe ATP depletion which leads to retraction of sinusoidal endothelial cells and Kuppfer cells, bleb formation in the microcirculation[5]. There is obstruction of sinusoid and microcirculatory disturbance due to bleb formation as well as accumulation of leukocytes and platelets leads to prolonged ischemia of hepatocytes, so-called, "no-flow phenomenon". In addition, due to the upregulation and generation of inflammatory mediators such as oxygen free radicals, cytokines and chemokines from the Kuppfer cells occupying sinusoid there is both a local and systemic inflammatory response after reperfusion[6]. Therefore, IRI of the liver is generally believed to cause severe hepatocellular injury as well as extrahepatic organ inflammation and injury that contributes to perioperative morbidity and mortality.

Reductions of NO· during ischemia-reperfusion of liver aggregates liver injury in both animals and humans[7,8]. In fact, decreased hepatic production of NO· from endothelial nitric oxide synthase or endothelial nitric oxide synthase (eNOS) (responsible for the constitutive production of NO·) within 60 min after reperfusion in human liver transplantation contributes to the ischemia-reperfusion graft injury[8]. In addition to reduced production, NO· is inactivated from the reactions with reactive oxygen species, such as superoxide radical leading to reduced bioavailability[9,10]. As a consequence, reduced NO· bioavailability leads to apoptosis, leukocyte adhesion, increase microcirculatory resistance, and mitochondrial dysfunction[10]. Not surprisingly, the restoration of NO· concentrations lessens liver ischemia injury *via* reversing the most of the previously mentioned adverse actions. Additional studies support the finding that eNOS is essential for reduction liver graft injury during liver ischemia-reperfusion. Injury to the liver was decreased in the wild type when compared to mice where eNOS was knocked out. When eNOS expression was exogenously increased or NO· donors enhanced protection was realized[11,12]. In addition, established NO· concentrations resulting from inflammation are generally greater due to more robust inducible nitric oxide synthase (iNOS) expression. At the present time the role of iNOS in liver protection is not well known. In a rat liver ischemia-reperfusion model, iNOS enzyme activity was significantly increased in parallel with increased iNOS mRNA expression after reperfusion, which suggests that induction of iNOS has an important role in liver ischemia-reperfusion[13]. Counter to this observation, in a porcine ischemia-reperfusion model, is that portal injection of aminoguanidine, a selective iNOS inhibitor, decreased IRI[14]. Additionally, when iNOS was knocked out in mice and then exposed to warm liver ischemia-reperfusion, they incurred more injury when compared to wild types. While the injury was greater in the iNOS deficient animals, iNOS mRNA was also undetectable in the wild types. While iNOS is crucial in increasing net NO· concentrations and contributing to liver injury resulting from ischemia-reperfusion, further work is needed.

Additional hepatoprotective studies thought to due to endogenous NO· production have been published. Nitric oxide-mediated protection has been shown to inhibit apoptosis depending on concentration *via* inhibition

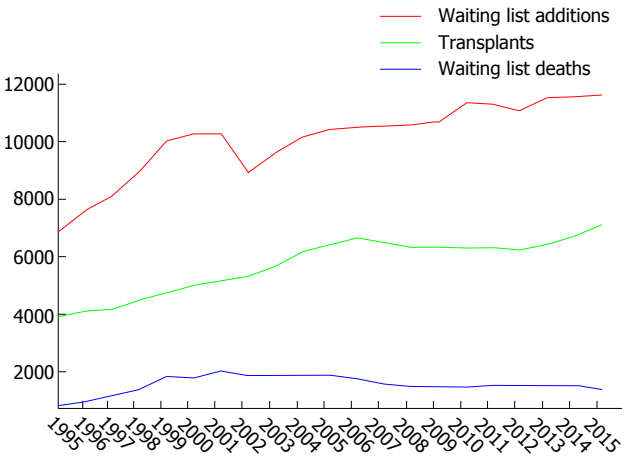

Figure 1 Number of transplants, waiting list additions and waiting list deaths in the United States between 1995 and 2015. Number of waiting list additions and deaths are based on the candidates and candidate who is listed more than one place is counted as one candidate. Data are available from: URL: https://optn.transplant.hrsa.gov/data/.

of caspase proteases *via* S-nitrosylation[15] (Figure 2). Reduced compartmental concentrations of NO· inhibits apoptosis while increasing concentrations yields toxic reactive nitrogen species such as peroxynitrite or oxygen radicals that have shown to cause apoptosis and necrosis[16]. Other proposed mechanisms of how NO· protects include down-regulation of nuclear factor kappa B[17], mitochondrial complex I inhibition that is reversible, and reductions in mitochondrial calcium accumulation[18]. Controversy continues in regards to how NO· may mitigate inflammation and injury. In fact, Jaeschke *et al*[19] demonstrated that NOS inhibition did not contribute to hepatic injury at the time of reperfusion. Additionally, the inhibition of NO· did not influence neutrophil function as related to migration or adhesion[20]. Nonetheless, most evidence whether lab-based or clinical, demonstrates favorable effects of NO· during liver ischemia-reperfusion. It is difficult to reconcile the results, but no doubt diversity exists in the role of NO· in different cell types, as well as differing cellular compartmental NO· concentrations, timing of administration, and duration of NO· exposure. Also laboratory methods applied may have some role on this conflicting results.

IMPACT OF EXOGENOUS NO· DELIVERY IN ATTENUATING LIVER IRI

Administration of inhaled NO· has demonstrated efficacy both in animal and human studies[21-25]. NO· inhalation decreases pulmonary and systemic vascular resistance with resultant improvements in tissue oxygenation increases in renal blood flow, and glomerular filtration rate[26-28]. Moreover, inhaled NO· has been demonstrated to exert extra-pulmonary or peripheral effects to the microvasculature as measured by enhanced perfusion and anti-inflammatory properties during post-reperfusion period[21,22,29,30]. Due to this seminal work, administration of inhaled NO· has undergone more extensive assessment as an anti-inflammatory therapy in humans subjected to predictable ischemia-reperfusion[23,30-32]. How extra-pulmonary effects of inhaled NO· remains unclear but generally it is believed in transformation of unstable NO· to relatively stable, NO·-containing intermediate upon entering in the circulation, and then recycled back to NO· at targeted remote location[31]. Study with a feline ischemia-reperfusion model suggested the intermediate molecule may be plasma S-nitrosothiols (*e.g.,* S-nitrosoalbumin), while studies in both mice and humans points to nitrite as a possible intermediate[31,33,34]. A protective role for nitrite in ischemia-reperfusion is also demonstrated by direct administration of nitrite in murine hepatic ischemia-reperfusion models and together with the demonstration of nitrite conversion to NO· under ischemic location[33,35]. NO·-containing molecules in the blood that are labile under biological conditions and can also be formed with the inhalation of NO· (*via* nitrosylation or S-nitrosation reactions). These also includes S-nitrosothiols in the red blood cell, ferrous-nitrosylhemoglobin and C- or N-nitrosamines[31,36-38].

Specifically, inhaled NO· (80 ppm, co-administered with 50% oxygen and 50% nitrous oxide, approximately 5 h) was administered preemptively to healthy patients undergoing lower extremity surgery requiring approximately two hours of tourniquet-induced ischemia and continued until the completion of the surgery[32]. Administration of inhaled nitric oxide (80 ppm) reduces the expression of CD11b/CD18, P-selectin, and NF-κB. Also there are associated increase in plasma levels of nitrate/nitrite, and reduced oxidative stress. In this health cohort inhaled NO· administered at 80 ppm significantly reduced inflammation from ischemia-reperfusion of lower extremity. Therefore, under conditions of impaired NO· metabolism, inhaled NO· may be an effective therapy to replenish systemic NO·, thus mitigates injury. A subsequent randomized controlled clinical trial evaluated the effects of preemptive inhaled NO· inrecipients (n = 20) undergoing liver transplantation[39]. Again, inhaled NO· (80 ppm, approximately 4 h) *vs* placebo was randomly administered to the recipients after patients were anesthetized and stopped upon case completion. Patients who received inhaled NO· significantly demonstrated shorter hospital stay and enhanced recovery of graft function (alanine transaminase and aspartate aminotransferase, prothrombin time and activated partial thromboplastin time) by approximately 2-3 d when compared to the placebo group. The intraoperative transfusion of platelets was reduced by 50% in recipients who received inhaled NO·. As would be expected plasma nitrite levels were significantly increased in inhaled NO· group compare when compared to placebo. Commonly cited untoward side effects such as the formation of critical levels of met hemoglobin, nitrogen dioxide or bleeding complications were not observed. Lang *et al*[39] then compared a placebo group of patients who received 80 ppm of inhaled NO· during the operative phase of liver transplantation. Patients receiving NO· had better allograft function and

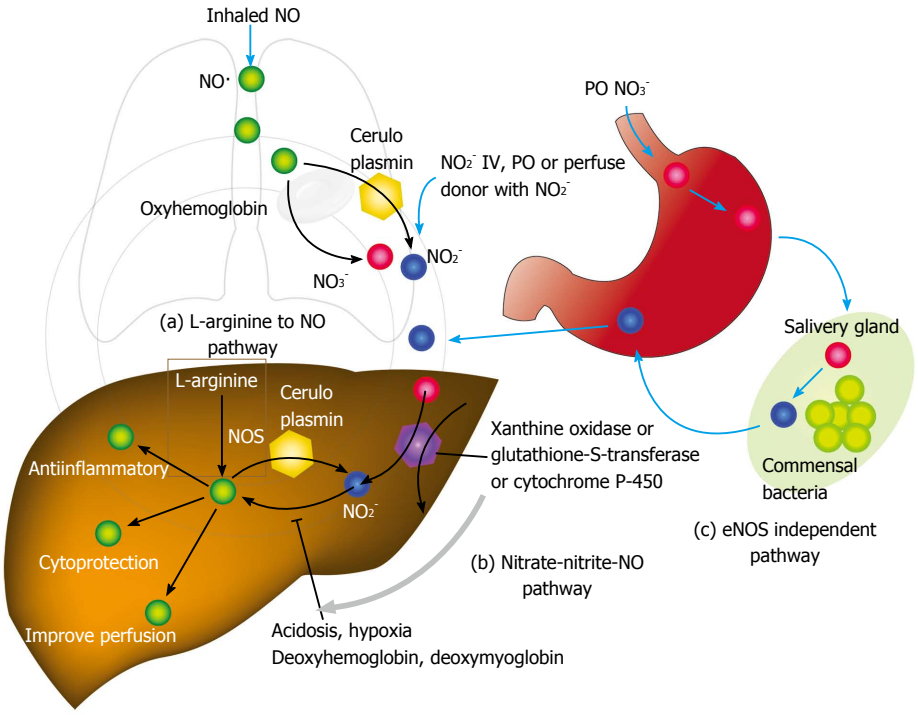

Figure 2 Delivery of nitric oxide to donor liver graft in liver transplantation. Preemptive treatment with inhaled nitric oxide can attenuate ischemia-reperfusion injury *via* modulation of a myriad of inflammatory, cellular and vascular mechanisms. IV: Intravenous; NO·: Nitric oxide; NO_2^-: Nitrite; NO_3^-: Nitrate; eNOS: Endothelial nitric oxide synthase; PO: Per oral.

reduced hepatobiliary complications 9 mo following the initial operation. Also, inhaled NO· significantly increased concentrations of serum nitrate, nitrite, and nitrosylhemoglobin. Consistent with previous data, nitrite was hypothesized to be critical to the findings of allograft protection. No adverse metabolic or hematologic effects were observed between groups. Mean costs of inhaled NO· was $1020 per transplantation. Use of inhaled NO· has also been tested in models of a non-heart beating donor model and steatotic liver, and demonstrated the injury attenuation and enhanced microcirculatory perfusion[40,41]. These studies are in line with other studies demonstrating inhaled NO· mitigates injury when utilized prior to predictable IRI. Replenishing NO· maybe more important in extended criteria donors which appear even susceptible to ischemia (cold and warm)-reperfusion.

Methemoglobinemia is a well-documented side effect of high dose supplemental inhaled NO·. Methemoglobin (MetHb) is rapidly formed by the oxidation of nitrosylhemoglobin, which is a byproduct from the binding of NO· to hemoglobin. This has been shown to occur in a dose and time-dependent fashion. MetHb has a fewer hemes to bind oxygen despite that methemoglobin has a higher affinity to oxygen compared to hemoglobin (1500 times higher affinity compared to carbon monoxide)[42], thus diminishing the oxygen carrying capacity of the blood. MetHb level of 10% of total hemoglobin cause clinically apparent cyanosis, and MetHb level of 35% cause headache, weakness, and dyspnea, and MetHb level of more than 70% are fatal. As aforementioned, clinically significant methemoglobinemia has not been reported when low-dose inhaled NO· is used. Only few case reports of methemoglobinemia has been reported when inhaled NO· was used in high dose (> 80 ppm)[43-45]. However it is worth mentioning that two cases of methemoglobinemia associated with low dose inhaled NO· due to delivery failure have been reported[46]. In both cases, methemoglobin reductase levels were confirmed to be normal, excluding of heredity methemoglobin reductase deficiency. Authors have speculated that variable (phasic) main flow provided from mechanical ventilator caused periodical accumulation of NO· in the inspiratory limb of airway circuit, leading to variable inhaled NO· concentration. Incorporated slow-response chemiluminescent analyzer was unable to detect this fluctuation of inhaled NO·. This fluctuation of NO· was also shown in lung model. Yamaguchi *et al*[47] showed that inhaled NO· was more concentrated when it was administered more distally in the inspiratory limb of the circuit as well as administered with lower flow rates. They speculated that delivered NO· was diluted by backflow in the NO· tubing from the higher pressure in the circuit in the early inspiratory phase of ventilation. This concentrated NO· in NO· tubing was delivered in the early expiratory phase, leading to fluctuation in NO· concentration. Therefore, inhaled NO· treatment requires caution during administration and other form of supplementation of NO· may be favored in terms of avoiding life-threatening methemoglobinemia.

Other possible complication is the generation of cytotoxic oxidant, "peroxynitrite" by rapidly reacting with superoxide anion[48]. Peroxynitrite can induce lipid

peroxidation and inhibit mitochondrial respiration[49,50]. Indeed lung damage has been reported after inhaled NO· administration[51]. Hydrogen gas discover to have an anti-oxidative effect by scavenging peroxynitrite and other hydroxyl radicals[52]. Hydrogen gas has been shown to ameliorate lipopolysaccharide-induced[53], ventilator-associated[54], and hyperoxia-induced acute lung injury[55]. Therefore co-administration of hydrogen gas has been investigated to enhance lung protection by NO·.

Underlying mechanisms of how inhaled NO· decreases injury remains speculative. Nitrite, an oxidative product of NO· metabolism, seems to play a protectant role, however[33,34,56-58]. Thus, consistent with this line of thinking, sodium nitrite has been shown to alleviate acute injury from ischemia-reperfusion in both murine heart (decreased myocardial infarct size) and liver (decrease apoptosis in hepatocytes)[33]. Nitrite-mediated protection seems to involve biochemical pathways that connect ischemia to nitrite reduction to NO· production, therefore exerting cytoprotection by multiple possible mechanisms. In a model of murine liver transplantation, harvested syngeneic liver grafts were perfused with Lactated Ringers, and University of Wisconsin solution, and sodium nitrite supplemented solution during cold preservation period. Several recipients were treated with or without nitrite *via* an intraperitoneal injection. Liver injury demonstrated by enzyme release was significantly mitigated with both nitrite-supplemented solutions. The protective role of nitrite against cold ischemic-induced injury was more robust with longer preservation periods. Cell morphology and architecture was better preserved with grafts treated with nitrite. Hepatocyte cell death/necrosis was significantly reduced in the nitrite supplemented liver grafts. Liver grafts with extended cold preservation times demonstrated both improved tissue histology and liver function after reperfusion when treated with either the nitrite-supplemented preservation solution or in just the nitrite-treated recipients. Surprisingly, combination treatment of both liver graft and recipient did not demonstrate protection. Further clinical studies in the use of inhalation of NO· or injection of NO· donors for extended criteria donor may have a large clinical impact given that there is a surge in use of extended criteria donors to expand donor pool and warrants further investigation.

Other potential route of NO· donor administration is per gastrointestinal tract. In fact, dietary intake of nitrate is major source of NO· donor in mammals[59]. Dietary nitrate is abundant in many vegetables and water. Ingested nitrate is absorbed from intestine. One quarter of absorbed nitrate is concentrated in saliva and metabolized to nitrite by commensal bacteria[60]. Inorganic nitrite is metabolized to NO· in the presence of gastric acid[61-63]. This production pathway of nitric oxide is independent of eNOS (eNOS - independent NO· production) and accounts for majority of nitrite and nitrate in mammalian body[62,63]. Absorbed nitrite, nitrate, or NO· from small intestine is directly delivered to liver through portal vein (Figure 2). Therefore, per oral administration of NO· donor can be a potential route of administration, especially post-transplant period.

Additional drugs that donate NO· have been thoroughly assessed[64-67]. Only two types of these drugs feasible to use clinically: Nitrates, and sodium nitroprusside. Nitrates, such as nitroglycerin are widely used to treat patients suffering from coronary ischemia and/or myocardial infarction due to the pronounced venodilatory effect that assists in reducing venous return and myocardial oxygen demand. Several formulations are available commercially including a slow release oral form, an ointment, a transdermal patch, a nebulizer, and lastly intravenous formulations. A major limitation of organic nitrates is tachyphylaxis due to sustained usage. Nitroglycerin releases NO· *via* the enzyme mitochondrial aldehyde dehydrogenase[68]. Sodium nitroprusside is another commercially available drug that is an NO· donor. Sodium nitroprusside's release of NO· is complex and but its net effect is to significantly diminish mRNA expression of a few pro-inflammatory mediators that promote hepatic injury[12]. Lastly, enhanced eNOS up-regulation confers hepatoprotection during IRI and may allow for another therapeutic option. When agents that increase eNOS expression such as trimetazidine, 5-amino-4-imidazole carboxamide riboside or activated protein C, are added to liver preservation solutions, hepatic allograft protection is afforded[12].

CONCLUSION

IRI has been well characterized the liver especially as it relates to liver resections and liver transplantation. The contribution of NO· deficiency is a newer finding and may have a central role in the pathogenesis of this injury. Replenishing the liver with NO· *via* either by inhalation, inhaled or intravenous nitrate or *via* other donor drugs represents a pragmatic means of mitigating injury. Clinical studies incorporating inhaled NO· provide solid evidence in mitigating injury from IRI. Inhaled NO· has demonstrated repeated efficacy without any demonstrable metabolic or hematological toxicities. Costs of routine NO· administration during liver transplantation is negligible when the entire spectrum of care is considered. Therefore, NO· has a potential to be a good therapeutic option for organ resuscitation in liver transplantation, especially for the extended criteria (marginal quality) donors, but further investigation is still warranted for routine clinical use.

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P- Reviewer: Boucek CD, Okumura T, Samhan-Arias AK, Zhao Q
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DOI: 10.4254/wjh.v8.i34.1497

World J Hepatol 2016 December 8; 8(34): 1497-1501
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Case Control Study

Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation

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Author contributions: Alsaad AA and Wadei HM contributed to the design of the study, acquisition, analysis and interpretation of the data, writing and reviewing the manuscript, and approved the final version of the manuscript for submission.

Institutional review board statement: We obtained approval from the Mayo Clinic Institutional Review Board (IRB) to conduct this study.

Informed consent statement: Exempted, demonstrated as minimal risk study.

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

Data sharing statement: Consent was not obtained but the presented data are anonymized and risk of identification is low.

Manuscript source: Invited manuscript

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Received: July 1, 2016
Peer-review started: July 4, 2016
First decision: August 10, 2016
Revised: September 2, 2016
Accepted: October 22, 2016
Article in press: October 24, 2016
Published online: December 8, 2016

Abstract

AIM

To determine the accuracy of fractional excretion of sodium (FeNa) in the diagnosis of hepatorenal syndrome (HRS).

METHODS

Eighty-eight liver transplantation candidates with renal dysfunction and/or proteinuria were included in the study sample. The baseline characteristics of the patients were obtained. All the 88 patients underwent iothalamate glomerular filtration rate testing, 24-h urine collection for urinary sodium and protein excretions, random urine for sodium and creatinine testing, and percutaneous kidney biopsy. FeNa was calculated using the equation [(urine sodium × serum creatinine)/(serum sodium × urine creatinine)] × 100%. Diuretic use was recorded among the participants. Patients on renal replacement therapy were not included in the original sample.

RESULTS

Seventy-seven (87%) of the 88 patients had FeNa < 1%. FeNa < 1% was present in 10/10, 10/12, 11/13, 12/15 and 34/38 in patients with HRS, acute tubular necrosis, membranoproliferative glomerulonephritis, minimal histological findings (⩽ 30%) and advanced (⩾ 30%-40%) interstitial fibrosis and/or glomerulosclerosis, respectively (P = 0.4). FeNa < 1% was 100% sensitive and 14% specific in diagnosing HRS. Receiver operating characteristic curve confirmed the poor accuracy of FeNa < 1% in diagnosing HRS (area under the curve = 0.58, P = 0.47). Calculated positive predictive value

and negative predictive value for FeNa < 1% in HRS diagnosis were 46% and 100%, respectively. When used as a continuous variable, FeNa did not correlate with kidney biopsy findings (P = 0.41).

CONCLUSION

FeNa < 1% was common in cirrhotic patients with renal dysfunction and it did not differentiate between HRS and other causes of renal pathologies. HRS diagnosis should be avoided in patients with FeNa > 1%.

Key words: Fractional excretion of sodium; Hepatorenal syndrome; Renal dysfunction; Liver transplantation; Urinary sodium excretion; Accuracy

Core tip: In this retrospective analysis of patients with advanced end-stage liver disease, we describe three main concepts. First, our data indicates that fractional excretion of sodium (FeNa) < 1% is a common finding in this group of patients irrespective of the etiology of their renal dysfunction. Second, our study suggests that FeNa < 1% cannot differentiate hepatorenal syndrome (HRS) from other causes of renal pathology. And third, we statistically measured the performance of FeNa < 1% in patients with HRS using kidney biopsy findings as golden diagnostic standard.

Alsaad AA, Wadei HM. Fractional excretion of sodium in hepatorenal syndrome: Clinical and pathological correlation. *World J Hepatol* 2016; 8(34): 1497-1501 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1497.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1497

INTRODUCTION

Kidney dysfunction is common in patients with end-stage liver disease (ESLD). It is estimated that nearly 20% to 25% of patients with ESLD will have some type of kidney dysfunction during their course of disease[1]. The spectrum of kidney disease can range from reversible kidney injury like acute kidney injury [whether prerenal azotemia or acute tubular necrosis (ATN)] to permanent and chronic kidney damage (CKD) and fibrosis. CKD could be either from causes unrelated to the liver disease such as diabetes, or related to the liver disease such as hepatitis C virus infection.

Hepatorenal syndrome (HRS) is a form of prerenal azotemia that is unique to patients with liver cirrhosis and ascites that is diagnosed after excluding other causes of renal impairment[2,3]. HRS occurs in nearly 8% of patients with ascites and 75% of patients with HRS will require dialysis[2].

Early studies have demonstrated that kidneys procured from HRS patients had normal renal histology and exhibited immediate allograft function after transplantation[4]. Pathophysiologically, patients with HRS experience severe vasoconstriction that involves various vascular beds including the kidneys with subsequent reduction in renal blood flow, glomerular filtration rate, and reciprocal increase in proximal tubular sodium re-absorption[5]. Indeed, urinary sodium excretion is low in patients with HRS[6,7]. Classic teaching has utilized FeNa cut-off of < 1% or > 1% to differentiate between prerenal (including HRS) and intrinsic renal dysfunctions especially those caused by ATN. While this FeNa cut-off has been used as discriminatory tool discriminatory tool in cirrhotic patients presenting with elevated serum creatinine, its accuracy has not been tested in diagnosing HRS against a gold standard such as kidney biopsy. Our program utilizes kidney biopsy in evaluating selected liver transplant candidates with renal dysfunction.

In this study, we sought to determine the accuracy of FeNa < 1% in HRS diagnosis using histological data as a gold standard for comparison.

MATERIALS AND METHODS

Settings and participants

After obtaining Mayo Clinic Institutional Review Board approval, we retrospectively reviewed the electronic medical record of 88 patients with ESLD who were undergoing LT evaluation at Mayo Clinic in Jacksonville, Florida. All 88 patients had renal dysfunction defined as an iothalamate glomerular filtration rate (GFR) of less than 40 mL/min per 1.73 m^2 or the presence of proteinuria or hematuria. Patients with fulminant hepatic failure were not included.

