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Polycystic liver disease: Classification, diagnosis, treatment process, and clinical management

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Abstract

Polycystic liver disease (PLD) is a rare hereditary disease that independently exists in isolated PLD, or as an accompanying symptom of autosomal dominant polycystic kidney disease and autosomal recessive polycystic kidney disease with complicated mechanisms. PLD currently lacks a unified diagnostic standard. The diagnosis of PLD is usually made when the number of hepatic cysts is more than 20. Gigot classification and Schnelldorfer classification are now commonly used to define severity in PLD. Most PLD patients have no clinical symptoms, and minority with severe complications need treatments. Somatostatin analogues, mammalian target of rapamycin inhibitor, ursodeoxycholic acid and vasopressin-2 receptor antagonist are the potentially effective medical therapies, while cyst aspiration and sclerosis, transcatheter arterial embolization, fenestration, hepatic resection and liver transplantation are the options of invasion therapies. However, the effectiveness of these therapies except liver transplantation are still uncertain. Furthermore, there is no unified strategy to treat PLD between medical centers at present. In order to better understand recent study progresses on PLD for clinical practice and obtain potential directions for future researches, this review mainly focuses on the recent progress in PLD classification, clinical manifestation, diagnosis and treatment. For information, we also provided medical treatment processes of PLD in our medical center.

Key words: Polycystic liver disease; Autosomal dominant polycystic kidney disease; Autosomal recessive polycystic kidney disease; Isolated polycystic liver disease; Diagnosis; Treatment

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Core tip: Polycystic liver disease (PLD) is a rare hereditary disease. However, there is no unified strategy in the treatment of PLD so far. In order to better understand recent progresses on clinical practice of PLD and contribute to potential directions for future researches, we conducted this review mainly focusing on recent progresses of PLD classification, clinical manifestation, diagnosis and treatments. For information, we also

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provided medical treatment process of PLD that is being used in our medical center.

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INTRODUCTION

Polycystic liver disease (PLD) is a rare hereditary disease and often defined as multiple diffuse cysts of the liver^[1]. It can independently exist in isolated PLD (PCLD), or as an accompanying symptom of autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD), while the mechanisms of cysts in PLD and PKD are complicated. In the one hand, they are both related to the primary cilia of biliary epithelial cells and the key proteins associated with cilia function, thus classified as fibrocystic diseases or ciliary diseases^[2]. In the other hand, some scholars have classified them as cholangiopathic disease due to the source of PLD cysts which is from congenital bile duct dysplasia through multiple mechanisms^[3]. Meanwhile, there is an opinion that the two mentioned above are actually associated with each other for there is a causality between fibrocystic malformation and dysgenesis of the biliary structures. In the limited treatments of PLD, drug therapy has gradually become a hot spot along with the deepening understanding on the pathogenesis of PLD, while there are still controversies in surgical treatments. In order to better understand recent progresses on PLD for clinical practice and contribute to potential directions for future researches, we conducted this non-systematic review mainly focusing on the recent progresses on PLD classification, clinical manifestation, diagnosis and treatments.

BRIEF SUMMARY OF EPIDEMIOLOGY AND MECHANISMS

Despite the genes associated with pathogenesis, the natural courses of various PLD in the liver are basically the same, showing as a continuous increase in the number and volume of cysts in liver^[4]. However, it is essential to clarify the various forms of PLD (Table 1).

PLD in ADPKD

ADPKD is the most common monogenic genetic disease in the kidneys, with a global incidence of about 0.25% to 1%^[5]. PLD is the most common extrarenal symptom of ADPKD, which involved with 94% ADPKD patients^[6]. Mutations in two genes (PKD1 and PKD2) cause the development of ADPKD. PKD1 is located at chromosome 16p13.3 with 80% of cases related to it, while PKD2 is located at chromosome 4q21-22, which is responsible for the remaining 5-10% of cases^[5]. PKD3 was once thought to be associated with ADPKD but excluded according to the recent family reanalysis^[7]. Additionally, GANAB, which is involved in protein folding, was also reported to be responsible for ADPKD^[5,8].

PLD in ARPKD

ARPKD is pretty rare with the incidence about 1:20000. It often occurs in children, of which 30% die from severe lung dysplasia and secondary respiratory failure, with renal collecting duct dilatation, bile duct dysplasia and portal fibrosis as the mainly clinical manifestations^[9]. At present, a mutation of *PKHD1* gene on the short arm of chromosome 6 encoding a fibrocystic protein, of which function is still not well-known, is found to be responsible for ARPKD. As well as PKD1 and PKD2, PKHD1 is also involved in the processes of forming the original cilia of liver and kidney, eventually causing cyst formation^[2].