All study patients had undergone renal biopsy after a stabilization of platelets count of less than 50000 × 10^6/L with platelets transfusion, and an international normalized ratio of less than 1.5 by fresh frozen plasma transfusion.

Data for GFR and 24-h urine collection for urinary sodium excretion, protein excretion, random urine sodium, and random creatinine were collected. Patients who underwent renal replacement therapy within the last 6 wk prior to evaluation were not included in the original sample, as their serum and urine electrolyte values will be modulated by dialysis. Diuretic use was recorded.

Kidney biopsy specimens were assessed using light microscopy, immunofluorescence and electron microscopy and were interpreted by an experienced nephropathologist as previously described[8]. Patients were grouped according to the primary kidney biopsy diagnosis into five main groups: HRS (normal kidney pathology), ATN, membranoproliferative glomerulonephritis (MPGN), minimal histological changes [defined as 10%-30% interstitial fibrosis (IF) and/or glomerulosclerosis (GS)] and advanced (> 30%) IF and/or GS.

FeNa was calculated using the equation: [(urine sodium x serum creatinine)/(serum sodium x urine creatinine)] × 100.

Patients with fulminant hepatic failure were not included in this analysis.

Table 1 Baseline characteristics of the 88 liver transplant candidates with renal dysfunction

Variable	
Age	60 ± 7
Male gender	57 (65)
Cause of ESLD	
HCV infection	40 (45)
NASH	13 (15)
Alcoholic cirrhosis	12 (14)
Cryptogenic cirrhosis	10 (11)
Other	13 (15)
MELD score	17.5 ± 5.8
History of diabetes	35 (40)
History of hypertension	40 (45)
Iothalamate GFR mL/min per 1.73 m^2	28 ± 14
Serum creatinine (mg/dL)	1.9 ± 0.9
Serum Na (mEq/dL)	137 ± 5
24-h urinary protein excretion (mg/d)	87 (0-13625)
24-h urinary Na excretion (mEq/d)	56 (0-238)
24-urine protein > 150 mg/d	35 (40)
Hematuria	40 (45)
Diuretic use	64 (72)
FeNa < 1	77 (87)
Kidney Biopsy	
HRS	10 (11)
ATN	12 (14)
MPGN	13 (15)
Minimal histology	15 (17)
⩾ 30%-40% IF/GS	38 (43)

Data presented as number (percent), mean ± SD or median (range). ESLD: End-stage liver disease; HCV: Hepatitis C virus; NASH: Non-alcoholic steatohepatitis; MELD: Model of end stage liver disease; GFR: Glomerular filtration rate; Na: Sodium; FeNa: Fractional excretion of sodium; HRS: Hepatorenal syndrome; ATN: Acute tubular necrosis; MGPN: Membranoproliferative glomerulonephritis; IF: Interstitial fibrosis; GS: Glomerulosclerosis.

Outcome measures

The primary outcome was to calculate the sensitivity, specificity, positive predictive value (PPV) and (NPV) of FeNa < 1% in diagnosing HRS. Secondary outcome was to measure the correlation between FeNa as a continuous variable and the kidney biopsy diagnosis.

Statistical analysis

Using SPSS software version 22, (Cary, NC), we analyzed the data of the 88 LT patients. Continuous variables were presented as mean ± SD; categorical variables were presented as number (%). The sensitivity, specificity, PPV and NPV in HRS diagnosis were calculated. A receiver operating characteristic (ROC) curve was constructed to assess the diagnostic accuracy of FeNa < 1% in diagnosing HRS.

RESULTS

Table 1 summarizes the baseline characteristics of the 88 liver transplantation patients. The mean ± SD age of the cohort was 60 ± 7 years and the majority were of male gender (65%). Seventy two percent of the patients were using at least one diuretic at time of FeNa calculation. Out of the 88 LT candidates, 77 (87%) had FeNa < 1%. As demonstrated in Table 1, FeNa < 1% was present in 10/10, 10/12, 11/13, 12/15 and 34/38 in patients with HRS, ATN, MPGN, minimal histological changes (10%-30% fibrosis) and advanced (⩾ 30%) IF and/or glomerulosclerosis (GS), respectively (P = 0.4).

Primary outcome measure

FeNa < 1% was 100% sensitive and 14% specific in diagnosing HRS. Calculated PPV and NPV for FeNa < 1% in the setting of HRS were 46% and 100%, respectively. Figure 1 represents the result of the ROC curve assessing the performance of FeNa < 1% in HRS diagnosis. As demonstrated in Figure 1, FeNa < 1% showed poor accuracy in diagnosing HRS with an area under the curve (AUC) of 0.58, P = 0.47. To assess the effect of diuretic use on the performance of FeNa < 1% in HRS diagnosis, we compared urinary sodium indices between patients on diuretics (n = 64) and those not on diuretic (n = 24) treatment. There was no observed difference in the 24-h urinary sodium excretion, FeNa (as a continuous variable) and number of patients with FeNa < 1% between those using and not using diuretics at time of FeNa calculation (P > 0.3 for all). Also, the sensitivity (100%), specificity (12.5%), PPV (14%) and NPV (100%) of FeNa < 1% in diagnosing HRS did not differ when patients not using diuretics were excluded from the analysis.

Secondary outcome measure

We then correlated FeNa as continuous variable with the kidney biopsy finding. Although FeNa was lowest in HRS patients it did not differentiate between HRS and other renal pathologies (Figure 2).

There was also no correlation between the degrees of the IF (r = 0.07, P = 0.54) or GS (r = 0.2, P = 0.07) and the 24-h urinary sodium excretion (Figures 3 and 4, respectively). This lack of correlation was not affected by diuretic use (data not shown).

DISCUSSION

Our study indicates that the majority of ESLD patients with renal dysfunction of unknown etiology or duration have a low 24-h urinary sodium excretion and a FeNa < 1% irrespective of renal pathology on kidney biopsy. Using kidney biopsy findings as a gold standard, we were able to determine the accuracy of FeNa < 1% in diagnosing HRS. Our results defined the sensitivity, specificity, PPV and NPV of FeNa < 1% in HRS as 100%, 14%, 46% and 100%, respectively. These results suggest that a FeNa > 1% excludes HRS diagnosis but a FeNa < 1% is not useful in diagnosing HRS.

Previous studies confirm that cirrhotic patients without renal dysfunction have low urinary sodium excretion rates and increased renal tubular reabsorption due to the activation of various neuro-hormonal mechanism and subsequent increase in renal tubular sodium re-absorption[5,9]. In patient with renal dysfunction however, low urinary sodium excretion implies maintained tubular integrity and favors either prerenal azotemia or HRS

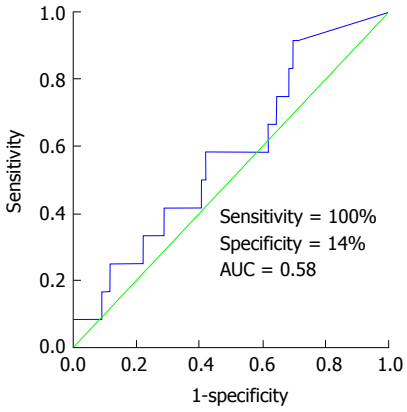

Figure 1 Receiver operator characteristics curve showing the poor accuracy of fractional excretion of sodium < 1% in diagnosing hepatorenal syndrome syndrome with area under the curve of 0.58, *P* = 0.47-0.58. AUC: Area under the curve.

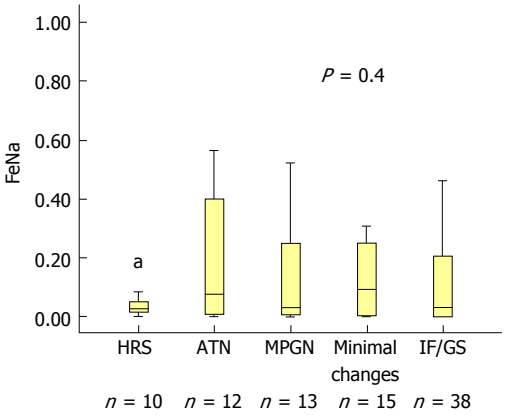

Figure 2 Correlation between fractional excretion of sodium as a continuous variable and kidney biopsy diagnosis. Although FeNa was lowest in HRS patients, it did not differentiate between HRS and other renal pathologies (*P* = 0.41). FeNa: Fractional excretion of sodium; HRS: Hepatorenal syndrome; ATN: Acute tubular necrosis; MGPN: Membranoproliferative glomerulonephritis; IF: Interstitial fibrosis; GS: Glomerulosclerosis.

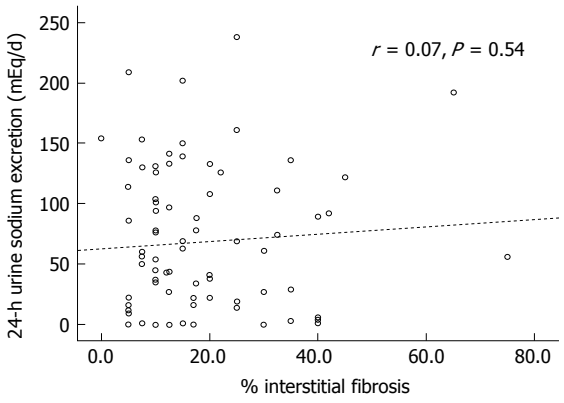

Figure 3 Scatter plot depicting the relationship between the percentage of interstitial fibrosis on kidney biopsy and 24-h urine sodium excretion. Correlation was overall poor (*r* = 0.07, *P* = 0.54).

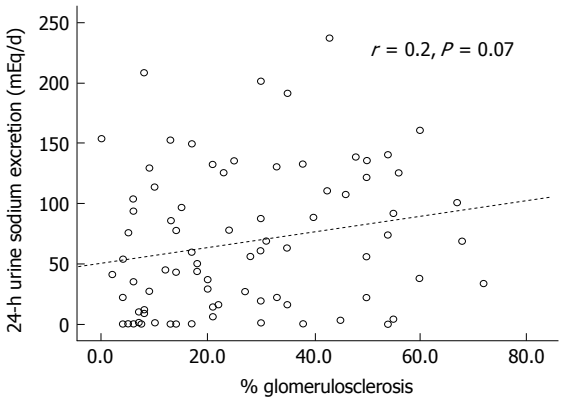

Figure 4 Scatter plot depicting the relationship between the percentage of glomerulosclerosis on kidney biopsy result and 24-h urine sodium excretion (*r* = 0.2, *P* = 0.07). Correlation was better than the one observed with percentage of interstitial fibrosis but still did not reach statistical significance.

diagnosis[5]. Results of this study challenge this understanding. Our findings indicated that urinary sodium excretion was similarly low in cirrhotic patients with renal dysfunction due to tubular injury (ATN), glomerular disease (MPGN), irreversible renal damage (advanced IF/GS) or normal renal histology (HRS). There was also no correlation between the degree of IF or GS and the 24-h urinary sodium excretion. These results indicate that a low urinary sodium excretion is present in the majority of cirrhotic patients with renal dysfunction and does not reflect an intact renal tubular integrity but rather reflects the avid renal sodium retention state in these patients irrespective of the cause of renal dysfunction. It is also important to mention that diuretic use did not affect urinary sodium indices or the performance of FeNa < 1% in HRS diagnosis which support the avid sodium retention state in these patients with advanced cirrhosis.

FeNa is more sensitive than urinary sodium concentration in detecting prerenal causes of renal dysfunction as the serum sodium level is factored into the equation. Previously published reports, however, indicated that although FeNa was lowest in patients with HRS, FeNa did not differentiate HRS and other causes of renal dysfunction including ATN[6,10]. These studies did not include kidney biopsy evidence of HRS (normal renal histology) and the diagnosis of HRS was made solely on clinical grounds[10,11].

The main strength of the current study is that we used kidney biopsy findings to diagnose HRS, ATN and other renal pathologies. By using histological data, we were able to confirm that FeNa < 1% is present in almost 90% of cirrhotic patients with renal dysfunction. Although FeNa was lowest in HRS and FeNa < 1% was present in 100% of patients with HRS, FeNa did not differentiate between HRS and other causes of acute or chronic renal dysfunction. Of note a previous prospective study demonstrated that FeNa < 1% was present in only 0% to 4% of patients with ATN and no history of liver disease[12]. In contrast, in the current study 10 of 12 patients (83%) with biopsy evidence of ATN had a FeNa < 1%. Previous studies also demonstrated that FeNa < 1% had a sensitivity and specificity of 58%-78% and 75%-81% in diagnosing prerenal azotemia, respectively, in subjects without liver disease and varied according to diuretic use[13]. The results of the current study indicate

that the sensitivity and specificity of FeNa < 1% in the diagnosis of HRS is much different than in non-cirrhotic patients with prerenal azotemia and that they are not affected by diuretic use. This difference is likely due to the intense renal vasoconstriction manifesting in cirrhotic patients with subsequent increase in renal sodium reabsorption and the diuretic resistant state these patients develop with worsening liver disease.

The performance of FeNa < 1% in diagnosing HRS was overall poor but the test had high sensitivity and high NPV (both 100%), indicating that in patients with negative test results (*i.e.,* FeNa > 1%) HRS diagnosis should be excluded.

The current study is limited by the small number of patients particularly those with normal biopsy findings and HRS diagnosis which could have affected the results. Another important limitation of the study is the lack of detailed information on dietary sodium intake and doses and class of the diuretic medications used. We also measured FeNa at a single time point prior to the kidney biopsy to minimize the selection bias. Future studies should address if serial measurements of FeNa in a given patient will confer similar results.

In conclusion, the current study indicates that FeNa < 1% is common finding in patients with ESLD and renal dysfunction and has a poor accuracy in diagnosing HRS. Our results also indicate that HRS diagnosis should be avoided in patients with FeNa > 1%. Further studies with large number of patients are needed to confirm the findings of this study.

COMMENTS

Background

Kidney dysfunction is common in patients with end-stage liver disease (ESLD). It is estimated that nearly 20% to 25% of patients with ESLD will have some type of kidney dysfunction during their course of disease.

Research frontiers

Classic teaching has utilized fractional excretion of sodium (FeNa) cut-off of < 1% or > 1% to differentiate between prerenal [including hepatorenal syndrome (HRS)] and intrinsic renal dysfunctions especially those caused by acute tubular necrosis. While this FeNa cut-off has been a useful discriminatory tool in cirrhotic patients presenting with elevated serum creatinine, its accuracy has not been tested in diagnosing HRS against a gold standard such as kidney biopsy.

Innovations and breakthroughs

The authors program utilizes kidney biopsy in evaluating selected liver transplant candidates with renal dysfunction.

Applications

The authors measured FeNa at a single time point prior to the kidney biopsy to minimize the selection bias. Future studies should address if serial measurements of FeNa in a given patient will confer similar results.

Peer-review

In patients with end stage cirrhosis and renal dysfunction (glomerular filtration rate < 40) the estimated FeNa < 1 could not discriminate between HRS and intrarenal kidney injury. The findings are quite relevant, since FeNa is used most often to exclude intrarenal disease in cirrhotic patients.

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P- Reviewer: Chok KSH, Lenz K **S- Editor**: Qi Y **L- Editor**: A
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DOI: 10.4254/wjh.v8.i34.1502

World J Hepatol 2016 December 8; 8(34): 1502-1510
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

Resection margin influences the outcome of patients with bilobar colorectal liver metastases

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Author contributions: Di Carlo S collected the data and drafted the manuscript; Yeung D collected the data and assisted in drafting of the manuscript; Gomez D analyzed the data, designed and supervised the study; Mills J, Zaitoun A and Cameron I provided analytical oversight and supervision.

Institutional review board statement: This study has been registered and approved by the Clinical Audit Department, Nottingham University Hospitals NHS Trust.

Informed consent statement: Since this is a retrospective study, individual patient consent was not required, and all local ethical guidelines with respect to retrospective studies in this Trust were adhered to.

Conflict-of-interest statement: All the authors have approved the manuscript and there is no conflict of interest to declare.

Data sharing statement: The statistical methods of this study were reviewed and performed by Gomez D, who is competent in SPSS statistical software.

Manuscript source: Invited manuscript

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Received: July 12, 2016
Peer-review started: July 13, 2016
First decision: August 26, 2016
Revised: September 16, 2016
Accepted: October 22, 2016
Article in press: October 24, 2016
Published online: December 8, 2016

Abstract

AIM

To evaluate the outcome of patients with bilobar colorectal liver metastases (CRLM) and identify clinico-pathological variables that influenced survival.

METHODS

Patients with bilobar CRLM were identified from a prospectively maintained hepatobiliary database during the study period (January 2010-June 2014). Collated data included demographics, primary tumour treatment, surgical data, histopathology analysis and clinical outcome. Down-staging therapy included Oxaliplatin- or Irinotecan- based regimens, and Cetuximab was also used in patients that were *K-RAS* wild-type. Response

to neo-adjuvant therapy was assessed at the multi-disciplinary team meeting and considered for surgery if all macroscopic CRLM were resectable with a clear margin while preserving sufficient liver parenchyma.

RESULTS

Of the 136 patients included, thirty-two (23.5%) patients were considered inoperable and referred for palliative chemotherapy, and thirty-four (25%) patients underwent liver resection. Seventy (51.4%) patients underwent down-staging therapy, of which 37 (52.8%) patients responded sufficiently to undergo liver resection. Patients that failed to respond to down-staging therapy (n = 33, 47.1%) were referred for palliative therapy. There was a significant difference in overall survival between the three groups (surgery *vs* down-staging therapy *vs* inoperable disease, $P < 0.001$). All patients that underwent hepatic resection, including patients that had down-staging therapy, had a significantly better overall survival compared to patients that were inoperable ($P < 0.001$). On univariate analysis, only resection margin significantly influenced disease-free survival ($P = 0.017$). On multi-variate analysis, R0 resection ($P = 0.030$) and female ($P = 0.036$) gender significantly influenced overall survival.

CONCLUSION

Patients undergoing liver resection with bilobar CRLM have a significantly better survival outcome. R0 resection is associated with improved disease-free and overall survival in this patient group.

Key words: Colorectal liver metastases; Chemotherapy; Liver resection

Core tip: The management of colorectal liver metastases (CRLM) has evolved over the last decade. More patients are being subjected to potentially curative liver resection following down-staging therapy and the introduction of specialist multi-disciplinary team meetings. The introduction of biological agents has also increased resection rates. The current study analysed patients with bilobar CRLM referred to our centre. Patients that underwent liver resection had a significantly better survival outcome following our multi-disciplinary approach.

Di Carlo S, Yeung D, Mills J, Zaitoun A, Cameron I, Gomez D. Resection margin influences the outcome of patients with bilobar colorectal liver metastases. *World J Hepatol* 2016; 8(34): 1502-1510 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1502.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1502

INTRODUCTION

Hepatic resection is the only potentially curative treatment for patients with colorectal liver metastases (CRLM) and the 5-year survival rate is up to 50%[1,2]. Patients with extensive, bilateral disease present a surgical challenge in removing all macroscopic disease while preserving sufficient functional liver remnant. Studies have shown that 20%-30% of all patients with CRLM are resectable at the time of diagnosis[3], with bilobar distribution of metastases a major contributing factor for unresectability[4].

More recently, the introduction of biological agents and the improved efficacy of down-staging chemotherapy regimens to treat bilobar CRLM have increased the proportion of patients with initially unresectable disease to subsequently operable disease. In addition, neo-adjuvant chemotherapy can potentially treat systemic disease to lower the risk of distant spread, and allow the identification of patients with biologically aggressive tumours that progress on chemotherapy that would not benefit from liver surgery[5]. Down-staging chemotherapy regimens are more toxic than palliative regimens, and hence, it is essential that there is multi-disciplinary team approach in determining the management plan for these patients[6]. However, long term outcomes for these patients following down-staging therapy and liver resection are indeterminate.

The aim of this study was to evaluate the outcomes of patients with bilobar CRLM following multi-disciplinary therapy. The secondary aim was to identify clinico-pathological variables that influenced disease-free and overall survival in this group of patients.

MATERIALS AND METHODS

Patients

Patients with bilobar CRLM were identified from a prospectively maintained hepatobiliary database at Queen's Medical Centre (QMC), Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom during a 4-year period from January 2010 to June 2014. QMC is a tertiary referral center for Nottinghamshire and surrounding regions located in the north of East Midlands, United Kingdom. Pre-operative radiological assessment included a computed tomography (CT) scan of the thorax, abdomen and pelvis and magnetic resonance imaging (MRI) of the liver. Patients with indeterminate lesions, in particular lung nodules, and patients with synchronous presentation underwent a positron emission tomography scan. Synchronous presentation was defined as the presence of liver metastases when colorectal cancer was diagnosed. Prior to any treatment, all patients including patients referred from the surrounding regions were discussed in a specialist multidisciplinary team (MDT) meeting consisting of hepatobiliary surgeons, hepatologists, oncologists, radiologists and pathologists. Patients were selected for liver resection without any prior neo-adjuvant therapy if all macroscopic CRLM were resectable to achieve a clear margin while preserving sufficient liver parenchyma.

Collated data included patient demographics, type of surgical resection, histopathology analysis and clinical

outcome. This study has been registered and approved by the Clinical Audit Department, Nottingham University Hospitals NHS Trust. Since this is a retrospective study, individual patient consent was not required, and all local ethical guidelines with respect to retrospective studies in this Trust were adhered to.

Down-staged therapy and adjuvant chemotherapy

Patients scheduled for preoperative systemic chemotherapy had 3-6 mo of neo-adjuvant treatment. The regimens used were either Oxaliplatin based: Two weekly FOLFOX [5-fluorouracil (FU) 400 mg/m^2 bolus, and 2400 mg/m^2 over 46 h, Leucovorin and Oxaliplatin 85 mg/m^2] or three weekly CAPOX (Capecitabine 1000 mg/m^2 BD for 14 d and Oxaliplatin 130 mg/ m^2).

However, in patients tested and found to be *K-RAS* wild-type, two weekly FOLFIRI (Irinotecan 180mg/m^2, 5-FU 400 mg/m^2 bolus, and 2400 mg/m^2 over 46 h) was administered with concurrent Cetuximab (400 mg/m^2 cycle 1, then 250 mg/m^2 cycle 2 onwards).

The response to neo-adjuvant therapy was assessed after 3-6 mo of therapy by CT scan and repeat MRI of the liver if required. Patients were then re-discussed at the MDT and considered for surgery based on absence of new disease, tumour response and extent of disease. Patients deemed to have resectable disease were scheduled for a liver resection, 4-6 wk after their last cycle of chemotherapy. Resectable disease was defined as excision of all macroscopic CRLM to achieve a clear margin while preserving sufficient liver parenchyma based on pre-operative radiological imaging.

Following liver resection, chemotherapy was considered in patients with tumour present at the margin (R1 resection).

Surgery

Liver resection was performed using the Cavi-Pulse Ultrasonic Surgical Aspirator. Intra-operative ultrasound was performed to confirm the findings of pre-operative imaging and to assist in surgical planning. The number of hepatic Couinaud's[7] segments resected was determined by the procedure performed as stated in the Brisbane nomenclature[8]. Type of surgical procedure was dependent on the resection of all macroscopic disease and achieving a clear resection margin, while preserving sufficient remnant liver. The extent of hepatic resection in this study was classified into two groups; less than hemi-hepatectomy and hemi-hepatectomy or more radical resection. Pre-operative PVE was performed if the FRL volume was estimated to be 20% or less of the total liver volume. Liver-first approach was defined when the hepatic resection was performed first prior to colonic or rectal resection[9,10].

In patients where the liver-first approach was adopted, primary tumour resection was usually scheduled 4-8 wk following liver resection, or after completion of chemo-radiotherapy for patients with locally advanced rectal cancer. All patients underwent re-staging with a CT scan and MRI to ensure there was no evidence of liver recurrence or distant metastases. Colorectal resection was performed according to accepted oncological standards, with complete meso-rectal excision for rectal cancers and lymph node dissection for colonic cancers.

Histology

Histopathological data of the resected liver specimen were collated. This included: Tumour size in maximum diameter; tumour number; and status of resection margin. R0 resection was defined as no microscopic evidence of tumour at or within 1 mm of the margin. Lymphatic, peri-neural, biliary and vascular invasion were also determined[11].

Follow-up

Patients were followed up in specialist hepatobiliary clinics. Following initial post-operative review at one month, all patients were examined in the outpatient clinic at 3, 6, 12, 18 and 24 mo and annually thereafter. At each clinical review, carcino-embryonic antigen levels were measured. All patients in this study had a minimum follow-up of 6 mo following hepatic resection for CRLM.

Surveillance imaging included CT scan of the thorax, abdomen and pelvis. Patients underwent 6-monthly CT scan during the first two post-operative years, followed by annual CT scans thereafter. Liver MRI was used to characterise suspicious hepatic lesions demonstrated on CT. Development of symptoms of recurrence at any time-point prompted earlier review than scheduled.

Overall and disease-free survival was recorded, with disease-free survival being defined as the time from primary hepatic resection to the first documented disease recurrence on imaging. Overall survival was defined as the time interval between the date of commencement of neo-adjuvant/induction therapy and the date of death or most recent date of follow-up if the patient was still alive. Following detection of recurrent disease on surveillance imaging, all patients were discussed at the MDT meeting. Patients who had non-resectable disease were referred to the oncologists for consideration of palliative chemotherapy.

Statistical analysis

Categorical data was presented as frequency and percentage. The Kaplan-Meier method was used to assess the actuarial survival and disease-free survival, and presented as median (range). Univariate analysis was performed to assess for a significant difference in clinico-pathological characteristics that influenced disease recurrence and survival. A multivariate analysis was performed by Cox regression (Step-wise forward model) for variables significant on univariate analysis. Statistical analyses were performed using the SPSS for Windows™ version 16.0 (SPSS Inc, Chicago, Ill, United States), and statistical significance was taken at the 5% level. The statistical methods of this study were reviewed and performed by Gomez D, QMC, Nottingham, United Kingdom.

Table 1 Clinical data of patients with bilobar colorectal liver metastases in this study

Demographic, clinical and pathological factors All patients (n = 136) All surgery patients (n = 71)	Total (n)
Demographic factors	
Age > 65 yr	68
Male gender	99
Synchronous presentation	80
Down-staging therapy	70
Oxaliplatin-based regimen	60
Irinotecan-based regimen	10
Addition of biological agent	30
Surgical factors (n = 71)	
Hemi-hepatectomy or more	22
Histo-pathological factor (n = 71)	
Largest tumour size ⩾ 5 cm	11
Number of metastases < 4	44
Lymphatic invasion present	15
Vascular invasion present	28
Peri-neural invasion present	9
Biliary invasion present	25
Resection margin (R0)	40

RESULTS

Patients

During the study period, a total of 136 patients (Table 1) with bilobar CRLM were discussed in the unit's MDT, of which 34 (25.0%) patients underwent surgery with curative intent as their primary treatment (Figure 1). There were 32 (23.5%) patients that had extensive disease and were referred for palliative therapy.

Seventy (51.4%) patients were considered for down-staging therapy, in view to consider liver resection depending on response to therapy. Besides receiving either an Oxaliplatin-based (n = 60) or Irinotecan-based (n = 10) regimen, 30 (42.8%) patients also had biological agents as part of their down-staging treatment. Within the group of patients that received down-staging therapy, 37 (52.8%) patients had a response to their down-staging therapy and underwent hepatic resection, while the remaining patients [n = 33 (47.2%)] did not undergo surgical resection. These patients did not respond to their down-staging therapy, which included: (1) having new metastases; (2) disease progression; and (3) inability to remove all macroscopic liver disease whilst leaving sufficient remnant liver. This decision was based on MDT review of up to date radiological imaging following down-staging therapy.

Liver resection

Overall, there were 71 (52.2%) patients that underwent liver resection, of which twenty-two patients had a hemi-hepatectomy or more. The most common surgical procedures performed was multiple non-anatomical resections (n = 40, 56.3%). Twenty-one patients were female and the median age at the diagnosis was 65 (range: 44-84) years. Seven (9.8%) patients had portal vein embolization prior to liver resection. There were 35 patients with synchronous disease, of which 17 patients had a liver-first approach. There was no post-operative mortality.

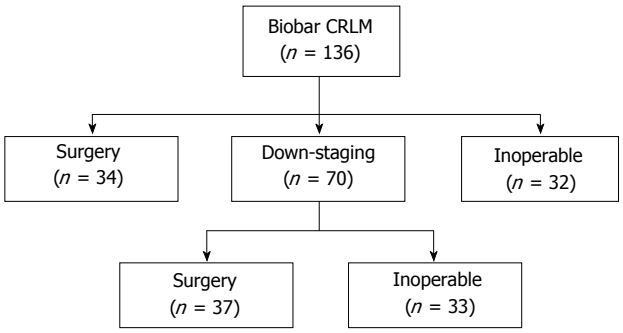


Figure 1 Outcome of patients with bilobar colorectal liver metastases in this study. CRLM: Colorectal liver metastases.

Survival outcome

The median overall survival for all patients in this study was 18 (1-48) mo (Figure 2). There was a significant difference in overall survival between the three groups (Surgery *vs* Down-staging therapy *vs* Inoperable disease, P < 0.001; Figure 3). All patients that underwent hepatic resection, including patients that had down-staging therapy, had a significantly better overall survival compared to patients that were inoperable [24 (6-48) mo *vs* 17 (1-43) mo; P < 0.001; Figure 4]. The disease-free survival for patients that underwent liver resection was 8 (range: 2-36) mo.

Prognostic factors influencing disease-free and overall survival

With respect to disease-free survival, patients with a clear (R0) resection margin following liver resection had a significantly better disease-free survival compared to patients with a R1 resection (P = 0.017; Table 2 and Figure 5).

Patients with a R0 resection (P = 0.022; Figure 6) and female gender (P = 0.024; Figure 7) has a significantly better overall survival compared to patients with a R1 resection and male gender on univariate analysis. On the multi-variate analysis, both R0 resection and female gender were independent predictors of improved overall survival (Table 3).

DISCUSSION

With the improvement in chemotherapy agents and the increased efficacy with the addition of biological agents, many centers have reported an increased number of patients being converted from initially unresectable, to resectable disease[12,13]. However, although there are an increased number of patients undergoing liver resection with curative intent, some authorities may suggest that these patients are unlikely to be cured[14]. Nevertheless, these patients have a better overall survival in comparison to patients treated with palliative systemic

Table 2 Statistical analysis of prognostic factors with respect to disease-free survival

Demographic, clinical and pathological factors	Survival [median (range) mo]	Uni-variate analysis
Demographic factors		
Age		0.099
< 65 yr (n = 43)	6 (3-36)	
⩾ 65 yr (n = 28)	12 (2-36)	
Gender		0.343
Male (n = 50)	6 (3-36)	
Female (n = 21)	5 (2-36)	
Presentation		0.755
Synchronous (n = 35)	6 (2-36)	
Metachronous (n = 36)	6 (3-36)	
Surgical factors		
Less than hemi-hepatectomy (n = 49)	6 (2-36)	0.760
Hemi-hepatectomy or more (n = 22)	6 (2-36)	
Histo-pathological factor		
Largest tumour size		0.813
< 5 cm (n = 60)	6 (2-36)	
⩾ 5 cm (n = 11)	9 (2-36)	
No. of metastases		0.538
< 4 (n = 44)	7 (2-36)	
> 5 (n = 27)	6 (3-36)	
Lymphatic invasion		0.256
Positive (n = 15)	6 (2-24)	
Negative (n = 56)	6 (2-36)	
Vascular invasion		0.775
Positive (n = 28)	6 (2-36)	
Negative (n = 43)	7 (2-36)	
Peri-neural invasion		0.115
Positive (n = 9)	6 (2-24)	
Negative (n = 62)	6 (2-36)	
Biliary invasion		0.919
Positive (n = 25)	6 (2-36)	
Negative (n = 46)	6 (2-36)	
Resection margin (R0)		0.017
R0 (n = 40)	8 (2-36)	
R1 (n = 31)	6 (2-36)	

Table 3 Statistical analysis of prognostic factors with respect to overall survival

Demographic, clinical and pathological factors	Survival [median (range) mo]	Uni-variate analysis	Multi-variate analysis	Risk ratio (confidence interval)
Demographic factors				
Age		0.173		
< 65 yr (n = 43)	20 (6-48)			
⩾ 65 yr (n = 28)	27 (7-48)			
Gender		0.024	0.036	3.172 (1.079-9.327)
Male (n = 50)	19 (6-48)			
Female (n = 21)	20 (11-48)			
Presentation		0.932		
Synchronous (n = 35)	23 (6-48)			
Metachronous (n = 36)	24 (6-48)			
Surgical factors				
Less than hemi-hepatectomy (n = 49)	22 (6-48)	0.947		
Hemi-hepatectomy or more (n = 22)	28 (7-48)			
Histo-pathological factor				
Largest tumour size		0.216		
< 5 cm (n = 60)	24 (6-48)			
⩾ 5 cm (n = 11)	28 (12-48)			
Number of metastases		0.674		
< 4 (n = 44)	24 (6-48)			
> 5 (n = 27)	24 (11-48)			
Lymphatic invasion		0.943		
Positive (n = 15)	24 (11-48)			
Negative (n = 56)	23 (6-48)			
Vascular invasion		0.367		
Positive (n = 28)	25 (6-48)			
Negative (n = 43)	23 (6-48)			
Peri-neural invasion		0.220		
Positive (n = 9)	12 (11-48)			
Negative (n = 62)	24 (6-48)			
Biliary invasion		0.608		
Positive (n = 25)	27 (11-48)			
Negative (n = 46)	22 (6-48)			
Resection margin (R0)		0.022	0.030	0.403 (0.178-0.917)
R0 (n = 40)	24 (6-48)			
R1 (n = 31)	22 (6-48)			

chemotherapy, with some authors reporting a median survival up to 45 mo[12,15]. In the present study, patients with bilobar disease who underwent surgery had a significantly better overall survival compared to patients who failed down-staged chemotherapy and/or treated with palliative chemotherapy.

Conversion rate

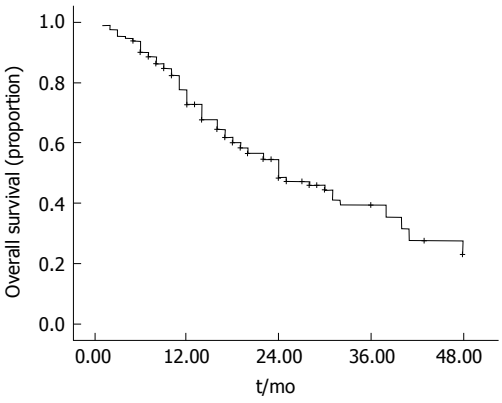
The addition of biological agents to current Oxaliplatin- and Irinotecan- based regimens has led to further improvements in response rates. In a large randomised control trial, Folprecht *et al*[16] showed an increased response rate up to 68% with the addition of Cetuximab. Similarly, Masi *et al*[17] observed that the addition of Bevacizumab to Oxaliplatin- and Irinotecan- based regimens increased the response rate up to 80%. In the present study, the unit's down-staging therapy protocol had a response rate and a conversion of unresectable to resectable disease of more than 50%. These results were consistent with data reported by the groups of Van Custem *et al*[18] and Bokemeyer *et al*[19] that observed a conversion rate of approximately 60% following down-staging chemotherapy.

Survival data

Adam *et al*[13] recently published their long-term survival results following down-sizing chemotherapy and hepatic resection in patients with CRLM and demonstrated that 24 (16%) of 148 patients were alive and disease-free with a minimum of 5-year follow-up. Several studies have shown the improvements in survival after the addition of anti-VEGF/EGFR[20-22]. Recently, a number of case series describing 10-year actual survivors after liver resection of CRLM have been published[23,24]. The present series demonstrated that hepatic resection for patients with bilobar CRLM had a median disease-free and overall survival of 8 and 24 mo, respectively.

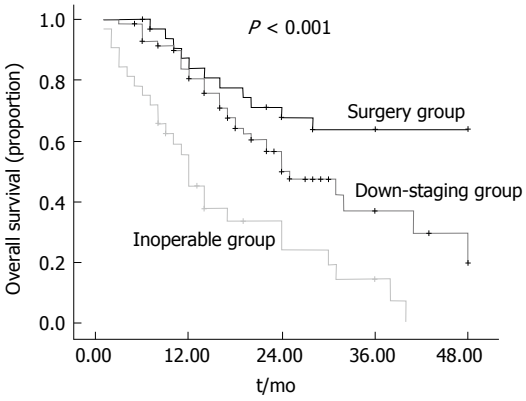
MDT approach

Definitions of resectable disease have evolved over time, with current consensus suggesting that disease should be considered technically resectable as long as complete macroscopic resection is feasible, whilst maintaining



Numbers at risk					
Patients	0	12	36	48	60
All (n = 136)	136	97	23	6	0

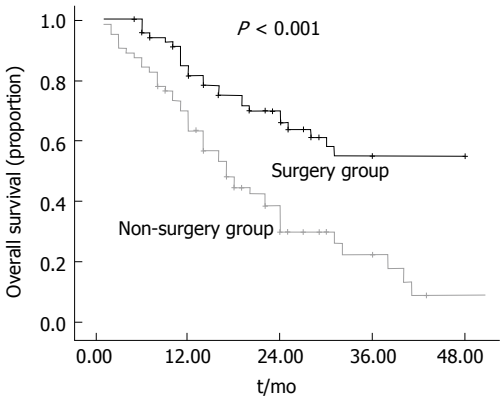
Figure 2 Overall survival of patients with bilobar colorectal liver metastases in this study. All patients (n = 136): 18 (1-48) mo.



Surgery (n = 34): 28 (7-48) mo
Down-staging therapy (n = 70): 18 (3-48) mo
Inoperable (n = 32): 11 (1-40) mo

Numbers at risk					
Patients	0	12	36	48	60
Surgery (n = 34)	34	32	14	4	0
Down-staging (n = 70)	70	49	6	2	0
Inoperable (n = 32)	32	16	3	0	0

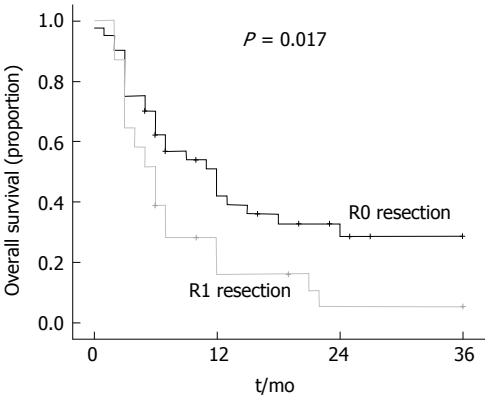
Figure 3 Difference in overall survival in patients that underwent surgery, down-staging therapy followed by surgery or palliative therapy and inoperable patients.



Surgery (n = 71): 24 (6-48) mo
No surgery (n = 65): 17 (1-43) mo

Numbers at risk				
Patients	0	12	36	48
Surgery group (n = 71)	71	66	18	6
Non-surgery group (n = 65)	65	40	6	0

Figure 4 Difference in overall survival in patients that underwent surgery following down-staging therapy compared to patients that either failed down-staging therapy or were treated with palliative therapy.



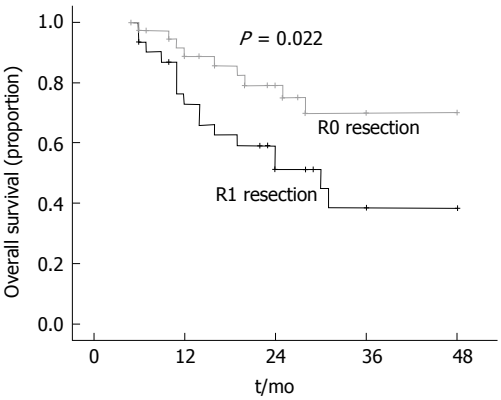
Numbers at risk			
Patients	0	12	36
R0 resection (n = 40)	40	17	5
R1 resection (n = 31)	31	7	1

Figure 5 Difference in disease-free survival in patients with R0 resection compared to patients with R1 resection.

sufficient future liver volume[25]. However, there remains concern that not all patients with technically resectable liver-limited metastases benefit from surgery; with approximately half of these patients will develop recurrences within three years of liver resection[26]. Therefore, it is crucial that the decision-making process around treatment strategies for metastatic colorectal cancer are made in a MDT environment that consists of specialist hepato-biliary surgeons, radiologists and oncologists that can define optimal patient management on a case by case basis. A recent study demonstrated that almost two-thirds of patients with tumours deemed unresectable by non-specialists were considered potentially resectable by a panel of specialist hepato-biliary surgeons based on radiological imaging[27].

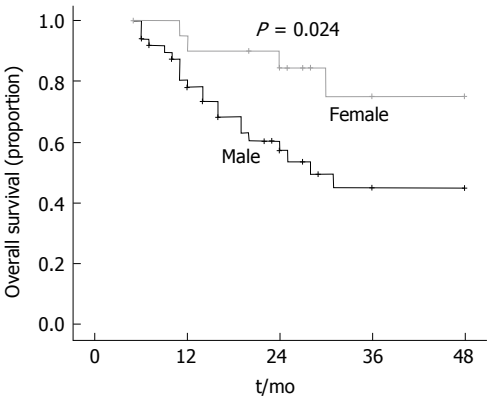
Prognostic factors

The role of margin status as a predictor of outcome following resection for CRLM is controversial. Bodingbauer *et al*[28] observed that resection margin and size of margin width did not correlate significantly with survival following resection for CRLM. In a series of 1019 patients, and co-investigators demonstrated that a resection margin > 1 cm was an independent predictor of survival following resection for CRLM[29]. Rees *et al*[1] also demonstrated that positive resection margins were an independent predictor of poorer survival. However,



Numbers at risk				
Patients	0	12	36	48
R0 resection (n = 40)	40	34	12	4
R1 resection (n = 31)	31	21	6	2

Figure 6 Difference in overall survival in patients with R0 resection compared to patients with R1 resection.



Numbers at risk				
Patients	0	12	36	48
Male (n = 50)	50	36	11	4
Female (n = 21)	21	20	9	2

Figure 7 Difference in overall survival in female patients compared to male patients following surgery for colorectal liver metastases.

Figueras *et al*[30] showed that a margin width < 1 cm in patients who underwent resection for CRLM did not significantly influence recurrent disease in a cohort of 609 patients. Homayounfar *et al*[31] demonstrated that R0 resection in patients with bilobar CRLM have improved survival rates following multi-modal therapy[32]. In the present series, a clear resection margin, defined as no microscopic evidence of tumour at or within 1 mm of the margin, was an independent predictor of both disease-free and overall survival. Due to the differences in results observed with respect to resection margin between published studies, it may be that only a selected group of patients undergoing resection for CRLM are influenced by a clear margin. In the present series that focused on patients with bilobar CRLM, that would be considered as having a high tumour burden, benefited from a R0 margin. This could be due to the fact that these patients have an aggressive tumour profile and it is crucial that complete tumour clearance is obtained. Hence, for these patients, "down-sizing" chemotherapy should certainly be considered prior to resection to aid in achieving a clear resection margin. Furthermore, many groups advocate a trial of neo-adjuvant chemotherapy in patients with a high tumour burden, as disease progression on chemotherapy would be a contraindication to surgery[33]. Nevertheless, with the increase use of chemotherapy, there is an increase in prevalence of patients undergoing hepatic resection with a background of chemotherapy-related injury, such as steato-hepatitis[34] and sinusoidal obstruction syndrome[35]. In such cases, the quality, rather than quantity of the remnant liver becomes an important issue to consider prior to extensive resection.

The present study also showed that female gender was an independent prognostic factor for improved overall survival. There are currently no other studies that have reported this finding.

There are limitations in this study. This is a retrospective study, and focused on a group of patients with bilobar liver metastases. These are patients with bad tumour biology and in most cases, will require down-staging therapy. Nevertheless, although these group of patients have a higher tumour burden; their prognosis can be improved with a MDT approach that focuses on multi-modal therapy.

Patients with bilobar CRLM treated with liver resection as a primary treatment or following down-staging therapy have a better overall survival compared to patients who failed down-staging therapy and/or treated with palliative chemotherapy. Obtaining a clear resection margin in these cases significantly influences outcome. In this group of patients, multi-modal therapy is crucial to achieve a better survival outcome.

COMMENTS

Background

Hepatic resection is the only potentially curative treatment for patients with colorectal liver metastases (CRLM) and the 5-year survival rate is up to 50%.

Research frontiers

The introduction of biological agents and the improved efficacy of down-staging chemotherapy regimens to treat bilobar CRLM have increased the proportion of patients with initially unresectable disease to subsequently operable disease. In addition, neo-adjuvant chemotherapy can potentially treat systemic disease to lower the risk of distant spread, and allow the identification of patients with biologically aggressive tumours that progress on chemotherapy that would not benefit from liver surgery.

Innovations and breakthroughs

In the present study, patients with bilobar disease who underwent surgery had a significantly better overall survival compared to patients who failed down-staged chemotherapy and/or treated with palliative chemotherapy.

Applications

Patients with bilobar CRLM treated with liver resection as a primary treatment or following down-staging therapy have a better overall survival compared to patients who failed down-staging therapy and/or treated with palliative chemotherapy.

Peer-review

This article is interesting but I think epidemiological data are more interesting

than univariate and multivariate analysis, which is the part highlighted by the authors. Structure of the manuscript is correct.

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P- Reviewer: Lorenzo D **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Li D

World Journal of Hepatology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i34.1511

World J Hepatol 2016 December 8; 8(34): 1511-1520
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Retrospective Study

On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B

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Supported by Major Science and Technology Special Project of China Twelfth Five-year Plan, Nos. 2013ZX10002004 and 2012ZX10002003.

Institutional review board statement: The study was approved by the Institutional Review Board of Peking University Health Science Center.

Informed consent statement: All recruited patients signed written informed consents.

Conflict-of-interest statement: The authors declare that they have no competing interests.

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

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Received: August 12, 2016
Peer-review started: August 13, 2016
First decision: September 13, 2016
Revised: September 26, 2016
Accepted: October 22, 2016
Article in press: October 24, 2016
Published online: December 8, 2016

Abstract

AIM

To investigate potential predictors for treatment response to nucleos(t)ide analogues (NAs) in hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) patients.

METHODS

Seventy-six HBeAg-positive CHB patients received 96-wk

NAs optimized therapy (lamivudine and adefovir dipivoxil) were studied retrospectively. Serum hepatitis B surface antigen, HBeAg, hepatitis B core antibody, hepatitis B virus (HBV) DNA and alanine aminotransferase levels were quantitatively measured before and during the treatment at 12 and 24 wk. Stepwise logistic regression analyses were performed to identify predictors for treatment response, and areas under the receiver operating characteristic curves (AUROC) of the independent predictors were calculated.

RESULTS

Forty-three CHB patients (56.6%) achieved virological response (VR: HBV DNA $\leqslant$ 300 copies/mL) and 15 patients (19.7%) developed HBeAg seroconversion (SC) after the 96-wk NAs treatment. The HBeAg level (OR = 0.45, P = 0.003) as well as its declined value (OR = 2.03, P = 0.024) at 24-wk independently predicted VR, with the AUROC of 0.788 and 0.736, respectively. The combination of HBeAg titer < 1.3 lg PEIU/mL and its decreased value > 1.6 lg PEIU/mL at 24-wk predicted VR with a sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of 85%, 100%, 100% and 83%, respectively, and the AUROC increased to 0.923. The HBeAg level (OR = 0.37, P = 0.013) as well as its declined value (OR = 2.02, P = 0.012) at 24-wk also independently predicted HBeAg SC, with the AUROC of 0.828 and 0.814, respectively. The HBeAg titer < -0.5 lg PEIU/mL combined with its declined value > 2.2 lg PEIU/mL at 24-wk predicted HBeAg SC with a sensitivity, specificity, PPV, NPV of 88%, 98%, 88% and 98%, respectively, and the AUROC reached 0.928.

CONCLUSION

The combination of HBeAg level and its declined value at 24-wk may be used as a reference parameter to optimize NAs therapy.

Key words: Response predictor; Quantitative detection; Hepatitis B e antigen; Hepatitis B virus DNA; Chronic hepatitis B; Nucleos(t)ide analogues

Core tip: Few studies have systematically evaluated quantitative hepatitis B surface antigen, hepatitis B e antigen (HBeAg), hepatitis B core antibody, hepatitis B virus DNA and alanine aminotransferase for predicting treatment response to nucleos(t)ide analogues (NAs) in HBeAg-positive chronic hepatitis B (CHB). In this study, on-treatment HBeAg level as well as its declined value at 24-wk were identified to be the best predictors not only for 96-wk virological response (VR) but also for HBeAg seroconversion (SC). The combination of HBeAg level and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively. Thus monitoring an early on-treatment HBeAg level and its decline may help to optimize NAs therapy for CHB patients.