PLD in PCLD

Unlike ADPKD and ARPKD, PCLD often does not involve the kidneys^[10]. In the previous studies of variant genes in PCLD, *PRKCSH* gene mutation accounted for the highest proportion of 15%, followed by SEC63 and LRP5. Meanwhile, GANAB is the first gene found to be associated with PCLD with a small proportion (approximately

Table 1 Brief summary of various polycystic liver disease

Disease	Genes mutation	Kidney involvement
ADPKD	<i>PKD1, PKD2, GANAB</i>	Yes
ARPKD	<i>PKHD1</i>	Yes
PCLD	<i>PRKCSH, SEC63, LRP5, GANAB, ALG8, SEC61B, PKHD1</i>	Usually not

ADPKD: Autosomal dominant polycystic kidney disease; ARPKD: Autosomal recessive polycystic kidney disease; PCLD: Isolated polycystic liver disease.

1%). However, there are still a big amount of cases where a pathogenic gene cannot be found. The products of *PRKCSH*, *SEC63* and *GANAB* genes are important proteins involved in the process of co-translational transport and maturation of glycoproteins in the endoplasmic reticulum^[11], while the unidirectional transmembrane molecules encoded by *LRP5* gene, with Frizzled receptors together, can bind to Wnt proteins, thereby initiating the Wnt signaling pathway and participating in the pathophysiological changes of PCLD^[12]. Furthermore, in the recent PCLD pathogenic gene research, mutations in three genes, *ALG8*, *SEC61B* and *PKHD1*, are also found to be involved in the development of PCLD, which together with the above-mentioned *PRKCSH*, *SEC63*, and *GANAB* genes can explain nearly 50% PLD cases^[13]. The α -1,3-glycosyltransferase encoded by the *ALG8* gene is an endoplasmic reticulum integral membrane protein^[14], and the *SEC61B* gene-encoded product is an important component of the SEC63 protein complex on the endoplasmic reticulum. Both the two genes play important roles in protein quality regulation^[15]. In addition, recent study^[16] showed cholangiocyte autophagy contributed to hepatic cystogenesis in PLD and represented as a potential therapeutic target.

CLINICAL MANIFESTATION

Although the volume of PLD liver increases by 1.8% per 6 to 12 mo^[17,18], most patients have no clinical symptoms regardless of the type of PLD. About 20% of patients develop obvious clinical symptoms including dyspnea, early satiety, abdominal distension, malnutrition, gastroesophageal reflux, back pain due to hepatomegaly pressing surrounding organs or cyst complications, which will seriously affect the quality of life^[19-21]. Moreover, patients suffering from PLD may develop hepatic venous outflow obstruction because of cystic mass effect, resulting in portal hypertension, ascites, variceal haemorrhage or splenomegaly^[22,23]. Gabow *et al*^[24] found that the risk factors for hepatic cyst symptoms in ADPKD patients were older age, female gender and multiple pregnancy history. Studies^[25,26] have also shown that in female, hepatic cysts grow rapidly under the influence of hormones, which may be related to the expression of estrogen receptors α and β ^[27]. Moreover, lower age was reported to be independently associated with larger liver volume in ADPKD females patients, whereas the higher age in male patients^[28]. The gender differences and related mechanisms should be investigated in future.

In most patients with PLD, liver function tests are usually normal because liver parenchyma is not completely destroyed^[29], however elevated γ -glutamyltransferase, alkaline phosphatase, aspartate aminotransferase and total bilirubin are reported in some serious cases^[30,31]. Elevation of γ -glutamyltransferase and alkaline phosphatase may be the result of biliary cell activation^[24,32], while the increase in total bilirubin can be seen in some cases of cystic compressing the bile duct. Furthermore, a study by Waanders *et al*^[33] found that 45% PLD patients showed an increase in CA19-9 with a degree of elevation positively correlated with polycystic liver volume. Besides, the possibility of cysts infection is needed to consider when detecting a significant increase of CA19-9, and decrease of CA19-9 can be seen following effective anti-infective treatments.

DIAGNOSIS

The diagnosis of PLD is usually made when the number of hepatic cysts is more than 20^[31]. However, patient with a family history of PCLD can be diagnosed when number of cysts more than 4^[10]. However, the type of PLD can be hard to distinguish. Because PCLD patients may have renal cysts while ADPKD or ARPKD patients may have hepatic cysts as the main clinical manifestations, the identification between them

without family history may be difficult and requires genetic analysis.