Gao YH, Meng QH, Zhang ZQ, Zhao P, Shang QH, Yuan Q, Li Y, Deng J, Li T, Liu XE, Zhuang H. On-treatment quantitative hepatitis B e antigen predicted response to nucleos(t)ide analogues in chronic hepatitis B. *World J Hepatol* 2016; 8(34): 1511-1520 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1511.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1511

INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem and an estimated 240 million persons are chronically infected worldwide, among which 20%-30% will develop cirrhosis and hepatocellular carcinoma (HCC)-the major complications of chronic hepatitis B (CHB)[1]. Antiviral treatment has been proved to be an effective and potent way to reverse the process of liver fibrosis or cirrhosis and decrease the incidence rate of liver complications[2]. However, due to the persistence of HBV covalently closed circular DNA (cccDNA) in the nucleus of infected hepatocytes, HBV cannot be completely eradicated by current antiviral drugs, and a long-term treatment are necessary for most patients. It is now clear that sustained viral suppression and hepatitis B e antigen (HBeAg) seroconversion (SC) are two important markers of treatment response for CHB patients receiving antiviral therapy, which is usually associated with a good long-term outcome[3]. Generally, after a 1-year course of the current available nucleos(t)ide analogues (NAs) or peginterferon (Peg-IFN) therapy, 7%-76% of patients achieved undetectable serum HBV DNA and 16%-32% developed HBeAg SC for patients with HBeAg-positive CHB[3]. Therefore, it is crucial to identify pre-treatment and early on-treatment biomarkers that can effectively predict long-term treatment response and use these biomarkers to choose appropriate antiviral drugs and treatment regimens to optimize therapy and improve efficacy.

Serum HBV DNA is the most widely used virological marker in the management of CHB patients[2,3]. A study from Zeuzem *et al*[4] reported that both baseline ALT $\geqslant$ 2 × upper limit of normal (ULN) (OR = 2.47, P = 0.0012) and non-detectable serum HBV DNA at treatment week 24 (OR = 2.61, P < 0.001) were associated with HBeAg SC after 2-year telbivudine (LdT) treatment, and among patients with non-detectable serum HBV DNA at 24-wk as well as favorable pretreatment characteristics [alanine aminotransferase (ALT) $\geqslant$ 2 × ULN and HBV DNA < 9 lg copies/mL], 52% obtained HBeAg SC at 2-year of therapy[4]. However, the detection of serum HBV DNA is costly and may not always objectively serve as a reliable indicator of sustained response to antiviral therapy[5]. Unlike HBV DNA, serum hepatitis B surface antigen (HBsAg), HBeAg and hepatitis B core antibody (anti-HBc) are classical serological markers for HBV infection and are used in clinical diagnosis routinely. The level of HBsAg was identified as an outcome predictor for Peg-IFN therapy among HBeAg-positive CHB patients[6]; however,

its predictive value in NAs treatment was inconsistent based on the reported data[7,8]. Serum HBeAg level was proposed to be a better outcome predictor for NAs treatment according to recent studies[8-12]. However, most of studies applied the semi-quantitative measurement of HBeAg, and some had a limited sample size or a short period of follow-up. Thus the predictive value of the quantitative HBeAg level needs to be further evaluated. In addition, benefiting from a newly developed double-sandwich anti-HBc immunoassay, anti-HBc quantification was identified as a novel biomarker for predicting treatment response[13]. Nevertheless, very few studies have systematically evaluated the predictive power of these biomarkers for NAs treatment response.

In the current study, HBeAg-positive CHB patients received 96-wk NAs optimized therapy [lamivudine (LAM) and adefovir dipivoxil (ADV)] were retrospectively investigated. Serum HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were quantitatively tested, and the baseline as well as early on-treatment levels of these parameters were analyzed using logistic regression model to assess their functions in predicting 96-wk virological response (VR) and HBeAg SC.

MATERIALS AND METHODS

Patients

We retrospectively analyzed a cohort of HBeAg-positive CHB patients who underwent the 96-wk LAM and ADV optimized therapy between 2011 and 2014 in China. The treatment was continued for CHB patients after week 96, and the data were not available from the patients after 96 wk. The inclusion criteria of patients enrolled for antiviral therapy were briefly summarized as follows: 18-65 years old, HBsAg positive for at least 6 mo, HBeAg positive and hepatitis B e antibody (anti-HBe) negative, 10^5 copies/mL $\leqslant$ HBV DNA $\leqslant$ 10^9 copies/mL, ALT $\geqslant$ 2 × ULN, and no history of antiviral therapy with NAs or interferon within previous six months. The patients were treated with LAM 100 mg/d, and ADV (10 mg/d) was added on when serum HBV DNA > 300 copies/mL at week 24 or a virological breakthrough (> 1 lg increase of serum HBV DNA from nadir or re-detectable after achieving an undetected level) occurred during the 96-wk treatment. Laboratory measurements were done every 12 wk before week 24, and every 24 wk from week 24 to week 96. The main endpoints were VR (defined as HBV DNA $\leqslant$ 300 copies/mL) and HBeAg SC at 96-wk. A total of 76 patients completed the 96-wk follow-up and finally included in the analyses. The study was approved by the Institutional Review Board of Peking University Health Science Center and conducted in accordance with the ethical standards of the Helsinki Declaration. The informed consents were obtained from recruited patients.

Laboratory measurements

HBV DNA was quantified by Roche COBAS TaqMan HBV test (Roche Diagnostics, Mannheim, Germany) with a linear range of 20-10^8 IU/mL (1 IU/mL = 5.82 copies/mL). Serological HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe) were measured by Chemiluminescent Microparticle ImmunoAssay using ARCHITECT i2000SR analyzer (Abbott Diagnostics, North Chicago, IL, United States). HBeAg level was quantified by World Health Organization (WHO) HBeAg reference standard (Paul-Ehrlich-Institute, Germany) also using ARCHITECT i2000SR analyzer[14]. Anti-HBc quantification was conducted by using a newly developed double-sandwich immunoassay (Wantai, Beijing, China) validated by WHO anti-HBc standards[15]. Biochemical tests (ALT, AST) were detected by the department of laboratory in four hospitals.

Statistical analysis

Categorical variables were compared using χ^2 or Fisher's exact tests. Continuous variables were compared using the Student's *t* test or Mann-Whitney test. Stepwise logistic regression analysis was performed to identify independent predictors for VR and HBeAg SC. The predictive value of the independent predictor was further evaluated using areas under the receiver operating characteristic curve (AUROC). The best cut-off value was determined in the condition of the highest Youden index (the sum of sensitivity and specificity minus 1). And the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated at the specified cut-off value. All analysis was done using SPSS 19.0 (SPSS, Chicago, IL, United States). A P value < 0.05 was considered as statistically significant.

RESULTS

Baseline characteristics

After the 96-wk NAs treatment, 56.6% (43/76) CHB patients achieved VR and 19.7% (15/76) patients developed HBeAg SC. Only 1 patient eliminated HBsAg. The baseline parameters such as age, HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were comparable between patients achieved HBeAg SC and those did not. Compared to patients without VR, those obtained VR had significantly higher baseline ALT level (247.95 ± 150.58 U/L *vs* 169.13 ± 156.56 U/L, P = 0.029), while HBsAg, HBeAg, anti-HBc, HBV DNA levels were not significantly different between two groups (Table 1).

Dynamic changes in serum levels of HBsAg, HBeAg, anti-HBc and HBV DNA during 96-wk treatment

Serum HBsAg, HBeAg, anti-HBc and HBV DNA levels were all significantly decreased from baseline to week 96 of therapy (HBsAg, 3.95 ± 0.83 lg IU/mL to 3.36 ± 0.86 lg IU/mL; HBeAg, 2.24 ± 1.31 lg PEIU/mL to 0.20 ± 1.13 lg PEIU/mL; anti-HBc, 4.77 ± 0.46 lg IU/mL to 3.45 ± 0.65 lg IU/mL; HBV DNA, 8.16 ± 1.34 lg copies/mL to 2.85 ± 1.36 lg copies/mL; all P < 0.001). Significant lower HBeAg and HBV DNA levels were found in patients with VR as compared with patients without VR at every

Table 1 Baseline clinical characteristics of patients with chronic hepatitis B according to treatment response after 96-wk nucleos(t)ide analogues therapy

Parameters	Overall	VR (+)	VR (-)	*P* value	SC (+)	SC (-)	*P* value
n (%)	76	43 (56.6)	33 (43.4)	-	15 (19.7)	61 (80.3)	-
Gender, female/male	20/56	15/28	5/28	0.053	5/10	15/46	0.491
Age, yr	32.63 ± 9.69	32.3 ± 9.83	33.06 ± 9.63	0.738	31.87 ± 11.6	32.82 ± 9.26	0.735
HBsAg, lg IU/mL	3.95 ± 0.83	3.96 ± 0.68	3.94 ± 1.00	0.881	3.93 ± 0.60	3.96 ± 0.88	0.385
HBeAg, lg PEIU/mL	2.24 ± 1.31	2.18 ± 1.38	2.32 ± 1.22	0.679	2.36 ± 1.41	2.21 ± 1.29	0.527
anti-HBc, lg IU/mL	4.77 ± 0.46	4.82 ± 0.43	4.71 ± 0.50	0.311	4.85 ± 0.44	4.75 ± 0.47	0.464
ALT, U/L	213.73 ± 157.17	247.95 ± 150.58	169.13 ± 156.56	0.029	216.49 ± 153.18	213.05 ± 159.38	0.94
ALT strata, ≥/< 5ULN	31/45	25/18	6/27	< 0.001	9/6	22/39	0.091
HBV DNA, lg copies/mL	8.16 ± 1.34	8.06 ± 1.45	8.3 ± 1.19	0.608	8.55 ± 0.91	8.07 ± 1.41	0.324
Genotype, C/non-C	53/23	31/12	22/11	0.61	10/5	43/18	0.773

VR (+)/VR (-), with/without virological response at week 96 (virological response: HBV DNA ≤ 300 copies/mL); SC (+)/SC (-), with/without HBeAg seroconversion at week 96. HBsAg: Hepatitis B surface antigen; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; VR: Virological response; SC: HBeAg seroconversion; ULN: Upper limit of normal.

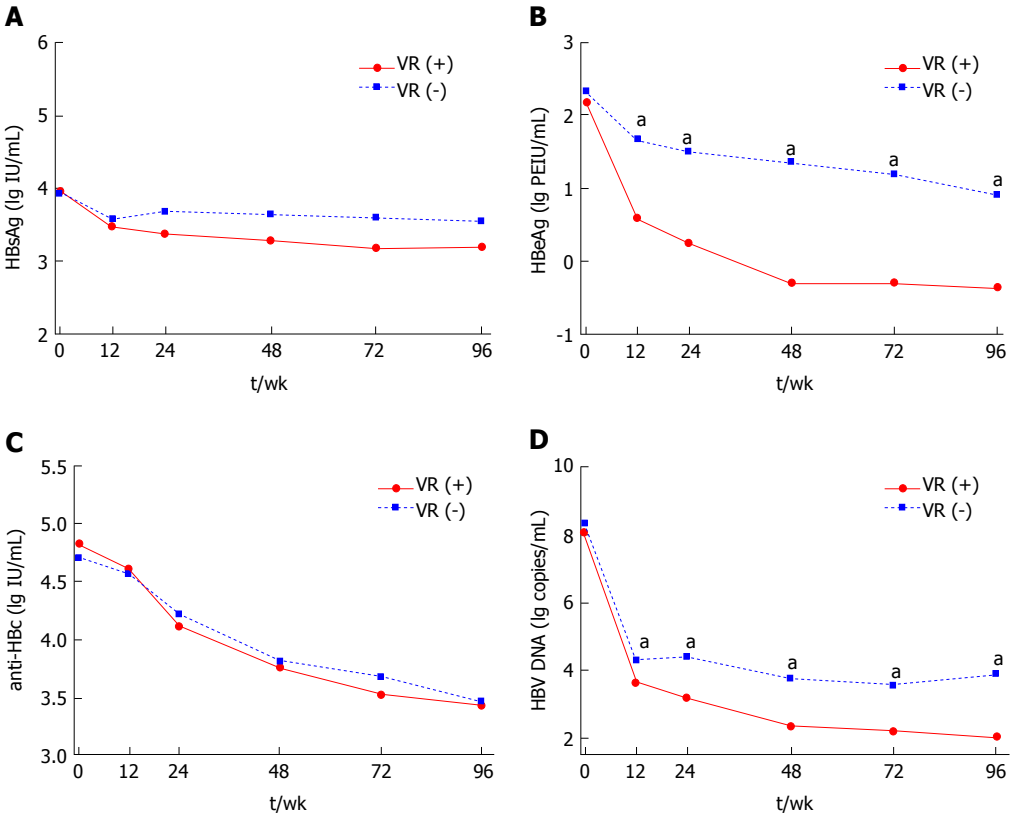

Figure 1 Dynamic changes of hepatitis B surface antigen (A), hepatitis B e antigen (B), hepatitis B core antibody (C) and hepatitis B virus DNA (D) levels from baseline to 96-wk in chronic hepatitis B patients received nucleos(t)ide analogues therapy stratified by virological response at 96-wk. [a]*P* < 0.05; VR (+): Virological response, HBV DNA ≤ 300 copies/mL; VR (-): Without virological response; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; anti-HBc: hepatitis B core antibody; HBV: Hepatitis B virus; VR: Virological response.

follow-up time-point except baseline ($P < 0.05$), while HBsAg and anti-HBc levels were comparable between two groups (Figure 1). Similarly, HBsAg and anti-HBc levels between patients with and without HBeAg SC were also comparable from baseline to week 96 (Figure 2A and C). However, HBeAg levels were significant lower in patients with HBeAg SC than in patients without HBeAg SC at every follow-up time-point except baseline ($P < 0.05$) (Figure 2B). And a significant difference in HBV DNA levels between patients with and without HBeAg SC was only observed at week 24, 72 and 96 ($P < 0.05$), as shown in Figure 2D.

Baseline and on-treatment parameters associated with 96-wk virological response

At baseline, ALT and ALT ≥ 5 × ULN were associated with VR according to univariate analysis, and multivariate analysis indicated that sex (OR = 3.76, 95%CI: 1.09-13.01, $P = 0.037$) and ALT ≥ 5 × ULN (OR = 7.09, 95%CI: 2.32-21.67, $P < 0.001$) independently predicted VR, re-

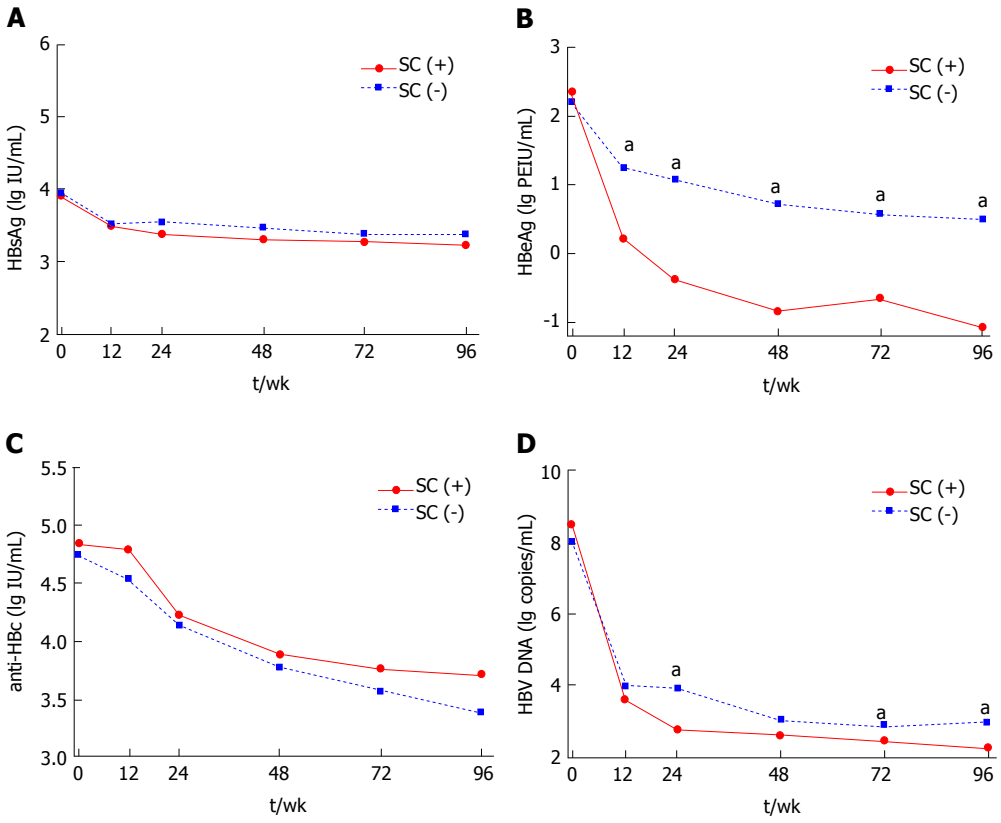

Figure 2 Dynamic changes of hepatitis B surface antigen (A), hepatitis B e antigen (B), hepatitis B core antibody (C) and hepatitis B virus DNA (D) levels from baseline to 96-wk in chronic hepatitis B patients received nucleos(t)ide analogues therapy stratified by hepatitis B e antigen seroconversion at 96-wk. [a]*P* < 0.05; SC (+): HBeAg seroconversion; SC (-): Without HBeAg seroconversion; HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; anti-HBc: hepatitis B core antibody; HBV: Hepatitis B virus; SC: HBeAg seroconversion.

spectively. At week 12, univariate analysis revealed that HBeAg, HBeAg decline, ALT decline, and HBV DNA were associated with VR, and multivariate analysis identified that HBeAg (OR = 0.62, 95%CI: 0.40-0.95, *P* = 0.03) and HBeAg decline (OR = 2.58, 95%CI: 1.25-5.33, *P* = 0.01) independently predicted VR, respectively. At week 24, HBeAg, HBeAg decline, ALT decline, HBV DNA and HBV DNA decline were associated with VR *via* univariate analysis, and multivariate analysis found that HBeAg (OR = 0.45, 95%CI: 0.27-0.77, *P* = 0.003) and HBeAg decline (OR = 2.03, 95%CI: 1.10-3.74, *P* = 0.024) independently predicted VR, respectively (Table 2).

Baseline and on-treatment parameters associated with 96-wk HBeAg seroconversion

At week 12, HBeAg, HBeAg decline, and HBV DNA decline were associated with HBeAg SC through univariate analysis, and HBeAg decline (OR = 2.47, 95%CI: 1.46-4.16, *P* = 0.001) independently predicted HBeAg SC *via* multivariate analysis. At week 24, univariate analysis presented that HBeAg, HBeAg decline, HBV DNA and HBV DNA decline were associated with HBeAg SC, and multivariate analysis identified that HBeAg (OR = 0.37, 95%CI: 0.17-0.81, *P* = 0.013) and HBeAg decline (OR = 2.02, 95%CI: 1.17-3.49, *P* = 0.012) independently predicted HBeAg SC, respectively (Table 2).

Based on the results of above analysis, both of the HBeAg level and its on-treatment declined value at 12-wk (or 24-wk) as independent predictors were further evaluated using AUROC for predicting 96-wk VR and HBeAg SC.

Predictive value of HBeAg titer as well as its declined value at week 12 and 24 for 96-wk virological response

At week 12, the HBeAg titer and its declined value predicted VR with an AUROC of 0.733 (95%CI: 0.617-0.849, *P* = 0.001) and 0.709 (95%CI: 0.590-0.827, *P* = 0.002), respectively, and the best cut-off value for the HBeAg titer and its decline was 0.8 lg PEIU/mL and 0.84 lg PEIU/mL, respectively. Twenty-two patients achieved HBeAg titer < 0.8 lg PEIU/mL as well as the declined value > 0.84 lg PEIU/mL at 12-wk, and among them 91% (20/22) reached VR, whereas only 29% obtained VR among 28 patients without meeting the above two standards. HBeAg titer combined with on-treatment decline at 12-wk predicted VR with an AUROC of 0.812 (95%CI: 0.687-0.936, *P* < 0.001) and the sensitivity, specificity, PPV, NPV was 71%, 91%, 91% and 71%, respectively.

At week 24, the HBeAg titer and its declined value predicted VR with an AUROC of 0.788 (95%CI: 0.683-0.892, *P* < 0.001) and 0.736 (95%CI: 0.620-0.851, *P* < 0.001), respectively, and the best cut-off value for the HBeAg titer and its decline was 1.3 lg PEIU/mL and 1.6 lg PEIU/mL, respectively. All the 22 patients with HBeAg titer < 1.3

Table 2 Baseline and on-treatment parameters associated with 96-wk virological response and hepatitis B e antigen seroconversion in chronic hepatitis B patients received nucleos(t)ide analogues therapy

Factors	VR				SC			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
Baseline								
Age	0.99 (0.95-1.04)	0.734	-	-	0.99 (0.93-1.05)	0.731	-	-
Sex, female/male	3 (0.96-9.38)	0.059	3.76 (1.09-13.01)	0.037	1.53 (0.45-5.20)	0.493	-	-
HBsAg (lg IU/mL)	1.04 (0.60-1.81)	0.879	-	-	0.96 (0.49-1.88)	0.904	-	-
HBeAg (lg PEIU/mL)	0.92 (0.65-1.31)	0.633	-	-	1.1 (0.70-1.72)	0.682	-	-
Anti-HBc (lg IU/mL)	1.68 (0.62-4.59)	0.308	-	-	1.6 (0.46-5.50)	0.459	-	-
HBV DNA (lg copies/mL)	0.87 (0.62-1.24)	0.451	-	-	1.38 (0.82-2.31)	0.221	-	-
ALT (U/L)	1.004 (1.000-1.007)	0.038	-	-	1 (0.99-1.01)	0.939	-	-
ALT strata, ≥/< 5ULN	6.25 (2.14-18.26)	< 0.001	7.09 (2.32-21.67)	< 0.001	2.66 (0.84-8.46)	0.098	-	-
Genotype, C/non-C	1.29 (0.48-3.46)	0.61	-	-	0.84 (0.25-2.80)	0.773	-	-
Week 12								
HBsAg (lg IU/mL)	0.83 (0.45-1.53)	0.551	-	-	0.95 (0.47-1.92)	0.882	-	-
HBsAg decline (lg IU/mL)	1.43 (0.72-2.84)	0.301	-	-	1.04 (0.46-2.35)	0.919	-	-
HBeAg (lg PEIU/mL)	0.5 (0.33-0.76)	0.001	0.62 (0.40-0.95)	0.03	0.5 (0.30-0.85)	0.011	-	-
HBeAg decline (lg PEIU/mL)	3.04 (1.55-5.98)	0.001	2.58 (1.25-5.33)	0.01	2.47 (1.46-4.16)	< 0.001	2.47 (1.46-4.16)	0.001
Anti-HBc (lg IU/mL)	1.14 (0.49-2.64)	0.764	-	-	2.56 (0.83-7.93)	0.102	-	-
Anti-HBc decline (lg IU/mL)	1.43 (0.45-4.59)	0.546	-	-	0.3 (0.06-1.51)	0.145	-	-
ALT (U/L)	0.99 (0.98-1.01)	0.221	-	-	1 (0.98-1.02)	0.876	-	-
ALT decline (U/L)	1.004 (1.00-1.01)	0.03	-	-	1 (0.99-1.01)	0.918	-	-
HBV DNA (lg copies/mL)	0.57 (0.36-0.90)	0.016	-	-	0.72 (0.42-1.25)	0.249	-	-
HBV DNA decline (lg copies/mL)	1.36 (0.90-2.03)	0.141	-	-	2.11 (1.16-3.84)	0.015	-	-
Week 24								
HBsAg (lg IU/mL)	0.61 (0.31-1.21)	0.155	-	-	0.84 (0.48-1.46)	0.535	-	-
HBsAg decline (lg IU/mL)	1.79 (0.94-3.42)	0.076	-	-	1.21 (0.62-2.37)	0.571	-	-
HBeAg (lg PEIU/mL)	0.39 (0.24-0.64)	< 0.001	0.45 (0.27-0.77)	0.003	0.28 (0.14-0.58)	< 0.001	0.37 (0.17-0.81)	0.013
HBeAg decline (lg PEIU/mL)	2.37 (1.41-3.99)	0.001	2.03 (1.10-3.74)	0.024	2.8 (1.64-4.78)	< 0.001	2.02 (1.17-3.49)	0.012
Anti-HBc (lg IU/mL)	0.73 (0.33-1.63)	0.448	-	-	1.29 (0.48-3.45)	0.608	-	-
Anti-HBc decline (lg IU/mL)	3.11 (0.99-3.79)	0.053	-	-	1.11 (0.31-3.99)	0.874	-	-
ALT (U/L)	0.99 (0.98-1.01)	0.477	-	-	0.99 (0.96-1.02)	0.516	-	-
ALT decline (U/L)	1.004 (1.00-1.01)	0.031	-	-	1 (0.99-1.01)	0.822	-	-
HBV DNA (lg copies/mL)	0.55 (0.37-0.80)	0.002	-	-	0.45 (0.23-0.86)	0.016	-	-
HBV DNA decline (lg copies/mL)	1.39 (1.02-1.88)	0.035	-	-	2.39 (1.39-4.11)	0.002	-	-

HBsAg: Hepatitis B surface antigen; HBeAg: Hepatitis B e antigen; Anti-HBc: Hepatitis B core antibody; ALT: Alanine aminotransferase; HBV: Hepatitis B virus; VR: Virological response (HBV DNA ≤ 300 copies/mL); SC: HBeAg seroconversion; ULN: Upper limit of normal.

lg PEIU/mL and the declined value > 1.6 lg PEIU/mL at 24-wk achieved VR, whereas only 17% of 23 patients without meeting the above two standards obtained VR. HBeAg titer combined with its decline at 24-wk strongly predicted VR with an AUROC of 0.923 (95%CI: 0.838-1.000, $P < 0.001$) and the sensitivity, specificity, PPV, NPV was 85%, 100%, 100% and 83%, respectively (Table 3).

Predictive value of HBeAg titer as well as its declined value at week 12 and 24 for 96-wk HBeAg seroconversion

At week 12, the HBeAg declined value predicted HBeAg SC with an AUROC of 0.767 (95%CI: 0.623-0.911, $P = 0.001$), and the best cut-off value for HBeAg decline was 1.8 lg PEIU/mL. The HBeAg declined value > 1.8 lg PEIU/mL at 12-wk predicted HBeAg SC with a sensitivity, specificity, PPV, NPV of 60%, 87%, 53% and 90%, respectively.

At week 24, the HBeAg titer and its declined value predicted HBeAg SC with an AUROC of 0.828 (95%CI: 0.712-0.944, $P < 0.001$) and 0.814 (95%CI: 0.676-0.953, $P < 0.001$), respectively, and the best cut-off value for the HBeAg titer and its decline was -0.5 lg PEIU/mL and 2.2 lg PEIU/mL, respectively. Eight patients achieved HBeAg titer < -0.5 lg PEIU/mL and the declined value > 2.2 lg PEIU/mL at 24-wk, among them 88% (7/8) achieved HBeAg SC; whereas only 2% of 51 patients who did not meet the above two standards obtained HBeAg SC. HBeAg titer combined with its declined value at 24-wk strongly predicted HBeAg SC with an AUROC of 0.928 (95%CI: 0.791-1.000, $P < 0.001$) and the sensitivity, specificity, PPV, NPV was 88%, 98%, 88% and 98% (Table 4).

DISCUSSION

Achieving a long-term suppression of serum HBV DNA through antiviral therapy is one of important targets of treatment for CHB patients. Several studies tried to investigate the value of an early on-treatment change of HBV markers such as HBeAg in predicting VR to NAs therapy. A study presented that the HBeAg titer decreased by 1 lg PEIU/mL at 12-wk predicted VR

Table 3 Predictive value of hepatitis B e antigen titer as well as its declined value at week 12 and 24 for virological response after 96-wk nucleos(t)ide analogues therapy

Factors	ROC		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	AUROC (95%CI)	*P* value				
Week 12						
HBeAg < 0.8 lg PEIU/mL	0.733 (0.617-0.849)	0.001	0.63 (0.48-0.78)	0.81 (0.67-0.96)	0.82 (0.68-0.96)	0.62 (0.47-0.77)
HBeAg decline > 0.84 lg PEIU/mL	0.709 (0.590-0.827)	0.002	0.65 (0.50-0.80)	0.75 (0.59-0.91)	0.78 (0.64-0.92)	0.62 (0.46-0.78)
Combined the above	0.812 (0.687-0.936)	< 0.001	0.71 (0.54-0.89)	0.91 (0.78-1.00)	0.91 (0.78-1.00)	0.71 (0.54-0.89)
Week 24						
HBeAg < 1.3 lg PEIU/mL	0.788 (0.683-0.892)	< 0.001	0.88 (0.78-0.98)	0.64 (0.46-0.81)	0.76 (0.63-0.88)	0.81 (0.65-0.97)
HBeAg decline > 1.6 lg PEIU/mL	0.736 (0.620-0.851)	< 0.001	0.55 (0.39-0.70)	0.94 (0.85-1.00)	0.92 (0.81-1.00)	0.62 (0.48-0.76)
Combined the above	0.923 (0.838-1.000)	< 0.001	0.85 (0.70-0.99)	1	1	0.83 (0.66-0.99)

HBeAg: Hepatitis B e antigen; VR: Virological response (HBV DNA ⩽ 300 copies/mL); AUROC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value.

Table 4 Predictive value of hepatitis B e antigen titer as well as its declined value at week 12 and 24 for hepatitis B e antigen seroconversion after 96-wk nucleos(t)ide analogues therapy

Factors	ROC		Sensitivity (95%CI)	Specificity (95%CI)	PPV (95%CI)	NPV (95%CI)
	AUROC (95%CI)	*P* value				
Week 12						
HBeAg decline > 1.8 lg PEIU/mL	0.767 (0.623-0.911)	0.001	0.6 (0.32-0.88)	0.87 (0.78-0.96)	0.53 (0.26-0.79)	0.9 (0.82-0.98)
Week 24						
HBeAg < -0.5 lg PEIU/mL	0.828 (0.712-0.944)	< 0.001	0.67 (0.40-0.94)	0.92 (0.84-0.99)	0.67 (0.40-0.94)	0.92 (0.84-0.99)
HBeAg decline > 2.2 lg PEIU/mL	0.814 (0.676-0.953)	< 0.001	0.73 (0.48-0.99)	0.9 (0.82-0.98)	0.65 (0.39-0.90)	0.93 (0.86-1.00)
Combined the above	0.928 (0.791-1.000)	< 0.001	0.88 (0.58-1.00)	0.98 (0.94-1.00)	0.88 (0.58-1.00)	0.98 (0.94-1.00)

HBeAg: Hepatitis B e antigen; SC: HBeAg seroconversion; AUROC: Area under the receiver operating characteristic curve; PPV: Positive predictive value; NPV: Negative predictive value.

(HBV DNA < 20 IU/mL) after 48-wk entecavir (ETV) therapy with a sensitivity, specificity, PPV, NPV of 67.6%, 87.9%, 86.2% and 70.7%, respectively[10]. However, the predictive value of HBeAg titer and its declined value at 12-wk for a long-term treatment response (⩾ 2 years) was not reported. In this study, we found that HBeAg titer < 0.8 lg PEIU/mL combined with its declined value > 0.84 lg PEIU/mL at 12-wk predicted VR after 96-wk LAM and ADV optimized therapy with a sensitivity, specificity, PPV, NPV of 71%, 91%, 91% and 71%, respectively, and accompanied with an AUROC of 0.812. In addition, although some studies indicated that the on-treatment HBeAg level or declined value at 24-wk was a predictor for NAs treatment response[9,12], the sample size was small or HBeAg was detected using a semi-quantitative method which cannot accurately quantify HBeAg level, thus limited the validity of the prediction. For example, Zhang *et al*[12] detected serum HBeAg semi-quantitatively and found that the on-treatment declined value of HBeAg (> 65%) at 24-wk was the best predictor for treatment response (HBeAg seroconversion and accompanied by undetectable serum HBV DNA) after 96-wk ETV therapy, and the PPV, NPV, AUROC was 83.3%, 93.6% and 0.885, respectively. However, the finding may have difficulty in applying clinical practice since the absent of accurate quantitative HBeAg levels. In our study, the HBeAg titer (OR = 0.45, *P* = 0.003) and its declined value (OR = 2.03, *P* = 0.024) at 24-wk were found to predict 96-wk VR with the AUROC of 0.788 and 0.736, respectively. Moreover, the combination of HBeAg titer < 1.3 lg PEIU/mL and its decrease > 1.6 lg PEIU/mL at 24-wk predicted 96-wk VR with a sensitivity, specificity, PPV, NPV of 85%, 100%, 100% and 83%, respectively, and the AUROC increased to 0.923, which had a better predictive value than the semi-quantitative method reported by Zhang *et al*[12]. With respect to the cost of semi-quantitative and quantitative tests of HBeAg, it is comparable between them. Twenty-two patients in our cohort matched the combination standard and they all achieved 96-wk VR. The results suggested that if patients reached HBeAg titer < 1.3 lg PEIU/mL and declined > 1.6 lg PEIU/mL at 24-wk, there will be a better viral suppression during the continued NAs therapy.

In addition to achieve long-term suppression in serum HBV DNA, HBeAg SC is another important indicator to evaluate the efficacy of antiviral therapy in HBeAg-positive CHB patients. In our study, the declined value of HBeAg at 12-wk (OR = 2.47, *P* = 0.001) independently predicted 96-wk HBeAg SC with an AUROC of 0.767, and for the predictive value of HBeAg declined value at 24-wk, an AUROC increased to 0.814. Lee *et al*[8] found that the decline of HBeAg at month 6 was a strongest predictor for HBeAg SC after 2 years of ETV treatment with an AUROC of 0.820 (*P* = 0.004), which was similar to our result (AUROC = 0.814). However, Lee's study used the declined value of HBeAg alone to predict treatment response and did not consider combining other indicators. Our data showed that both HBeAg titer as well

as its declined value at 24-wk were independent predictors for 96-wk HBeAg SC, and it strongly predicted HBeAg SC with an AUROC of 0.928 if combining HBeAg titer < -0.5 lg PEIU/mL and declined value > 2.2 lg PEIU/mL at 24-wk. A study from Shin *et al*[11] revealed that HBeAg titer < 0.62 lg PEIU/mL after 48 wk of ETV therapy was a strongest predictor for HBeAg SC at year 3 with an AUROC of 0.86 (P < 0.001), which was inferior to the combined prediction validity of HBeAg level and declined value at 24-wk in our study (AUROC = 0.928). Our study presented that 88% (7/8) of patients with HBeAg titer < -0.5 lg PEIU/mL and declined value > 2.2 lg PEIU/mL at 24-wk achieved 96-wk HBeAg SC, whereas only 2% (1/51) of patients without meting above standards obtained HBeAg SC, thus got a NPV of 98%. Among the 51 patients with unfavorable 24-wk HBeAg titer, 46 patients were added on ADV at 24-wk due to serum HBV DNA > 300 copies/mL. The results suggested that patients with HBeAg titer > -0.5 lg PEIU/mL as well as declined value < 2.2 lg PEIU/mL after 24 wk of LAM therapy will rarely achieve HBeAg SC during the following NAs treatment, even adding on ADV still cannot improve the efficacy. So the patients with unfavorable 24-wk HBeAg titer should be considered to switch to other drugs or use other regimens for a better treatment outcome.

Results of logistic regression analysis in the current study showed that no baseline parameters were associated with 96-wk HBeAg SC, which was consistent with Lee's report[8], who did not find correlations between baseline level of HBeAg (or HBsAg, HBV DNA) and HBeAg SC after 2 years of ETV therapy either. However, other studies showed that pre-treatment serum HBeAg was associated with HBeAg SC during ETV treatment[10,12]. The inconsistent results may due to that treatment period was relatively short or HBeAg was detected semi-quantitatively in the latter's studies[10,12]. Several recent studies revealed that B lymphocytes played a key role in the regulation of host immune responses to HBV, while anti-HBc was produced and secreted by hepatitis B core antigen-specific B lymphocytes, therefore the serum anti-HBc level may be a surrogate marker for the host immune response to HBV[16,17]. Fan *et al*[13] demonstrated that the baseline level of anti-HBc independently predicted HBeAg SC after 2 years of LdT and ADV optimized therapy (OR = 1.99, P = 0.001). However, our results presented that baseline anti-HBc was not associated with HBeAg SC or VR after 96-wk LAM and ADV optimized therapy (HBeAg SC, OR = 1.60, P = 0.459; VR, OR = 1.68, P = 0.308). The discrepancy may due to the little difference of baseline anti-HBc level between patients with and without HBeAg SC (0.10 lg IU/mL) in our study, while the difference in baseline anti-HBc was 0.24 lg IU/mL in Fan's study. Besides, previous studies identified that the elevation of ALT was contributed to T lymphocyte mediated hepatolysis occurred in CHB patients, therefore baseline ALT level may reflect T lymphocyte immune response to HBV which is related to the outcome after antiviral treatment[18]. Zeuzem *et al*[4] reported that the pre-treatment ALT level was associated with VR and baseline ALT $\geqslant$ 2 × ULN could independently predict non-detectable serum HBV DNA after 2 years of LdT treatment (OR = 2.00, P = 0.0071). In agreement with these findings, we also found that baseline ALT $\geqslant$ 5 × ULN independently predicted 96-wk VR (OR = 7.09, P < 0.001) with the AUROC of 0.700 (P = 0.003, data not shown), which suggested that patients with a higher pre-treatment ALT level maybe situated in a better immune status and will have an active virological response to NAs treatment.

All the parameters (HBsAg, HBeAg, anti-HBc and HBV DNA) presented continuous descent during the 96-wk treatment. Serum HBV DNA dropped significantly from baseline 8.16 lg copies/mL to 96-wk 2.85 lg copies/mL (P < 0.001) and 56.6% (43/76) patients obtained HBV DNA $\leqslant$ 300 copies/mL in our cohort, showing a similar data to the GLOBE study[19], in which 55.6% HBeAg-positive patients achieved HBV DNA < 300 copies/mL with serum HBV DNA declined by 6.1 lg copies/mL after 2-year LdT treatment. The antiviral effect of NAs agents was reducing viral replication by the inhibition of HBV DNA polymerase, but having limited impacts on the level of HBsAg[20]. In our study, HBsAg decreased slowly by 0.59 lg IU/mL after 96-wk therapy. Heathcote *et al*[21] also reported that the mean HBsAg level in HBeAg-positive CHB patients decreased by 0.66 lg IU/mL after 2 years of tenofovir disoproxil (TDF) treatment, which was similar to our data. In addition, anti-HBc level decreased from baseline 4.77 lg IU/mL to 96-wk 3.45 lg IU/mL in our cohort, with an average decrease of 1.32 lg IU/mL. In Fan's report[13], 1.26 lg IU/mL of an average decrease of anti-HBc level was observed in HBeAg-positive CHB patients after 104-wk LdT and ADV optimized therapy (baseline 4.20 lg IU/mL to 104-wk 2.94 lg IU/mL). The average declines in anti-HBc levels were comparable between the two studies, although the baseline anti-HBc level was relatively higher in our study. Concerning the dynamic change of HBeAg, Shin *et al*[11] reported that HBeAg level reduced from baseline 2.23 lg PEIU/mL to 0.96 lg PEIU/mL after 96-wk ETV therapy and 17.1% (14/82) achieved HBeAg SC. In the present study, HBeAg level decreased from baseline 2.24 lg PEIU/mL to 96-wk 0.20 lg PEIU/mL and 19.7% (15/76) obtained HBeAg SC, which presented comparable HBeAg reduction and HBeAg SC rate as compared to Shin's study.

Comparing dynamic changes in HBeAg level between patients with and those without 96-wk VR (or HBeAg SC), our results showed that baseline HBeAg levels were comparable between two groups. Further, HBeAg levels in patients obtained 96-wk VR (or HBeAg SC) were significantly lower than those without VR (or HBeAg SC) from 12-wk to the end of follow-up (P < 0.05, as shown in Figures 1B and 2B). With respect to dynamic changes of HBV DNA levels, the same trend of decline was observed. The patients achieved 96-wk VR had a significant lower HBV DNA level at every follow-up time-point except baseline when compared with those

without VR ($P < 0.05$), while the significant differences in HBV DNA levels between patients with and without HBeAg SC were only observed at week 24, 72 and 96 (Figures 1D and 2D). These results indicated that HBeAg may have a better predictive value than HBV DNA for treatment response in HBeAg-positive CHB. At the same time, our finding showed that both the HBV DNA level as well as its declined value at 24-wk (or 12-wk) were significantly associated with 96-wk VR and HBeAg SC when performed univariate analysis ($P < 0.05$). However, HBV DNA levels and its declines would not associate with 96-wk VR and HBeAg SC anymore if all parameters including HBeAg levels and its declines were enrolled in multivariate analysis. Other studies also pointed out that HBeAg levels as well as its declines may maintain a better predictive value than HBV DNA to predict NAs treatment response in HBeAg-positive CHB patients[10,11]. HBeAg is generated by transcription and translation of HBV cccDNA, and previous studies had reported that serum HBeAg level was significantly correlated with intrahepatic HBV cccDNA ($r = 0.507$, $P = 0.010$)[22]. Thus, the decline in serum HBeAg level may reflect the reduction of HBV cccDNA and represented a good treatment outcome. In addition, viral persistence and the development of CHB was associated with viral manipulation and evasion of the host's immune system, while HBeAg has been reported to attenuate the host immune response to the nucleocapsid protein and down-regulate the innate and adaptive immune responses[23,24]. Therefore, the decline in HBeAg level might weaken this effect and thus the patients may present a better immune control for HBV infection.

There were some limitations in our study. Firstly, LAM and ADV used in the cohort are no longer the first-line antiviral drugs. However, NAs drugs have a similar antiviral mechanism, and HBeAg SC rates in CHB patients are comparable between ETV (or TDF) therapy and an optimized therapy with LAM and ADV. Hence, we propose that the combination parameter of HBeAg level and its declined value at 24-wk might be used as a reference parameter to predict the efficacy of ETV or TDF treatment. Secondly, treatment endpoint evaluated in our study was on-treatment response. For virological response, off-treatment response may be more important than on-treatment response.

To our knowledge, this is the first report that identified the combination of on-treatment quantitative HBeAg level and its decline as the predictor of positive response to long term NAs therapy among HBeAg-positive CHB patients. In particular, the combination of HBeAg titer and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively. This combination predictor was identified through a retrospective investigation based on the cohort of HBeAg-positive CHB patients received LAM and ADV optimized therapy. We will conduct a prospective study to evaluate and confirm the predictive validity of the combination parameter in the cohort of ETV or TDF therapy in the future, and anticipate that the combination of on-treatment HBeAg level and its declined value could serve as a reference parameter to guide NAs therapy.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Shumei Yun, from Missouri Department of Health and Senior Services, Jefferson City, MO, United States for proofreading and editing the manuscript.

COMMENTS

Background

The antiviral effect of current available nucleos(t)ide analogues (NAs) or peginterferon drugs are not satisfied. To improve the efficacy, it is crucial to explore the pre-treatment and early on-treatment biomarkers to effectively predict long-term treatment response. However, very few studies have systematically evaluated the predictive power of quantitative hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antibody (anti-HBc), hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) for NAs treatment response.

Research frontiers

In the current study, serum HBsAg, HBeAg, anti-HBc, HBV DNA and ALT were quantitatively tested during the 96-wk NAs therapy, and the baseline as well as early on-treatment levels of these parameters were comprehensively analyzed to assess their functions in predicting 96-wk virological response (VR) and HBeAg seroconversion (SC).

Innovations and breakthroughs

This is the first report that the combination parameter of on-treatment quantitative HBeAg level and its declined value was found to predict 96-wk treatment response to lamivudine and adefovir dipivoxil optimized therapy for HBeAg-positive chronic hepatitis B (CHB) patients. In particular, the combination of HBeAg titer and its decline at 24-wk strongly predicted 96-wk VR and HBeAg SC with the AUROC of 0.923 and 0.928, respectively.

Applications

The combination variable of on-treatment HBeAg level and its declined value may serve as a reference parameter to optimize NAs therapy for HBeAg-positive CHB patients.

Peer-review

HBV is a major cause of chronic liver disease including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Reactivation of HBV is closely related to acute exacerbation of HBV carriers which sometimes leads to liver failure. Introduction of NAs dramatically changed the landscape of HBV treatment that is useful weapon to suppress HBV replication. NAs can only suppress HBV replication but not eradicate HBV. Therefore several problems remains including setting of endpoint and adequate cessation of NAs.

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P- Reviewer: Inoue K **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Li D

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DOI: 10.4254/wjh.v8.i34.1521

World J Hepatol 2016 December 8; 8(34): 1521-1528
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Observational Study

Seroprevalence of hepatitis B surface antigen in pregnant women attending antenatal clinic in Honiara Solomon Islands, 2015

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Author contributions: Getahun A prepared the proposal, designed the study, drafted and revised the manuscript; Panda N identified the topic and supervised the study implementation; Baekalia M, Puiahi E, Tahani D and Manongi D performed the laboratory tests; Lee A reviewed and edited the manuscript and provided technical support; Khan S design the study, analyzed and interpreted the data; all authors contributed to the write up and the revision of the manuscript.

Supported by University Research Publication Committee (URPC), Fiji National University, No. ACT339; and Hepatitis B Free (HBF) Ltd, Australia, No. 25 167 817 389.

Institutional review board statement: The study was reviewed and approved by the College Research and Ethics Committee of Fiji National University (FNU) and the National Health Research and Ethics Committee of MHMS, Solomon Islands (HRC14/28).

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

Conflict-of-interest statement: The study team declared no conflict of interest.

Data sharing statement: The de-identified dataset is available from the corresponding author at aneley.getahun@fnu.ac.fj.

Manuscript source: Invited manuscript

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Received: June 27, 2016
Peer-review started: June 29, 2016
First decision: August 5, 2016
Revised: September 9, 2016
Accepted: October 17, 2016
Article in press: October 18, 2016
Published online: December 8, 2016

Abstract

AIM

To determine the seroprevalence of hepatitis B surface antigen (HBsAg) among pregnant women attending antenatal clinic in Honiara, Solomon Islands.

METHODS

This descriptive cross-sectional study was carried out in seven area health centers in Honiara. From March to June

2015, identification of eligible pregnant women in each site was conducted using systematic random sampling technique. A total of 243 pregnant women who gave written informed consent were enrolled. Standardized tool was used to record demographics, obstetric history and serology results. HBsAg and hepatitis B e antigen (HBeAg) were tested using point-of-care rapid diagnostic test. All HBsAg positive samples were verified using enzyme-linked immunosorbent assay.

RESULTS

The mean age of participants was 26 ± 6 years. The overall hepatitis HBsAg prevalence was 13.8% with higher rate (22%) reported in women between 30-34 years of age. Majority of HBsAg positive participants were Melanesians (29 out for 33). None of the pregnant women in the 15-19 years and ⩾ 40 years tested positive for HBsAg. There was no statistically significant difference in HBsAg prevalence by age, ethnicity, education and residential location. The overall HBeAg seroprevalence was 36.7%. Women between 20-24 years of age had the highest rate of 54.5%. Low level of knowledge about hepatitis B vaccination was reputed. Overall, 54.6% of participants were not aware of their hepatitis B vaccination status and only 65.2% of mothers reported their child had been vaccinated.

CONCLUSION

Hepatitis B is a disease of public health importance in Solomon Islands and emphasize the need for integrated preventative interventions for its control.

Key words: Hepatitis B; Chronic hepatitis; Hepatitis B surface antigen; Hepatitis B e antigen; Seroprevalence; Pregnant women; Solomon Islands

Core tip: The objective of this study was to determine the prevalence of chronic hepatitis B infection in a cohort of antenatal women in Honiara. The overall hepatitis HBsAg and hepatitis B e antigen (HBeAg) prevalence was 13.8% and 36.7%, respectively. Our study for the first time reported HBeAg prevalence in pregnant women. Furthermore, the study revealed low level of knowledge about hepatitis B vaccination whereby 54.6% of participants were not aware of their vaccination status. Hepatitis B is a disease of public health importance in Solomon Islands and emphasize the need for efficient delivery of integrated services for its prevention and control.

Getahun A, Baekalia M, Panda N, Lee A, Puiahi E, Khan S, Tahani D, Manongi D. Seroprevalence of hepatitis B surface antigen in pregnant women attending antenatal clinic in Honiara Solomon Islands, 2015. *World J Hepatol* 2016; 8(34): 1521-1528 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1521.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1521

INTRODUCTION

Hepatitis B virus (HBV) infection occurs worldwide. It is estimated that over two billion people have been infected with 360 million individuals who remain chronically infected[1]. There are around 600000 deaths every year due to the consequences of hepatitis B[2]. Hepatitis B is highly endemic in Africa, South-East Asia and the Pacific (excluding Japan, Australia and New Zealand), the Amazon Basin and parts of the Middle East, central Asia and some countries in Eastern Europe. In these areas, about 70% to 90% of the population are exposed to the virus during the first four decades of life, and eight to 20% of people then become chronically infected[3,4]. It is estimated that around 45% of the world population live in areas where there are high levels of hepatitis B infection[4].

Hepatitis B virus is a DNA virus classified within the family hepadnaviridae. It is transmitted by percutaneous and permucosal exposure to infected blood and other body fluids, mainly semen and vaginal fluid[5]. The predominant mode of transmission varies depending on the endemicity of the disease in a given population. In areas of high endemicity, HBV is mostly acquired in early childhood from mother to child at birth or from person to person in early childhood. Exposure at an earlier age increases likelihood of progress to chronic infection. In low endemic areas, sexual transmission is the predominant route[1,2].