Currently there are mainly two clinical classifications on PLD: Gigot classification^[34] (Table 2) and Schnelldorfer classification^[35] (Table 3). Both of them include the number and size of cysts and the remaining liver parenchyma volume as the criteria for typing, while the latter also considers the inflow and outflow of pre-retained liver segments, which is more conducive to the choice of treatment. There was a Qian classification^[19] relying on the number of cysts and the presence of symptomatic hepatomegaly (Table 4), however it is seldom used now because of oversimplification and, more importantly, having no contribution to selection of treatments.

TREATMENTS

Most patients with asymptomatic PLD do not need any treatment, while minority need only when incapacitating symptoms and a lower quality of life caused by hepatomegaly or complications such as cyst rupture, infection, bleeding, or hepatic venous outflow obstruction^[21,36]. At present, the treatments of PLD are divided into three main categories: drug therapy, percutaneous therapy and surgical therapy.

Drug therapy

Somatostatin analogues: Although there is currently no approved effective treatment for PLD, recent progresses in somatostatin analogues have been achieved with positive results^[37]. Somatostatin is a neurohormone with a wide range of effects through combining with somatostatin receptor (SSTR). There are five subtypes of SSTR (SSTR-1 to SSTR-5), which are expressed in varied tissues of human body. Somatostatin analogues such as octreotide, lanreotide and *etc.* are able to interact with the SSTR on the surface of the cyst wall to reduce the cAMP level of the bile duct epitheliums, inhibit the secretion of cyst fluid and hyperplasia of the bile duct cells, therefore inhibiting the growth of hepatic cysts^[38,39].

Multiple controlled trials^[17,18,40,41] showed that the liver volume of the group using somatostatin analogues was significantly reduced comparing to the control group. For octreotide, Caroli *et al.*^[40] administered 40 mg of octreotide per month and found that the experimental group had a significant reduced liver volume 71 ± 57 mL within 6 mo. Meanwhile, Hogan *et al.*^[17] gave the same dosage of octreotide to patients with severe ADPKD and PLD and the liver volume decreased by $4.95\% \pm 6.77\%$ within a year ($P = 0.048$). In some patients with symptomatic PLD, octreotide were reported for significantly slowing disease progression, reducing symptoms and improving quality of life for 4 years^[42]. For lanreotide, the liver volume of PLD patients using lanreotide decreased by 2.9% on average in 6 mo, and 4.0% in a year ($P = 0.01$)^[18]. And more recently, lanreotide also showed positive effects on decreasing liver volume in patients with ADPKD and PLD, compared with control arm^[43]. For the pattern of effectiveness, the 120 mg lanreotide group benefited more than the 90 mg lanreotide group and the control group^[41]. In another study, Temmerman *et al.*^[44] increased the therapeutic dose of lanreotide non-responder from 90 mg to 120 mg, which led to stopping liver volume growing. These studies have shown that the efficacy of lanreotide may be dose-dependent.

Pasireotide is a more stable somatostatin analogue than octreotide, with a half-life of 12 h and it is currently used to treat Cushing's syndrome. Unlike octreotide and lanreotide, pasireotide can combine with all the SSTR subtypes to function, except SSTR-4^[45]. Studies^[46] have shown that pasireotide is more effective in relieving hepatorenal cyst formation than octreotide in the PKD mouse model. Further clinical data are required to confirm its efficacy.

On the contrary, a research^[47] systematically reviewed seven PLD drug treatment studies from January 1966 to August 2014 and found that though the use of somatostatin analogues significantly reduced liver volume in 6 mo, however the improvement of patient quality of life and relief of clinical symptoms were very limited. In addition, there are some controversies about duration of efficacy and effect of cessation of treatment (also called drug holiday). Some studies^[48] have also shown that the efficacy of somatostatin analogue therapy can only last for 2 years, and cessation of treatment would lead to disappearance of effect or even a rebound effect^[42,49,50]. However, a study^[43] showed the benefit to reduce liver volume from lanreotide still persisted 4 mo after cessation of the drug. Meanwhile, second cycle of somatostatin analogues after a drug holiday would still be as effective as the first in reducing liver volume^[50]. This issue should be investigated in future clinical practicing.