Vaccination is the most cost effective way for the prevention of hepatitis B. The World Health Organization (WHO) recommends the inclusion of hepatitis B vaccine in routine immunization programs in all countries[1]. Four doses of vaccines are given, the first one within 24 h after birth followed by three vaccines in infancy. The complete vaccine series induces protective antibody levels in more than 95% of infants, children and young adults. Protection lasts at least 20 years and is likely lifelong[1].

The Solomon Islands is located in the southwest Pacific, stretching about 1700 km from the eastern tip of Papua New Guinea to the northern-most islands of Vanuatu. The Island chain is comprised of nine administrative provinces: Guadalcanal, Malaita, Western, Rennell Bellona, Central, Makara Ulawa, Choiseul, Isabel and Temotu. According to the 2009 census, total population is estimated at 515870 of which 80% live in rural areas[6]. In 2013, Solomon Islands was ranked 157 out of 187 countries and territories on the Human Development Index. The country is one of the world's least developed countries and the 2013 Human Development Index was below the average for countries in the low human development group was well as below the average for countries in East Asia and the Pacific region[7].

The current WHO model for Ante Natal Care (ANC) recommend that all pregnant women have at least four ANC assessments by a skilled attendant. WHO also encourages countries to develop national guidelines to

outline the essential minimum packages of ANC based on the local epidemiology and priorities[8]. In Solomon Islands, ANC is based on the Manual of Obstetrics and Gynecology for Doctor, Midwives and Nurses in Solomon Islands, 2005[9]. This recommends early ANC assessment (after 2-3 missed period) to determine expected date of delivery and identify and treat common conditions such as anemia and syphilis. The first ANC visit includes thorough history taking, physical examination, administering first dose of tetanus toxin, and bloods for hemoglobin, malaria and syphilis and urine for glucose and protein. Antenatal screening for hepatitis B is not part of the routine care.

Hepatitis B infection is hyper endemic in Solomon Islands. A large study conducted in Honiara central hospital in 1994 reported overall Hepatitis B surface antigen (HBsAg) prevalence of 19.6% which ranged from 14.6% in females to 23.4% in males[10]. Surveillance reports and prevalence studies in population sub-groups such as pregnant women and blood donors also reported high level of chronic infection, with 14.5%-16% in pregnant women and 25% in blood donors[11,12]. HBV C3 and D4 are the two prevalent subgenotypes in Solomon Islands and in the Pacific region[13-16], Utsumi *et al*[13], further reported genotype to be specific with ethnicity where genotype was C were predominant in Melanesians while genotype was D was common among Micronesians. The prevalence of hepatitis B e antigen (HBeAg) was higher among carrier of HBV subtype C compared to carriers of subtype B however it was not statistically significant. In this study there was no statistically significant difference between carriers of the two genotypes in terms of sex, liver function text (AST, serum albumin and total bilirubin) and ant-HBe seroprevalence. Previous study in Solomon Islands reported significantly higher prevalence of HBeAg among carriers of genotype C which could be associated with severe hepatic inflammation and complications[14]. However, these studies were not designed to further evaluate the relationship between genotype and clinical progression as they were cross-sectional prevalence studies.

There is paucity of information on the prevalence of complications of HBV infection such as cirrhosis and primary hepatocellular carcinoma in Solomon Islands. Historically, the island reported a high incidence of liver cancer among males[17]. According to the 2014 WHO report, liver cancer was the single most common cause of cancer in males[18]. Mortality from HBV related complications in Solomon Island remains unknown. Hepatocellular carcinoma is common in neighboring Melanesian islands of Fiji[19] and Papua New Guinea[20].

The clinical course of chronic HBV infection generally does not change during pregnancy and chronic infection is not implicated in increased maternal morbidity or mortality[21,22]. Most pregnant women with chronic HBV infection are asymptomatic and often detected during routine ANC screening. Pregnancy related compilations and perinatal outcomes of chronic HBV are not well elucidated. Some studies reported gestational diabetic, antepartum hemorrhage, preterm labour and lower Apgar score to be associated with chronic infection[23,24]. Recent large scale studies from the United States and China revealed no association between maternal HBV infection and the risk of fetal growth retardation, pregnancy induced hypertension or preeclampsia[24,25]. In Solomon Islands, the impact of chronic HBV infection on pregnancy outcomes has not been investigated.

Hepatitis B vaccine was introduced in the national immunization program in 1990-1991 and is recommended for infants at birth, 6, 10 and 14 wk of age[26]. In 2009, the coverage of hepatitis B vaccine was 45% at birth and 81% for $\geqslant$ 3 vaccines[27].

Ongoing transmission at birth and in early childhood is likely to continue to contribute to the significant burden of disease. This early exposure in life increases risk of chronic infection and its complications of liver cirrhosis and its sequelae, liver cancer and early death. There are no recent seroprevalence studies to document the current burden of disease. Therefore, this study aims to contribute to a clearer understanding of the current status of chronic infection in a cohort of antenatal women in Honiara, Solomon Islands.

MATERIALS AND METHODS

A descriptive, cross-sectional study was carried out in seven area health centers (Kukum, Mataniko, Rove, Vura, Mbokonavera, White River and Mbokona) providing ANC in the catchment areas of Honiara City Council. Ethical clearance was obtained from the College Research and Ethics Committee of Fiji National University and the National Health Research and Ethics Committee of Ministry of Health and Medical Services, Solomon Islands (HRC14/28).

All pregnant women who presented for the first antenatal visit were eligible for the inclusion. Using the one sample population proportion formula, the sample size required was estimated as 239 (based on 16% prevalence of HBV among pregnant women[12], 95%CI, 5% margin error, and 15% non-respondent). Enrolment of eligible pregnant women in each area health center was conducted proportionally based on the monthly average of first ANC bookers using systematic random sampling technique. Potential study participants were invited to participate and those that gave written consent were enrolled. A total of 243 pregnant women were enrolled between March to June 2015. Information was collected using standardized proforma data collection tool which included demographics (age, ethnicity, residential location, education level and occupation), obstetric and medical history as well as HBsAg and HBeAg serology results.

One milliliter of blood that was collected for routine ANC testing was aliquoted and stored at -4 ℃ in the national referral hospital laboratory. The specimens were thawed back to room temperature for testing according to the manufacturer's instructions[28]. All samples were tested for HBsAg with Standard Diagnostics Bioline,

the HBsAg testing kit (30 Tests/kit, Cat. No. 01FK10W, Standard Diagnostics, Inc, South Korea). This is a point of care qualitative immunochromatography testing strip method for the detection of HBsAg. Further testing for HBsAg was performed on all positive sera and 5% of randomly selected nonreactive samples using Murex HBs version 3 enzyme linked immunosorbent assay (ELISA) - horseradish peroxidase conjugated kit which have a specificity of 99.97% and sensitivity of 100% respectively (DiaSorin, S.p.A. United Kingdom branch). Duplicate samples including positive and negative controls were included and the procedure was carried out in accordance to the manufacturer's instructions[29] with the washing step done manually. Samples that were positive on both rapid test kits and the ELISA were considered HBsAg positive From the 33 positive samples for HBsAg, 30 samples were analyzed for HBeAg using the ABON HBV combo test kit (ABON Biopharm, Hangzhou Co., Ltd). The remaining 3 samples were insufficient for further testing. The testing procedure and interpretation was carried out according to the manufacturer's instructions. Positive results were indicated by two red bands; one in the test region and other in the control region. Negative results were indicated by one red band on the control region.

The data was entered into Microsoft Excel spreadsheet and analyzed using Statistical Package for the Social Sciences software version 22. A descriptive analysis was used to determine the demographic, obstetric and medical profile of study participants. The overall HBsAg and HBeAg prevalence was calculated as well as determination by age group, ethnicity and location of residence. Results are presented as proportion, means with standard deviation. χ^2 test and Fishers' exact tests were used to compare the proportions between hepatitis B seropositive *vs* sero-negative and demographic variables. Results were considered statistically significant at $P < 0.05$.

RESULTS

A total of 243 pregnant women attending their first antenatal visit were enrolled in the study. Three pregnant women with incomplete information were subsequently excluded from analysis. The data from remaining 240 were used for analysis. The mean age of participants was 26 ± 6 years (range 16 to 45). Majority of participants were Melanesians (91%), Polynesians and Micronesian represented 5.4% and 3.3% respectively. Most pregnant women (62.1%) achieved secondary or tertiary level education, with 7.9% reporting no education. Majority of the pregnant women who took part in the study were unemployed (58.7%). Nearly half of the study participants (46%) were peri-urban dwellers (Table 1).

The average presentation for first ANC visit was in the 6 mo of pregnancy. Most women presented for the first time in their second trimester (58.2%). Majority of women were not aware of their hepatitis B vaccination status (54.6%), with only 4.6% reporting prior vaccination. The median number of children was 1 per participant, with most had at least one child (58.3%). Women with children under the age of 5 years (47.9%) were asked about the hepatitis B vaccination of their child/children. Of these, 65.2% of mothers said their child/children had been vaccinated, 27% were uncertain and the remaining 7.8% not having received hepatitis B vaccine.

A total of 33 sera tested positive for HBsAg, with a sero-prevalence of HBsAg among study participants of 13.8%. Highest rate of hepatitis B infection was seen in participants between the ages of 30-34 year (22%). None of the pregnant women in the 15-19 years (n = 33) and ⩾ 40 years (n = 2) tested positive for HBsAg. Majority of HBsAg positive participants were Melanesian (29 out for 33). In this study the highest rates of sero-prevalence were reported among Polynesians (23.1%) followed by Melanesians (13.2%). No statistically significant difference in HBsAg prevalence by age group, ethnicity, education level and residential location is seen (Table 2). A total of 44 samples (33 positive and 11 representing 5% of the negative results) were tested with Murex HBs version 3 ELISA for quality assurance. There was 100% concordance in results.

Of the 33 HBsAg positive pregnant women, 30 were tested for HBeAg. The overall prevalence of HBeAg was 36.7%. Higher prevalence was recorded among women between 20-24 years old (54.5%) followed by 25-29 years old (27.3%). All the HBeAg positive women were from Melanesian ethnic group and 54.5% reside in urban areas.

DISCUSSION

The urgency to address the needs of hepatitis B associated disease and resultant suffering is now being actively addressed with particular attention to those countries with high rates of chronic infection. The hyper prevalence of hepatitis B in the Pacific islands is well accepted but remains poorly defined with gaps in recent data on disease burden. There are complex reasons for this and this study contributes to current understanding in a select cohort of people in Solomon Islands.

We report hepatitis B sero-prevalence rate of 13.8% in this descriptive cross-sectional study of pregnant women attending for their first antenatal visit in seven area health centres in Honiara. Data preceding this dates back to 2008, with comparable rates of 13.7% (41/298) reported amongst a similar antenatal cohort in Honiara, Gizo and Munda[12]. Their study determined HBsAg using ELISA (Determine and Serodia). Slightly higher rates of 15.8% were reported amongst women aged 15 to 24 years *vs* 11.9% in women aged 25-44 years[12]. Our study report similar rates of sero-prevalence based on rapid point of care tests in a similar antenatal cohort and hence have similar biases. These studies are both likely to underestimate the burden of disease due to convenience sampling bias in select age in the female population presenting to health care facilities. However,

Table 1 Demographic characteristics of study participants (*n* = 240)

Demographic profile	*n* (%)
Age group	
15-19	33 (13.6)
20-24	82 (33.7)
25-29	50 (23.9)
30-34	41 (16.9)
35-39	24 (9.9)
⩾ 40	2 (0.8)
Ethnicity	
Melanesian	219 (91.3)
Polynesian	13 (5.4)
Micronesian	8 (3.3)
Education level	
No education	19 (7.9)
Primary	72 (30)
Secondary	127 (52.9)
Tertiary	22 (9.2)
Occupation	
Unemployed	141 (58.8)
Employed (private/government)	77 (32.1)
Student	20 (8.3)
Unknown	2 (0.8)
Residential location	
Peri-urban	111 (46.30
Urban	104 (43.3)
Rural	25 (10.4)

Table 2 Comparison of hepatitis B surface antigen prevalence by selected socio-demographic variables

	Total	HBsAg positive *n* (%)	HBsAg negative *n* (%)	*P*-value
Age group				-
15-19	33	0	33 (100)	
20-24	82	13 (15.9)	69 (84.1)	
25-29	58	7 (12.1)	51 (87.9)	
30-34	41	9 (22.0)	32 (78.0)	
35-39	24	4 (16.7)	20 (83.3)	
⩾ 40	2	0	2 (100)	
Ethnicity				0.513
Melanesian	219	29 (13.2)	190 (86.8)	
Polynesian	13	3 (23.1)	10 (76.9)	
Micronesian	8	1 (12.5)	7 (87.5)	
Education level				0.143
No education	17	5 (26.3)	14 (73.7)	
Primary	77	7 (9.7)	65 (90.3)	
Secondary	127	16 (12.6)	111 (87.4)	
Tertiary	22	5 (22.7)	17 (77.3)	
Occupation				-
Unemployed	141	16 (11.3)	125 (88.7)	
Employed	77	15 (19.5)	62 (80.5)	
Student	20	2 (10.0)	18 (90.0)	
Unknown	2	0	2 (100)	
Residential location				0.112
Urban	104	13 (12.5)	91 (87.5)	
Peri urban	111	13 (11.7)	98 (88.3)	
Rural	25	7 (28.0)	18 (72.0)	

HBsAg: Hepatitis B surface antigen.

it does not distract from the high rate of 13.8%. Other previous data are limited to small studies, in select populations. Study on healthy blood donors reported higher prevalence of HBsAg of 19.6% and 22.3%[10,11]. Further national random representative sero-surveys are needed to provide a much more accurate assessment of disease burden in Solomon Islands.

We report a trend with increased rates seen with increasing age with peak prevalence of 22% in the 30-34 year group. The increased rates with increasing age may reflect ongoing new infections through sexual contact or other routes including health services. This is proceeded by further decline in the older age group (35-39 years), and no cases seen over the age of 40 years. This decline in the older age group may represent spontaneous sero-conversion over time and nil cases due to small sampling size. No HBsAg positive patients are noted in the youngest cohort (15-19 years). This is likely due to the efforts childhood vaccination program. This draws further attention in the need to ensure high rates of birth dose and vaccination coverage to improve herd immunity and hence overall prevalence over time. Initial effort to assess current vaccination coverage rates as well as addressing the barriers to delivery such as cold chain, birth outside health care facilities and lack of awareness are required.

Amongst the ethnic groups, the highest rates were seen amongst the Polynesian cohort with a prevalence of 23.1% compared with Melanesian and Micronesian (13.2% and 12.5% respectively), lack of statistical significance may be attributed to total numbers recruited for this study, with the largest sampling size from the Polynesian cohort. Similar reports of difference in hepatitis B in ethnic groups are reported from 1994 with highest rates of hepatitis B seen in Micronesians (28.1%), followed by Melanesians (20%) and then Polynesians (8.4%)[10]. This ethnic variation is well reported from other parts of the Pacific islands and further understanding of this relevance will assist in the contribution to the understanding of the disease, mode of transmission, disease progress and management strategies. Further work into this is clearly warranted.

Although the prevalence of hepatitis B is noted to be higher in those from rural settings (28%) as compared to those from urban and periurban settings (12.5% and 11.7%), this does not reach statistical significance. There is likely to be a number of factors contributing to this difference including vaccination coverage as well as ongoing risk of horizontal transmission modality and access to health care. Hepatitis B rate is also likely to have geographical variations in different islands. Improved understanding to address this gap is warranted with majority of the population (80.2%) in Solomon Islands living in rural areas where access to clean water and sanitation is not reliable[7]. These resource barriers are likely contributors to higher rates of hepatitis B, and ongoing risks of new infection.

Our study for the first time reports the prevalence of HBeAg among pregnant women in Solomon Islands. The overall prevalence of 36.7% is comparable to the rates reported among mothers and the general population. Furusyo *et al*[10], reported an overall prevalence of 41.3% among 315 HBsAg positive adult patients attending

general outpatients and blood donors. The prevalence did not differ by sex however patients from Melanesian ethnic groups had significantly higher HBeAg seropositive compared to the other two ethnic groups. Another study among mothers of children who received vaccination reported HBeAg prevalence of 40.7%[30]. Subsequently in 2001, seroprevalence of 35% was reported among 206 blood donors with chronic hepatitis[14]. All studies reported a progressive decline in HBeAg sero-prevalence with increasing age. Wilson *et al*[31], reported high prevalence of HBeAg among pregnant women in the Pacific region which ranged from 48% in Kiribati to 70% in Fiji. HBeAg determines infectivity. High prevalence of HBeAg in pregnant women coupled with low up take of birth dose vaccine in Solomon Island increase the risk for vertical transmission of HBV to their newborns.

Only 4.6% of women screened had received previous hepatitis B vaccination. This low rate represents an opportunity to increase awareness and improve vaccination coverage. More than half the women were not aware of their vaccination status. Despite the routine introduction of childhood vaccination for hepatitis B in 1990-1991, only 65.2% of women with children under the age of 5 were able to report that their child/children had received vaccination. One in three mothers was not aware of their child's vaccination status. This gap in awareness about the vaccination requires attention, with opportunities for education for community as well as health care workers. Birth dose vaccination coverage as well as completion of the three doses remains a significant challenge in resource poor setting and efforts to evaluate this in Solomon islands and address the specific barriers is needed. In particular, challenges include remote settings, lack of cold chain, engagement of health care workers and competing needs of antenatal care, as well as access to vaccines. Solomon Islands have one of the lowest birth dose coverage (45%) in the WHO-Western Pacific Region[27]. There are potential solutions to address these gaps that require resource allocation and prioritization with burden compounded by the remote settings. Hence, programs that explore integration into currently systems are should be considered. Even with optimal vaccination delivery, the protective coverage of vaccination is 70% in those born to positive mothers *vs* 81% to those born to hepatitis B negative mothers[31]. Hence, efforts to address this ongoing risk of vertical transmission and its associated high risk of progression to chronic lifelong infection are needed.

The screening tool used for this study was a point of care test (Standards Diagnostics). The 5% of negative samples tested by Murex HBsAg version 3 ELISA method is considered as quality control in the study. According to testing kit evaluation made by independent studies (including WHO), on a number of commercial HBsAg rapid tests, the Murex HBsAg version 3 ELISA is able to detect 0.13-0.21 IU/mL surface antigen concentration with a clinical sensitivity of 100% by one study[32,33]. Standard Diagnostics testing kits allows for rapid detection of the HBsAg which is an antigen associated with hepatitis B. This antigen usually becomes positive very soon after infection and persists if the person is unable to develop protective antibodies, indicating chronic infection if present after 6 mo. These rapid point of care test kits are utilized mostly in resourced limited settings, are cheap, easy to use and interpret and requires less laboratory skills and does not need instruments. On the other hand, ELISA which is the most preferred screening technique with accuracy of 99.9% is time consuming, laborious and needing proficient skills to perform[34,35]. Thus rapid HBsAg tests serves as the common testing methods in the Solomon Islands for screening of antenatal mothers, blood donors and patients.

HBV viral load testing remain outside the scope of most of these resource poor settings both in terms of cost of equipment, consumables as well as training for laboratory staff. Additional information is required on the performance characteristics of these rapid tests in terms of their current on field performance and factors that may affect it including cut off viral load, contribution of diversity in hepatitis B variants and the effect of hepatitis B therapy. Other tests that are potentially of use include the use of hepatitis B surface antibody tests and core antibody tests which could add value to the overall understanding of the viral replication status and hence infectivity with potential role in the monitoring of patients who are found to be positive on screening. Further, rapid tests may not be able to detect occult hepatitis B infection, this remaining an area of further study. Although these rapid tests have a clear role, the need for additional laboratory services needs to be considered. Models including the establishment of reference labs could be explored.

The cost effectiveness of routine screening for HBsAg has not been fully investigated. Routine screening of pregnant women for hepatitis B has not been included in the WHO optimum service package for ANC. This could be due to concern over its cost effectiveness and support for mathematical modeling for funding purposes would help clarify. Currently, some PICs countries have included routine screening for Hepatitis B in their ANC package[36-39]. In Fiji and French Polynesia, screening is recommended during the first ANC visit and newborns of seropositive mothers are given immunoglobulin as well as birth dose vaccines at birth followed by three subsequent vaccine doses[36,37]. Similarly in Vanuatu, universal screening of pregnant women for HBsAg was commenced in Port Vila hospital in 2013[39]. In Solomon Islands, introduction of routine screening of pregnant women for HBsAg before childbirth would have two benefits. First, it will enable prompt identification of newborns of positive mothers for the provision of vaccine immediately after birth and subsequent follow up for completion of vaccination. Secondly, in high endemic areas it provides opportunity to immunize HBsAg negative pregnant women (if they have not been immunized) who are at high risk of infection in the community. Identification, screening and vaccination of contacts of positive patients could also be implemented. This targeted immunization coupled with ongoing promotion of current universal

immunization program could provide an opportunity to increase uptake of birth dose and overall hepatitis B vaccine coverage in infants.

Clearly, allocation of resources to allow this both in terms of cost and supporting services related to its delivery is required but need commitment from government. Solomon Islands could provide an example of models of care with an integrated program including awareness, prevention, screening, diagnostics, therapy and management through engagement and education. Specific local needs and resources require attention with focus on efficient delivery of care integrating hepatitis B programs into currently existing program as not to unnecessarily to the existing burden of stretched resources. This could form part of a national strategic plan for the delivery of hepatitis B services in Solomon Islands.

This study found a high rate of HBsAg and HBeAg prevalence among pregnant women in Honiara, with low level of awareness and vaccination uptake among women and their children. The challenges in hepatitis service in Solomon Islands are both unique and similar to those of the many of islands and atolls of the Pacific. Availability and access to care can be addressed by small steps and integration of currently available resources and programs without the need for introduction of separate programs and its associated funding requirements and complex strategies. Rapid point of care tests for screening and diagnosis are also available for further study. The WHO treatment and care guidelines in 2015 provide a framework with direction to further promote momentum on a broader scale to address the multiple facets needed to support hepatitis related service delivery in the resource poor setting[40].

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to all pregnant women who gave consent and participated in the study, without their participation this study would not have been completed. The research team would also like to thanks the nurses in the seven area health centers and Honiara City Council health division staff and who kindly supported the study.

COMMENTS

Background

The Solomon Islands is located in the Southwest Pacific and has total population of 515870. Hepatitis B surface antigen (HBsAg) prevalence of 19.6% was reported among the general population in 1994. Surveillance reports and prevalence studies in population sub-groups such as pregnant women and blood donors also reported high level of chronic infection, with 14.5%-16% in pregnant women and 25% in blood donors. Hepatitis B vaccine was introduced in the national immunization program in 1990-1991 and is recommended for infants at birth, 6, 10 and 14 wk of age. In 2009, the coverage of hepatitis B vaccine was 45% at birth and 81% for $\geqslant$ 3 vaccines.

Research frontiers

Hepatitis B infection is hyper endemic in Solomon Islands. Ongoing transmission at birth and in early childhood is likely to continue to contribute to the significant burden of disease. However, there are no recent seroprevalence studies to document the current burden of disease. The research hotspot is to contribute a clearer understanding of the current status of chronic HBV infection in a cohort of antenatal women and provide evidence based information for national HBV prevention and control strategies.

Innovations and breakthroughs

The hyper prevalence of hepatitis B in Solomon Island is well accepted but remains poorly defined with gaps in recent data on disease burden. This study enrolled 240 pregnant women attending antenatal care in Honiara and found a high rate of HBsAg and hepatitis B e antigen (HBeAg) prevalence with low level of awareness and vaccination uptake among pregnant women and their children respectively.

Applications

This study found a high rate of HBsAg and HBeAg prevalence among pregnant women which suggests the increased risk of perinatal transmission of HBV. Moreover, the study provided background information on hepatitis B disease burden and described the various challenges and proposed integrated approach for HBV control In Solomon Islands.

Peer-review

The study design, material-methods are appropriate for the aim. The results are typical for a HBV endemic population but somewhat limited. It would have been more interesting if anti-HBs and anti-HBc total results of the study group were also provided.

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P- Reviewer: García-Elorriaga G, Panduro A, Sayiner AA
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Li D

World Journal of Hepatology

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Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4254/wjh.v8.i34.1529

World J Hepatol 2016 December 8; 8(34): 1529-1534
ISSN 1948-5182 (online)

ORIGINAL ARTICLE

Prospective Study

Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina

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Author contributions: Dominguez C and Fernandez JL designed the study; Dominguez C, Romero E and Graciano J performed the research; Dominguez C and Fernandez JL analyzed the data; Dominguez C and Fernandez JL wrote the paper; Viola L revised the manuscript for final submission.

Institutional review board statement: The study was reviewed and approved by the Guemes Sanatorio institutional review board (BSAS, Argentina).

Clinical trial registration statement: Our study is not a clinical trial.

Informed consent statement: The protocol was approved by our institutional review board and patients gave the usual written informed consent for hospitalization, No additional procedures other than those indicated by the physicians, based on routine practice and international standards, were performed. Considering this fact, our institutional reviewers considered that another special consent was not required.

Conflict-of-interest statement: The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Data sharing statement: There is no additional data sharing available.

Manuscript source: Invited manuscript

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Received: July 12, 2016
Peer-review started: July 14, 2016
First decision: August 22, 2016
Revised: September 20, 2016
Accepted: October 17, 2016
Article in press: October 18, 2016
Published online: December 8, 2016

Abstract

AIM

To study the prevalence, characteristics, risk factors and mortality at 28 d of acute-on-chronic liver failure (ACLF).

METHODS

A total of 100 cirrhotic patients admitted to our hospital for more than one day were included during the period between June 2013 and December 2015. We used the European Association for the Study of the Liver-Chronic Liver Failure-Consortium diagnostic criteria for ACLF, considering it as the acute decompensation of cirrhosis associated with the presence of one or more organ failure. For the diagnosis of organic failure the Chronic Liver Failure-Sequential Organ Failure Assessment score was used. Our population was divided into patients with and without ACLF. Clinical characteristics, presence of precipitating events, potential risk factors for developing ACLF and causes of mortality were analyzed. Mortality at 28 d was evaluated.

RESULTS

Twenty-nine patients (29%) developed ACLF criteria. Alcoholism, detected in 58 patients (58%), was the

major etiological agent of cirrhosis. Bacterial infections were recognized as a precipitating event in 41.3% of cases and gastrointestinal bleeding in 27.5%. No precipitating event was identifiable in 27.5% of patients with ACLF. Comparing patients with and without ACLF, statistically significant risk factors were: Child Pugh score 10.2 ± 2.1 *vs* 8.4 ± 1.6 ($P < 0.0001$), MELD score 20.7 ± 8.5 *vs* 12.3 ± 4 ($P < 0.0001$), presence of ascites 27 (93%) *vs* 43 (60.5%) ($P = 0.001$), leukocytosis 15300 ± 8033 per cubic millimeter *vs* 10770 ± 5601 per cubic millimeter ($P < 0.0001$), and high plasma levels of C reactive protein values 50.9 ± 46.4 mg/L *vs* 28.6 ± 23.4 mg/L ($P < 0.0019$). Mortality rate was 62% (18 patients) *vs* 5.6% (4 patients), respectively ($P < 0.0001$).

CONCLUSION

We observed that the ACLF is a frequent entity in this group of patients and has a significantly higher mortality rate.

Key words: Acute-on-chronic liver failure; Acute liver decompensation; Cirrhosis; Ascites; Mortality

Core tip: Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that is gaining acceptance in recent times. It is characterized by an acute impairment of an underlying chronic liver disease with high short-term mortality, produced by the development of organic failures and associated with precipitating event. However, little is known about the development and progression of this syndrome. Guided by the European Association for the Study of the Liver-Chronic Liver Failure-Consortium diagnostic criteria and the CANONIC study, we could establish that the prevalence of ACLF in our center was 29%, and that Child Pugh advanced stage, MELD score, presence of ascites and inflammation parameters were significant risk factors for ACLF.

Dominguez C, Romero E, Graciano J, Fernandez JL, Viola L. Prevalence and risk factors of acute-on-chronic liver failure in a single center from Argentina. *World J Hepatol* 2016; 8(34): 1529-1534 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1529.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1529

INTRODUCTION

Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that includes the acute deterioration of a chronic liver disease, usually associated with a precipitating event, the development of one or more organ failure and high short-term mortality.

The term ACLF was initially coined in 1995[1]. There are more than thirteen different definitions up to date. Until worldwide diagnostic criteria are accepted, two consensual definitions are commonly used[2]. The first, belonging to the Asian Pacific Association for the Study of the Liver, considers that the ACLF is an "acute hepatic insult manifesting as jaundice and coagulopathy, complicated within four weeks by ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed chronic liver disease"[3]. According to the second definition, developed in a joint symposium of the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases, ACLF is an "acute deterioration of pre-existing chronic liver disease, usually related to a precipitating event, and associated with increased mortality at three months due to multisystem organ failure"[4].

Recently, an European consortium exclusively dedicated to the study of liver failure in patients with chronic liver disease (EASL-CLIF-Consortium) conducted the CANONIC study with the aim to define the ACLF and be able to identify those cirrhotic patients with a high risk of short-term mortality. Based on the analysis of 1343 cirrhotic patients, the EASL-CLIF-Consortium proposed as diagnostic criteria the acute decompensation of the liver disease (defined by the development of ascites, encephalopathy, gastrointestinal bleeding or bacterial infection) associated with the presence of one or more organ failure. The organ failure was defined by the Chronic Liver Failure-Sequential Organ Failure Assessment (CLIF-SOFA) score (Table 1) and a mortality at 28 d higher than 15%[5,6].

Acute decompensation of cirrhosis is the leading cause of hospitalization in cirrhotic patients[7]. In many of these patients complications develop in the absence of organic failure, but in others they are associated with impaired function of kidneys, liver or other organs. The last group of patients, falling within the definition of ACLF, are those with a high risk of short term mortality.

The CANONIC study showed that ACLF is an extremely relevant and very common syndrome, with a prevalence of around 30%, differing from a mere acute decompensation by the presence of organ failure, the mortality rate 15 times higher, the clinical characteristics, the association with precipitating events and the parameters of systemic inflammation[8-10].

Due to the lack of a worldwide accepted definition and diagnostic criteria, many aspects of this syndrome, such as prevalence, natural history, precipitating factors, clinical features and pathophysiological mechanisms remain unknown[11,12].

The aims of our study were to determine the prevalence of ACLF in the cirrhotic patients of our institution using the diagnostic criteria established by the CANONIC study, to describe the clinical characteristics of ACLF, to assess the risk factors for developing ACLF, and to evaluate the mortality at 28 d, comparing the cases with and without ACLF.

MATERIALS AND METHODS

In this prospective observational study we analyzed

Table 1 Chronic liver failure-sequential organ failure assessment score

Organ/system	0	1	2	3	4
Liver (bilirubin, mg/dL)	< 1.2	⩾ 1.2 to ⩽ 2	⩾ 2 to < 6	⩾ 6 to < 12	⩾ 12
Kidney (creatinine, mg/dL)	< 1.2	⩾ 1.2 to < 2	⩾ 2 to < 3.5	⩾ 3.5 to < 5 or dialysis	⩾ 5 or diálysis
Cerebral (HE grade)	No HE	I	II	III	IV
Coagulation (RIN, platelet count)	< 1.1	⩾ 1.1 to < 1.25	⩾ 1.25 to < 1.5	⩾ 1.5 to < 2.5	⩾ 2.5 or platelet count ⩽ 20000 per cubic millimeter
Circulation (mean arterial pressure, mmHg), inotropic drugs (μg/kg per minute)	⩾ 70	< 70	Dopamine ⩽ 5 or dobutamine or terlipressin	Dopamine > 5 or E ⩽ 0.1 or NE ⩽ 0.1	Dopamine > 15 or E > 0.1 or NE > 0.1
Lungs (SpO_2/FiO_2)	> 512	> 357 a ⩽ 512	> 214 a ⩽ 357	> 89 to ⩽ 214	⩽ 89

The text in bold indicates the diagnostic criteria for organ failure. HE: Hepatic encephalopathy; E: Epinephrine; NE: Norepinephrine; FiO_2: Fraction of inspired oxygen; SpO_2: Pulse oximetric saturation.

patients with cirrhosis, diagnosed by a previous liver biopsy or by indirect signs (clinical examination, laboratory, imaging and endoscopy), who were hospitalized for more than one day in the Sanatorio Güemes, which is one of the biggest high complexity medical centers in Argentina, located in Buenos Aires City, with a capacity of 480 beds.

The protocol was approved by our institutional review board and patients gave the usual written informed consent for hospitalization, no additional procedures other than those indicated by the physicians, based on routine practice and international standards, were performed. Considering this fact, our institutional reviewers considered that another special consent was not required.

Patients were recruited between June 2013 and December 2015. Data were obtained from medical records, including previous episodes of decompensation (ascites, encephalopathy, spontaneous bacterial peritonitis, esophageal varices, variceal bleeding or hepatocellular carcinoma), physical examination, laboratory analysis, presence of potential precipitating factors (infections, active alcohol intake, gastrointestinal bleeding), and etiology of cirrhosis.

For the diagnosis of organic failure the CLIF-SOFA score was used (Table 1). Our population was divided into patients with and without ACLF. Within the group with ACLF the type and number of affected organs were analyzed and divided in 3 grades. ACLF grade 1 included patients with single kidney failure; patients with single failure of the liver, coagulation, circulation, or respiration who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL and/or mild to moderate hepatic encephalopathy; and patients with single cerebral failure, who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL. ACLF grade 2 included patients with failure of two organs and ACLF grade 3 included patients with failure of three or more organs.

After discharge, the mortality at 28 d was evaluated by monitoring on an outpatient basis or by telephone calls when patients did not attend the visit.

Clinical characteristics of each group, presence of precipitating events, potential risk factors for developing ACLF and causes of mortality were analyzed. Within the analyzed clinical parameters, the West-Haven scale for encephalopathy grades was used[13]; ascites was classified in mild (mild ascites only detectable by ultrasound), moderate (moderate ascites evident by moderate symmetrical distension of abdomen) and severe (large or gross ascites with marked abdominal distension)[14]; circulation dysfunction implied arterial hypotension (mean arterial pressure below 70 mmHg) or requirement of inotropic drugs; and respiratory failure implied the need for mechanical ventilation.

Laboratory data included a complete blood analysis allowing the calculation of MELD and Child-Pugh scores. Inflammation parameters were evaluated by white blood cell count and C-reactive protein (CRP).

Both the clinical parameters and the laboratory results were recorded when patients were enrolled, when they showed some intercurrent or organic decompensation, and at discharge or previously to death.

Statistical analysis

For statistical analysis, the χ^2 test or the Fisher test were used for dichotomous variables as appropriate. For continuous variables the Student *t* test was used. For risk factors, the OR with their respective 95%CI were calculated as association measures.

RESULTS

A total of 100 patients were included, of which 67 were male (67%) and 33 female (33%).The mean age was 60 ± 11 years and mean Child-Pugh score was 9 ± 1.9. Regarding to the etiology of cirrhosis, alcohol was found in 58 patients (58%), followed by hepatitis C infection and cryptogenic disease (Table 2).

The total of patients who fulfilled criteria for ACLF was 29 (29%), 10 of them (34.4%) were grade 1, 5 (17.3%) grade 2 and 14 (48.3%) grade 3 (Table 3). Seventeen patients (59%) had criteria for ACLF at admission to the hospital and 12 (41%) developed it during hospitalization, with an average time of presentation of 10 d. Renal failure was the prevalent organ failure for ACLF grade 1. For ACLF grade 2, coagulation failure was the prevalent finding followed by renal and respiratory failure. For ACLF grade 3, the prevalence of all organ failures was high with a significant impact in the circulatory and respiratory

Table 2 Cirrhosis etiology

Etiology	*n* (%)
Alcohol	58 (58)
Alcohol + hepatitis C virus	5 (5)
Hepatitis C virus	13 (13)
Nonalcoholic steatohepatitis	4 (4)
Cryptogenic	12 (12)
Autoimmune hepatitis	4 (4)
Primary biliary cirrosis	1 (1)
Primary biliary cirrhosis + autoimmune hepatitis	1 (1)
Hepatitis B virus + alcohol	1 (1)
Hemochromatosis	1 (1)

Table 3 Prevalence of acute on chronic liver failure *n* (%)

ACLF	Grade 1	Grade 2	Grade 3
Patients	10 (34.4)	5 (17.3)	14 (48.3)
Mortality	3 (30)	2 (40)	13 (92)

ACLF: Acute-on-chronic liver failure.

Table 4 Type and number of organ failure *n* (%)

Organs failure	ACLF 1	ACLF 2	ACLF 3
Renal	7 (70)	2 (40)	10 (71)
Cerebral	1 (10)	1 (20)	12 (85)
Coagulation	1 (10)	3 (60)	8 (57)
Liver	1 (10)	1 (20)	2 (14)
Circulatory	0 (0)	1 (20)	14 (100)
Respiratory	1 (10)	2 (40)	14 (100)

ACLF: Acute-on-chronic liver failure.

Table 5 Precipitating events of acute-on-chronic liver failure

Potential precipitating events of ACLF	*n* (%)
Bacterial infection	12 (41.3)
Gastrointestinal hemorrhage	8 (27.5)
Renal failure secondary to acute diarrhea	1 (3.4)
No precipitating event	8 (27.5)

ACLF: Acute-on-chronic liver failure.

system (Table 4).

Analyzing the possible precipitating factors in patients with ACLF, an infectious cause was recognized in 12 (41.3%), being pneumonia the main source of infection, and gastrointestinal bleeding in 8 (27.5%). One patient (3.4%) developed ACLF after a renal failure secondary to acute diarrhea. There was not an evident precipitating factor in 8 cases (27.5%) (Table 5). In the group of patients without ACLF, we observed the following clinical events: Gastrointestinal bleeding in 27 patients (38%), bacterial infections in 20 (29%), other causes such as constipation in 5 (7%) and no event in 19 (26%).

When patients with and without ACLF were compared, we observed, respectively: Male 23 (79%) *vs* 44 (62%) [$P = 0.11$, OR = 2.36 (95%CI: 0.78-7.43)], age 60 ± 11 years *vs* 60 ± 11 years ($P = 1.00$), active alcohol intake in the last 3 mo 9 (31%) *vs* 22 (31%) [$P = 1$, OR = 1.00 (95%CI: 0.23-2.79)], Child Pugh 10.2 ± 2.1 *vs* 8.4 ± 1.6 ($P < 0.0001$), MELD score 20.7 ± 8.5 *vs* 12.3 ± 4 ($P < 0.0001$), previous episodes of ascites 18 (62%) *vs* 29 (41%) [$P = 0.07$, OR = 2.37 (95%CI: 0.89-6.33)], previous episodes of encephalopathy 9 (31%) *vs* 10 (14%) [$P = 0.08$, OR = 2.74 (95%CI: 0.87-8.69)], presence of esophageal varices 18 (62%) *vs* 37 (52%) [$P = 0.38$, OR = 1.5 (95%CI: 0.57-3.99)], prior variceal hemorrhage 4 (13.7%) *vs* 10 (14%) [$P = 1.00$, OR = 0.97 (95%CI: 0.23-3.84)], presence of ascites during hospitalization 27 (93%) *vs* 43 (60.5%) [$P = 0.001$, OR = 8.79 (95%CI: 1.80-8.10)], white blood cell count 15300 ± 8.033 per cubic millimeter *vs* 10770 ± 5.601 per cubic millimeter ($P < 0.0001$), natremia 133.3 ± 6.9 mEq/L *vs* 135.1 ± 5.3 mEq/L ($P = 0.16$), and CRP values 50.9 ± 46.4 mg/L *vs* 28.6 ± 23.4 mg/L ($P < 0.0019$) (Table 6).

Twenty patients were hospitalized in the intensive care unit, 14 received mechanical ventilation and none had artificial liver support because it is not available at our center. ACLF resolved or improved in 11 patients (38%) during hospitalization: 7 patients (70%) in grade 1, 3 (60%) in grade 2 and only 1 (7%) in grade 3. In the group of ACLF, 18 patients (62%) died, due to septic shock 10, type 1 hepatorenal syndrome 3, shock without focus 3, upper gastrointestinal bleeding 1 and bronchoaspiration 1. The mortality was 30% in ACLF grade 1, 40% in grade 2 and 92% in grade 3. In the group without ACLF, 4 patients (5.6%) died, due to infection 3 and cardiac failure 1.

DISCUSSION

ACLF is a syndrome different from traditional decompensated cirrhosis, not only because of the presence of organ failure and high mortality rate but also because of the alcoholic etiology of cirrhosis, the prevalence of some specific triggers such as bacterial infection and the higher level of systemic inflammation[15,16]. To recognize ACLF allows to identify those patients at high risk for death due to organ failure and the CANONIC study provided much more precise diagnostic criteria[4,5,15]. So, we followed these criteria in our center and we found a prevalence of 29%, similar to the 30.9% found in the CANONIC study[5,10]. It is interesting to point out that cirrhotic patients may develop ACLF during their stay in the hospital, with an incidence of 14.4%. This figure is quite higher than the 10.8% observed in the CANONIC study[5].

It is noteworthy that 65.8% of our patients who developed ACLF had more than one organ involved (grades 2 and 3). This finding differs from the results of the CANONIC study showing that 64.3% of patients had only one organ involvement[5]. A possible explanation for this discrepancy may be that our patients had advanced stages of cirrhosis (Child-Pugh C 72%) and high prevalence of alcoholism as etiology of the cirrhosis (58% *vs* 48.6% in the CANONIC study). An advanced disease

Table 6 Comparative results between groups with and without acute on chronic liver failure *n* (%)

	ACLF	No ACLF	*P* vaule	OR	95%CI
Age (yr ± SD)	60 ± 11	60 ± 11	1.00		
Male	23 (79)	44 (62)	0.11	2.3	0.78-7.43
Child Pugh (score ± DS)	10.2 ± 2.1	8.4 ± 1.6	< 0.0001		
MELD (score ± DS)	20.7 ± 8.5	12.3 ± 4	< 0.0001		
Active alcoholism	9 (31)	22 (31)	1.00	1	0.3-2.8
Prior ascites	18 (62)	29 (41)	0.07	2.3	0.9-6.3
Prior encephalopthy, *n* (%)	9 (31)	10 (14)	0.08	2.74	0.9-8.7
Esophageal varices	18 (62)	37 (52)	0.38	1.5	0.5-4
Ascites	27 (93)	43 (60.5)	0.001	8.8	1.8-58.1
Variceal hemorrhage	4 (13.7)	10 (14)	1	0.97	0.2-3.8
White cell count (n/mm^3 ± SD)	15.300 ± 10.770	8.033 ± 5.601	< 0.0001		
Serum sodium (mEq/L ± SD)	133.3 ± 6.9	135.1 ± 5.3	0.16		
CRP (mg/L ± SD)	50.9 ± 46.4	28.6 ± 23.4	0.002		
Mortality	18 (62)	4 (5.6)	< 0.0001		

OR: Odds ratio; 95%CI: Confidence interval 95%; SD: Standard deviation; CRP: C-reactive protein; ACLF: Acute-on-chronic liver failure.

may have been the trigger of irreversible pro- and anti-inflammatory mechanisms[10,17,18]. The commonest organ failure was the kidney failure (66%)[19,20]. The prevalence of circulatory and respiratory failure was high (51% and 58%) but significant only in patients with ACLF grade 3.

As expected by previous references, bacterial infections primarily and gastrointestinal bleeding secondly were the main precipitating events[5,21]. It is important to note that in 27.5% of cases we did not identify an evident precipitating factor to explain ACLF in 27.5% of cases, a fact that was previously observed by other authors[5].

We found that Child-Pugh score, MELD score, presence of ascites, elevated leukocyte count and high CRP values parameters were significant risk factors for the development of ACLF. Although Child-Pugh and MELD scores were not considered as risk factors, the statistical significance of ascites, kidney disfunction, hepatic encephalopathy, bilirubin, serum creatinine and international normalized ratio in the CANONIC study allows us to infere that our findings agree with these observations. The role of leukocyte count and CRP as inflammatory parameters were also emphasized by these authors[5,9].

As it was previously observed, mortality was significantly higher in our patients with ACLF. Mortality in our patients with ACLF grade 1 was higher when compared with the figures reported by Gustot *et al*[22] (30% *vs* 6% to 18%), but it was similar in patients with ACLF grade 2 and 3 (40% to 92% *vs* 42% to 92%). As it was also observed by these authors, mortality increased significantly when three or more organs were involved[5,10,22].

The main strength of our investigation is the prospective design that allowed a rigorous collection of data and its main weakness is that it was performed in a single center with a limited number of patients. Despite this limitation, we can draw several conclusions from our results. ACLF is a syndrome that occurs with high frequency in cirrhotic patients hospitalized for decompensated liver disease, reaching a prevalence of 29% in our centre. As noted in the literature, ACLF is a very dynamic syndrome. It resolved or improved in 38% of our patients, a figure lower than the 49% observed by Gustot *et al*[22]. Patients may enter the hospital with ACLF but they may also develop it during their stay, there are risk factors that may predict it development and mortality significantly increases when it occurs. Consequently, it is important to recognize this entity, to be aware of its development, to correct the precipitating factors and perhaps to install a more aggressive therapy, in order to reduce the high mortality[15,23-25]. To overcome the limitations of our study and to achieve a better knowledge of the epidemiology and clinical characteristics of ACLF in our country, it would be desirable to transfer our bounded experience to a multicenter prolonged study.

COMMENTS

Background

Acute-on-chronic liver failure (ACLF) is an increasingly recognized entity that includes the acute deterioration of a chronic liver disease, usually associated with a precipitating event, the development of one or more organ failure and high short-term mortality. However, little is known about the development and progression of this syndrome. This study aimed to determine the prevalence of ACLF and describe the characteristics of this syndrome; assess the risk factors and analyze the mortality at 28 d.

Research frontiers

Until the development of the CANONIC study there was no established definition of ACLF and the published definition were based only on expert opinions. In this study using the CANONIC diagnostic criteria, the authors describe the clinical characteristics, the prevalence and natural history of ACLF in cirrhotic patients of the authors' institution.

Innovations and breakthroughs

As suggested in the literature, the authors observed that the ACLF is a frequent entity in this group of patients and has a significantly higher mortality rate.

Applications

As ACLF is a frequent syndrome, it is important to recognize this entity, to be aware of its development and to install supportive measures in order to reduce the high mortality.

Terminology

Acute-on-chronic liver failure: Acute deterioration of cirrhosis associated with organ/s failure and short term mortality.

Peer-review

The paper is well written and includes information about a relevant topic.

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P- Reviewer: Bossen L, Lenz K **S- Editor**: Qi Y **L- Editor**: A
E- Editor: Li D

World Journal of Hepatology

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DOI: 10.4254/wjh.v8.i34.1535

World J Hepatol 2016 December 8; 8(34): 1535-1540
ISSN 1948-5182 (online)

CASE REPORT

Major hepatectomy using the glissonean approach in cases of right umbilical portion

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Author contributions: Ome Y clinically managed the patients, performed the operations, gathered the clinical data, designed the report and wrote the paper; Kawamoto K, Park TB and Ito T supervised the clinical practices and helped draft and revise the manuscript.

Institutional review board statement: The Kurashiki Central Hospital Institutional Review Board does not require approval for case reports. Ethics approval was not necessary for this case report.

Informed consent statement: Written informed consent was obtained from the patients.

Conflict-of-interest statement: None of the authors have conflicts of interest to declare.

Manuscript source: Invited manuscript

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Received: June 21, 2016
Peer-review started: June 24, 2016
First decision: August 11, 2016
Revised: September 11, 2016
Accepted: October 25, 2016
Article in press: October 27, 2016

Published online: December 8, 2016

Abstract

Right umbilical portion (RUP) is a rare congenital anomaly associated with anomalous ramifications of the hepatic vessels and biliary system. As such, major hepatectomy requires a careful approach. We describe the usefulness of the Glissonean approach in two patients with vessel anomalies, such as RUP. The first patient underwent a right anterior sectionectomy for intrahepatic cholangiocarcinoma. We encircled several Glissonean pedicles that entered the right anterior section along the right side of the RUP. We temporarily clamped each pedicle, confirmed the demarcation area, and finally cut them. The operation was performed safely and was successful. The second patient underwent a left trisectionectomy for perihilar cholangiocarcinoma. We secured the right posterior Glissonean pedicle. The vessels in the pedicle were preserved, and the other vessels and contents were resected. Identifying the vessels for preservation facilitated the safe lymphadenectomy and dissection of the vessels to be resected. We successfully performed the operation.

Key words: Right anterior sectionectomy; Right umbilical portion; Glissonean approach; Left trisectionectomy; Glissonean pedicle; Cholangiocarcinoma; Hepatocellular carcinoma

Core tip: Right umbilical portion (RUP) is a rare congenital anomaly, and its presence is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system. Major Hepatectomies for patients with this anomaly are complicated and require a careful approach. The Glissonean approach is acknowledged as a successful technique. The targeted Glissonean pedicle to

be resected or preserved is easily identified by clamping; thus, the Glissonean approach can be used in various situations of hepatic resection. This report describes the usefulness of the Glissonean technique, especially in cases with an anomaly, such as RUP.

Ome Y, Kawamoto K, Park TB, Ito T. Major hepatectomy using the glissonean approach in cases of right umbilical portion. *World J Hepatol* 2016; 8(34): 1535-1540 Available from: URL: http://www.wjgnet.com/1948-5182/full/v8/i34/1535.htm DOI: http://dx.doi.org/10.4254/wjh.v8.i34.1535

INTRODUCTION

Right umbilical portion (RUP) is a rare congenital anomaly, and its reported incidence ranges from 0.2% to 1.2%[1-6]. The presence of RUP is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system. During anatomical liver resection, only the vessels feeding the area intended for resection should be resected, whereas the other vessels should be preserved. Consequently, major hepatectomies for cases with RUP are complicated and require a careful approach and attention to the anomalous branching of those vessels. Only a few hepatectomy cases with RUP have been reported in the English literature. Here, we report two successful cases with RUP who safely underwent anatomical hepatectomy. We also describe the usefulness of the Glissonean approach.

CASE REPORT

Case 1

A 70-year-old man with hepatitis C presented with a liver tumour. He had a past medical history of distal gastrectomy for gastric ulcer, Graves' disease, and diabetes mellitus. Laboratory tests showed normal levels of carcino embryonic antigen (CEA), CA19-9 and alpha-fetoprotein (AFP) but elevated PIVKA-Ⅱ at 808 mAU/mL. The indocyanine green retention rate at 15 min was 12.9% and the Child-Pugh score was 5 points, Grade A. He was diagnosed with intrahepatic cholangiocarcinoma or combined hepatocellular and cholangiocarcinoma located in segment 8. A computed tomography (CT) scan also revealed that his gallbladder was attached to the left side of the liver; RUP was noted (Figure 1).

The patient underwent right anterior sectionectomy (Figure 2). Laparotomy showed that the gallbladder was attached to the round ligament. After the mobilization of the right lobe, the gallbladder was resected. Then, the right anterior Glissonean pedicles, which ramified along the right side of the RUP, were extrahepatically separated and encircled with tape. We temporarily clamped each pedicle and confirmed the demarcation area and blood flow *via* ultrasonography. The demarcation area was the same as the three-dimensional image visualization *via* preoperative simulation. The liver parenchyma was transected along the demarcation line using the Pringle manoeuvre. We finally ligated and cut the encircled right anterior Glissonean pedicles. The operation succeeded without injuring any of the vessels intended for preservation. The operation required 244 min, and the estimated blood loss was 776 mL.

Macroscopic findings showed an irregular mass, 25 mm in size. A histological examination revealed that the tumour was a poorly differentiated intrahepatic cholangiocarcinoma that invaded the intrahepatic portal vein. The patient was diagnosed as stage Ⅱ (T2N0M0). All of the surgical margins were negative. He recovered uneventfully and was discharged on postoperative day 6.

Case 2

A 70-year-old woman presented with general fatigue and intrahepatic bile duct dilatation. Tumour markers, such as AFP, PIVKA-Ⅱ and CEA, were normal, but CA19-9 was elevated at 843.6 U/mL. Other laboratory tests showed elevated ALP at 601 IU/L, elevated γ-GTP at 318 IU/L, and impaired serum albumin at 3.3 g/dL. Bilirubin was normal. The indocyanine green retention rate at 15 min was 4.6% and the Child-Pugh score was 6 points, Grade A. She was diagnosed with perihilar cholangiocarcinoma and RUP *via* ultrasound, CT and magnetic resonance cholangiopancreatography (Figure 3). The tumour involved the confluence of the left lateral, left medial, and right anterior hepatic ducts; the right posterior branch was intact.

The patient underwent left trisectionectomy with extrahepatic bile duct resection (Figure 4). First, Kocher's manoeuvre and lymphadenectomy around the pancreas head were performed. The distal common bile duct was transected at the level of the pancreas. Then, we performed lymphadenectomy in the hepatoduodenal ligament. The gallbladder was dissected and we secured and encircled the right lateral Glissonean pedicle with tape. The portal vein, the hepatic artery, and the hilar plate were separated from the other structures just proximal to the secured Glissonean pedicle. The vessels entering the pedicle were preserved and the other vessels and contents were resected. In the preoperative simulation, only one right posterior branch of the hepatic artery was identified. During the operation, however, two arteries were found entering the right posterior section. We preserved the vessels that nourished the right posterior section and resected the root of the left hepatic artery, the right anterior hepatic artery, and the common trunk of the left lateral portal vein and RUP; Next, the demarcation area was confirmed. The left side of the liver was fully mobilized, and the liver parenchyma was transected along the demarcation line; Finally, we cut the right posterior hepatic duct, and the specimen was removed. Hepaticojejunostomy to the right posterior bile duct and jejunojejunostomy were conducted, and the operation was successfully completed. The operative time was 697 min, and the estimated blood loss was 716 mL.

A histological examination showed moderately differen-

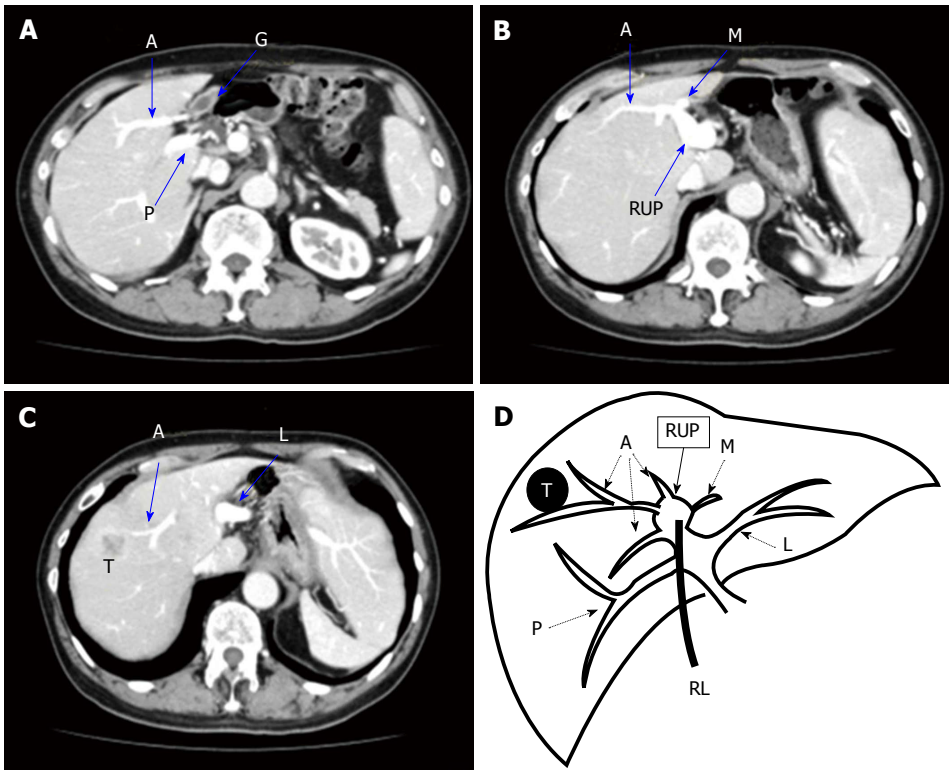

Figure 1 Case 1 enhanced computed tomography. A: Computed tomography shows the left-sided gallbladder and RUP; B: The right anterior and medial segmental portal branches ramify from the RUP after its trifurcation as well as the right posterior and left lateral branch; C: A 25-mm sized tumour peripherally enhanced in the arterial phase was detected in segment 8; D: Diagram of the intrahepatic portal vein branching and the location of the tumour. A: Right anterior portal vein; P: Right posterior portal vein; G: Gallbladder; M: Left medial portal vein; RUP: Right umbilical portion; L: Left lateral portal vein; T: Tumour; RL: Round ligament.

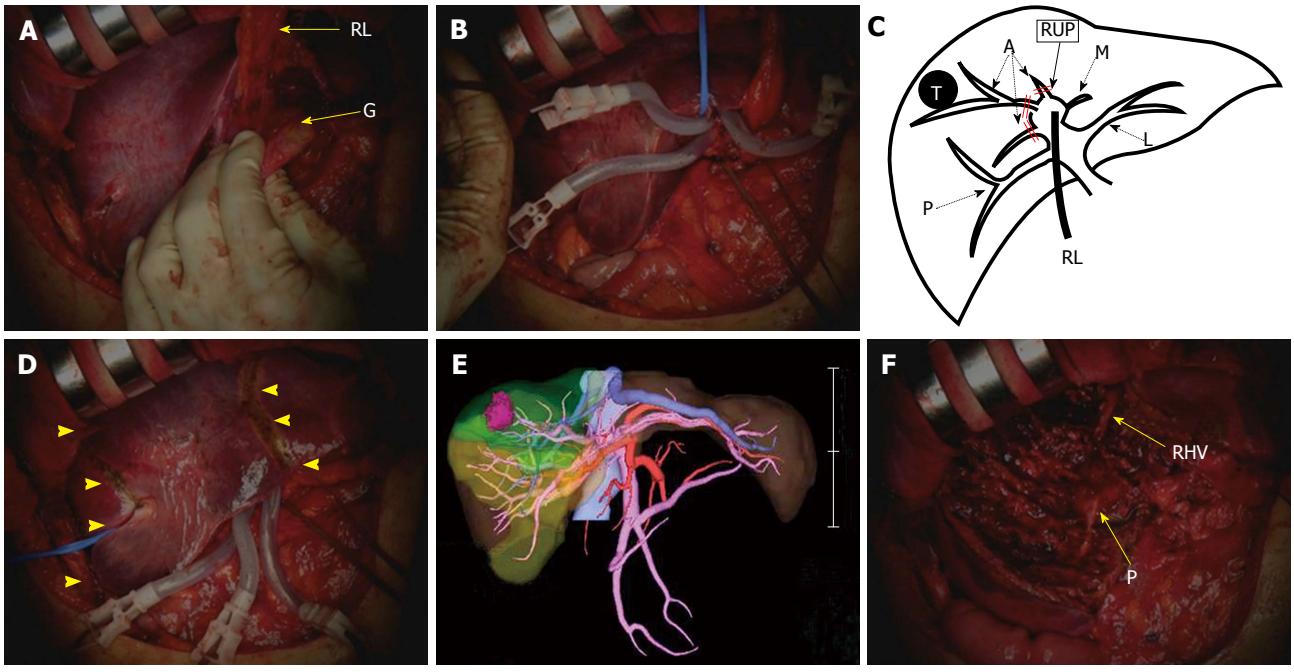

Figure 2 Case 1 operative findings. A: The gallbladder was attached to the round ligament; B: Three ramifications of the right anterior Glissonean pedicles were separated and clamped; C: Diagram of the clamped Glissonean pedicles (double line); D and E: The demarcation area (arrow head) was identified as in the preoperative simulation; F: The accomplishment of a right anterior sectionectomy. RL: Round ligament; G: Gallbladder; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein.

tiated cholangiocarcinoma, 30 mm in size that was invading the hepatic duct and the portal vein. Two lymph node metastases were revealed. The patient was diagnosed as stage ⅡB (T3N1M0). All of the surgical margins were

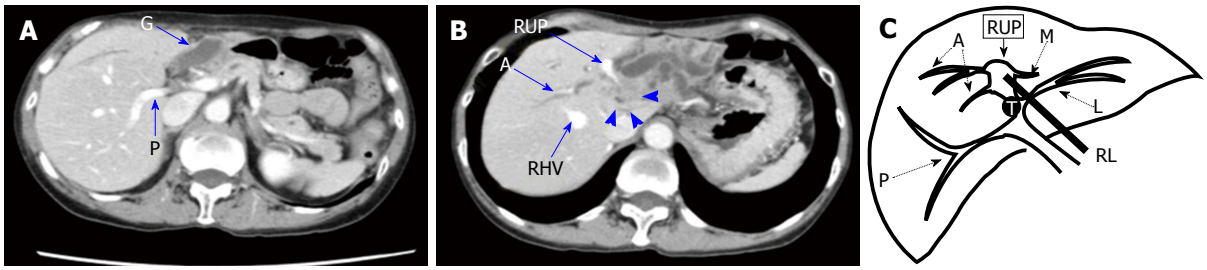

Figure 3 Case 2 enhanced computed tomography. A and B: CT shows the right posterior portal branch to be solely bifurcated, and the right anterior and medial segmental portal branches ramify from the RUP; B: A 25-mm sized mass (arrow head) is adjacent to the RUP. The RUP is almost occluded, and the intrahepatic distal bile duct is dilated (B); C: Diagram of the intrahepatic portal vein branching and the location of the tumour. RL: Round ligament; G: Gallbladder; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein.

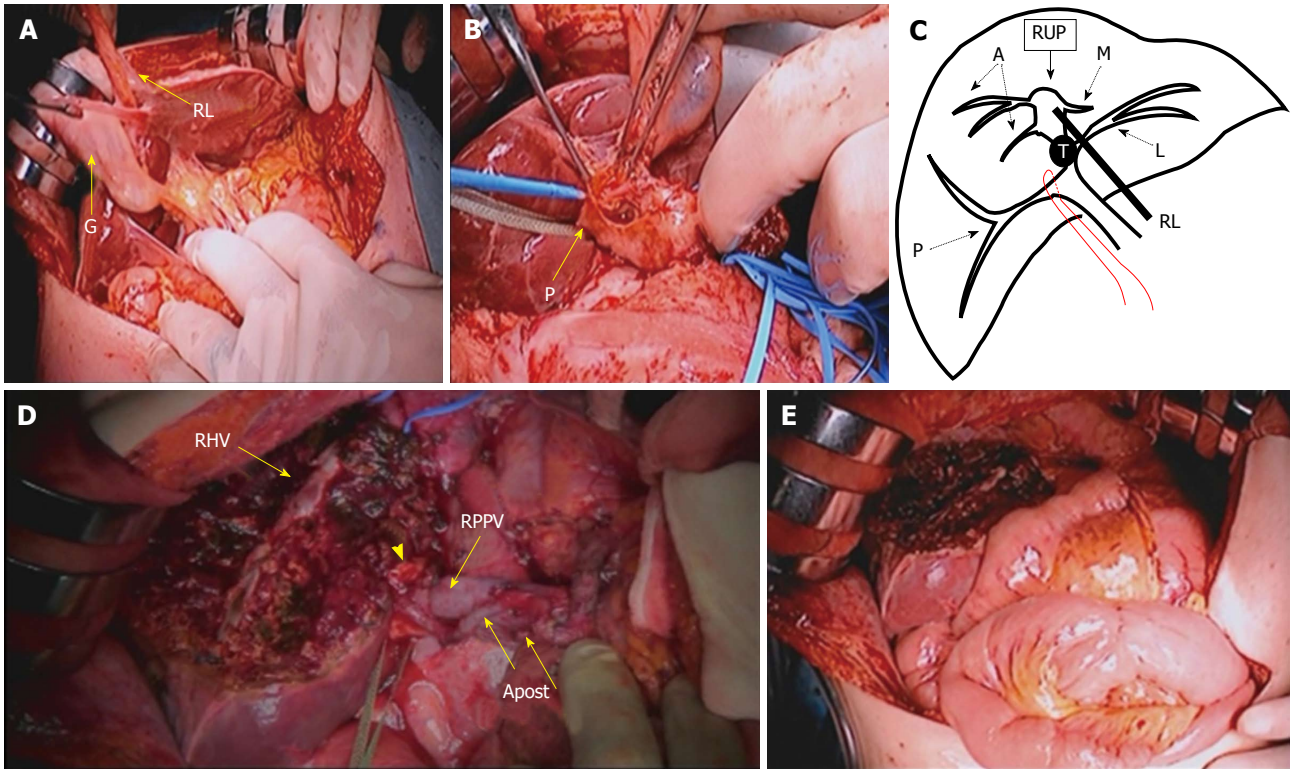

Figure 4 Case 2 operative findings. A: The gallbladder was attached to the round ligament; B: The right posterior Glissonean pedicle was encircled, and the vessels entering the right posterior Glissonean pedicle were identified; C: Diagram of securing the right posterior branch of the Glissonean pedicle; D: The accomplishment of left trisectionectomy; E: Hepaticojejunostomy was performed. RL: Round ligament; G: Gallbladder; A: Right anterior branch of the Glissonean pedicle; P: Right posterior branch of the Glissonean pedicle; M: Left medial branch of the Glissonean pedicle; RUP: Right umbilical portion; L: Left lateral branch of the Glissonean pedicle; T: Tumour; RHV: Right hepatic vein; RPPV: Right posterior portal vein; Apost: Right posterior hepatic artery; Arrow-head: Stump of the right posterior bile duct.

negative. The postoperative course was uneventful and this patient was discharged on postoperative day 13.

DISCUSSION

RUP, previously known as a left-sided gallbladder, is a rare congenital anomaly. However, we occasionally encounter it in our daily medical procedures (*e.g.*, cholecystectomy). RUP is an anatomical anomaly in which the umbilical portion exists between the right anterior and left medial section. The right-sided round ligament adheres to the RUP. Other theories exist regarding liver segmentation with RUP. One is that segment 4 is absent[5]. Another is that the right side of the RUP is comparable with the dorsal segment of the right anterior section and the left side of the RUP with the ventral segment of the right anterior section[7]. In this report, we defined RUP as the umbilical portion that exists between the right anterior and left medial section. Nagai *et al*[1] reviewed the literature concerning this anomaly and classified the type of portal branching according to bifurcation type and trifurcation type. Nineteen cases with RUP have undergone hepatectomy in the English-language literature[1,3,6,8-15] (Table 1). RUP is associated with anomalous ramifications of the hepatic artery, portal vein, and biliary system; thus, surgery for cases with

Table 1 The reported patients with right umbilical portion who underwent hepatectomy in the English-language literature

Ref.	Age (yr)	Sex	Disease	Surgical procedure	Type of intrahepatic portal venous branching
Uesaka *et al*[8]	53	Male	Liver metastasis of bile duct cancer	Right hepatectomy	Trifurcation type
Idu *et al*[9]	Unknown	Male	Perihilar cholangiocarcinoma	Left hepatectomy	Unknown
Nagai *et al*[1]	67	Male	Bile duct cancer	Right anterior sectionectomy, segmentectomy 1 and pancreatoduodenectomy	Trifurcation type
Nagai *et al*[1]	67	Male	Hepatocellular carcinoma	Segmentectomy 8, and partial resection of segment 1	Trifurcation type
Asonuma *et al*[3]	48	Male	Living donor	Left lateral sectionectomy	Unknown
Asonuma *et al*[3]	29	Male	Living donor	Left lateral sectionectomy	Unknown
Asonuma *et al*[3]	35	Female	Living donor	Left lateral sectionectomy	Bifurcation type
Kaneoka *et al*[10]	53	Male	Perihilar cholangiocellular carcinoma	Left hepatectomy and segmentectomy 1 with extrahepatic bile duct resection	Trifurcation type
Kaneoka *et al*[10]	61	Male	Extrahepatic bile duct cholangiocarcinoma	Left hepatectomy, segmentectomy 1, and pylorous-preserving pancreaticduodenectomy	Trifurcation type
Tashiro *et al*[11]	53	Male	Hepatocellular carcinoma	Partial hepatectomy	Trifurcation type
Hwang *et al*[12]	18	Male	Living donor	Right hepatectomy	Bifurcation type
Hwang *et al*[12]	24	Unknown	Living donor	Right posterior sectionectomy	Trifurcation type
Hwang *et al*[12]	39	Unknown	Living donor	Left hepatectomy leaving S4a	Bifurcation type
Hsu *et al*[6]	Unknown	Unknown	Hepatocellular carcinoma	Right hepatectomy	Trifurcation type
Hsu *et al*[6]	Unknown	Unknown	Hepatocellular carcinoma	Partial resection of left lateral section	Trifurcation type
Hsu *et al*[6]	Unknown	Unknown	Hepatocellular carcinoma	Left lateral sectionectomy	Bifurcation type
Abe *et al*[13]	70	Female	Liver metastasis of uterine cervical cancer	Right hepatectomy with extrahepatic bile duct resection	Bifurcation type
Sakaguchi *et al*[14]	76	Male	Liver metastasis of rectal cancer	Right posterior sectionectomy and partial resection of segment 1 and right anterior section	Trifurcation type
Almodhaiberi *et al*[15]	67	Male	Perihilar cholangiocarcinoma	Extended left lateral sectionectomy and segmentectomy 1 with extrahepatic bile duct resection	Trifurcation type
Case 1	70	Male	Intrahepatic cholangiocarcinoma	Right anterior sectionectomy	Trifurcation type
Case 2	70	Female	Perihilar cholangiocarcinoma	Left trisectionectomy with extrahepatic bile duct resection	Trifurcation type

RUP requires careful procedures, especially with regard to hepatic resection. Previous reports described the importance of the thorough preoperative and intraoperative recognition of the various anomalies associated with RUP to prevent operative accidents.

CT and three-dimensional imaging have been developed, and preoperative simulation is of great help. We must preoperatively evaluate and recognize the anatomy precisely in cases with this anomaly. However, some vessels go unrecognized during the preoperative survey but can be encountered during the procedure, as was observed in case 2. Thus, paying special attention during the operation is important.

The Glissonean approach is acknowledged as a potentially successful technique for liver surgery, and it is widely performed for liver resection. The ramification pattern of the hepatic artery, portal vein and bile duct in the hepatoduodenal ligament often varies across patients. However, the Glissonean pedicle peripheral to the hilar plate, which is wrapped by connective tissue and contains the hepatic artery, portal vein, and bile duct, enters its proper area and never contains branches that nourish other areas. Consequently, the Glissonean pedicle transection peripheral to the extrahepatic hilar plate is a safe and sure method that enables the cutting of the intended vessels without damaging the vessels to be preserved. Secondary and tertiary branches of the Glissonian pedicle peripheral to the hilar plate can usually be approached and transected extrahepatically. When the targeted Glissonean pedicle is transiently and selectively clamped, we can recognize the area to be resected. Surgeons do not have to consider any variations in the hepatoduodenal ligament. The Glissonean approach is a successful method, especially in cases with anomalous ramifications of the hepatic artery, portal vein and biliary system. The Glissonean pedicle to be resected was separated in case 1, whereas that to be preserved was encircled in case 2. The Glissonean approach can be used in various situations of hepatic resection and it contributes to a safe and secure liver surgery.

In conclusion, we successfully performed two major hepatectomies using the Glissonean approach in cases with RUP. The Glissonean approach is a useful method and contributes to a safe procedure for cases with an anomalous anatomy such as RUP.

COMMENTS

Case characteristics

A 70-year-old man with hepatitis C presented with a liver tumour without any symptoms; a 70-year-old woman presented with general fatigue and intrahepatic bile duct dilatation.

Clinical diagnosis
Intrahepatic cholangiocarcinoma or combined hepatocellular and cholangiocarcinoma of the right umbilical portion (RUP); perihilar cholangiocarcinoma of the RUP.

Differential diagnosis
Metastatic liver tumour; intrahepatic cholangiocarcinoma and inflammatory biliary stenosis.

Laboratory diagnosis
The level of tumour marker PIVKA-Ⅱ was elevated at 808 mAU/mL; other tumour markers were normal; the level of tumour marker CA19-9 was elevated at 843.6 U/mL; other tumour markers were normal.

Imaging diagnosis
A computed tomography (CT) scan showed RUP and a 25-mm sized tumour peripherally enhanced in the arterial phase in segment 8; a CT scan showed RUP and a 25-mm sized tumour in the left side of the perihilar region, which caused dilatation of intrahepatic distal bile duct and almost occluded the RUP.

Pathological diagnosis
A pathological examination showed a poorly differentiated intrahepatic cholangiocarcinoma invading the intrahepatic portal vein; the pathological findings revealed a moderately differentiated cholangiocarcinoma invading RUP.

Treatment
The patient was treated with right anterior sectionectomy; the patient was treated with left trisectionectomy.

Related reports
Only nineteen cases of hepatectomy among patients with RUP have been reported in the English-language literature.

Term explanation
RUP is a congenital anomaly in which the umbilical portion exists between the right anterior section and left medial section.

Experiences and lessons
This report emphasizes that the Glissonean approach is useful, especially in cases with anomalous ramifications of the hepatic artery, portal vein and biliary system such as RUP. This procedure contributes to a safe and secure liver surgery.

Peer-review
This paper is the first report about major hepatectomy using the Glissonean approach in cases with RUP, and demonstrates the safety and usefulness of the Glissonean approach for hepatectomy in cases with anomalies such as RUP, and this report is very important guidance for surgeons who perform major hepatectomy for cases with RUP.

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P- Reviewer: Bramhall S, Lau WYJ, Qin JM **S- Editor**: Qiu S
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