Mammalian target of rapamycin (mTOR): mTOR is a serine/threonine protein kinase belonging to the phosphatidylinositol 3-kinase-associated kinase (PI3K) family

Table 2 Gigot classification

	Number of cysts	Cyst size	Remaining areas of noncystic liver parenchyma
Gigot type I	< 10	Large (> 10 cm)	Large
Gigot type II	Multiple	Small, medium	Large
Gigot type III	Multiple	Small, medium	Few

and plays an important role in regulating signaling in many pathways. It mainly presents in two different complexes: Rapamycin target protein complex 1 (mTORC1) and rapamycin target protein complex 2 (mTORC2). mTORC1 is a growth regulator that can sense and aggregate trophic and environmental factors, while mTORC2 can promote cell survival, regulate cytoskeletal remodeling, ion transport and growth^[51]. mTOR inhibitor is a targeted drug currently used in the treatment of cancer, including sirolimus, everolimus, and *etc.* In the PKD animal model, they showed significant inhibition of cyst growth and delaying the progression of the disease^[52-55]. Qian *et al.*^[56] showed that sirolimus significantly reduced liver volume (11.9%) in ADPKD patients after renal transplantation ($P = 0.009$). However, in the clinical randomized controlled trials by Serra *et al.*^[57] and Walz *et al.*^[58] 18 mo of sirolimus and 2 years of everolimus were used, respectively, and had no significant effect on progression of renal cysts ($P = 0.26$, $P = 0.06$). Chrispijn *et al.*^[59] used different drug regimens for PCLD and ADPKD patients. The efficacy of everolimus-octreotide combination therapy was not significantly different in reducing liver volume compared with octreotide monotherapy ($P = 0.73$). On the other hand, as an immunosuppressive agent, mTOR inhibitors could increase the incidence of infection and malignant tumors as well as other side effects including dyslipidemia, thrombosis and lung diseases. Although most of them are moderate and may regress with lower doses, these side effects are unpredictable and idiosyncratic, which medics need to pay highly cautions to in clinical practice^[60].

In summary, though with acceptable safety profile, there is not enough evidence to prove that mTOR inhibitors can benefit PLD patients. Thus, the use of mTOR inhibitors for the treatment of PLD is not recommended until more systematic and comprehensive results are obtained.

Ursodeoxycholic acid: Ursodeoxycholic acid (UDCA), which is a Ca^{2+} agonist in hepatocytes and biliary epithelial cells, has been shown to delay the growth of hepatic cysts in PLD animal model experiments. The mechanism is to inhibit cystic hyperplasia of biliary epithelial cells by inhibiting the proliferation of cystic bile duct epithelium and decreasing cytotoxic bile acid levels in the liver without affecting apoptosis by the PI3K/AKT/MEK/ERK1/2 pathway^[61]. However, a multicenter randomized controlled trial^[62] showed that liver volume insignificantly increased by $4.6\% \pm 7.7\%$ in advanced PLD patients after 24 wk of UDCA treatment, with a liver volume increase of $3.1\% \pm 3.8\%$ in the control group ($P = 0.493$), but subgroup analysis showed significant delay on the growth of hepatic cysts in ADPKD patients ($P = 0.049$).

Vasopressin-2 receptor antagonist: Vasopressin-2 receptor (V2R) is localized in the renal tubular epithelium, which promotes vesicle secretion and cell proliferation by up-regulating cAMP level^[63]. Studies^[64] have shown that antagonizing V2R in the kidney can delay the growth of renal cysts and improve renal function in the PCK mouse model. In a randomized controlled trial^[65], the growth rate of renal cysts in the treatment group treated with tolvaptan was slower than the control group ($P < 0.001$). Meanwhile, even in advanced ADPKD patients, tolvaptan also showed protective effect on kidney function^[66]. Although V2R is theoretically not expressed in biliary epithelial cells, meaning V2R antagonists are not effective against hepatic cysts, successful V2R treatment in reducing liver volume in PLD patients have recently been reported^[67].

Percutaneous therapy

Cyst aspiration and sclerosis: This treatment is often used for patients with a single giant cyst, as the Gigot type I^[68]. Besides completely suction of fluid, the sclerosing agent will be injected into the cyst to destroy the epitheliums of the cyst wall. The most commonly used agent is ethanol, followed by ethanolamine oleate, minocycline, tetracycline, *etc.*^[68,69]. A retrospective study by Benzmira *et al.*^[70] collected 58 cases of hepatic cysts treated with puncture and ethanol sclerotherapy, and the cyst volume was reduced by an average of 94% and the symptom relief rate was 95%. In meta-

Table 3 Schnelldorfer classification

	Symptoms	Cyst characteristics	Areas of relative normal liver parenchyma	Isosectoral portal vein or hepatic vein occlusion of preserved sector
Type A	Absent or mild	Any	Any	Any
Type B	Moderate or severe	Limited Number with large cysts	> 2 sectors	Absent
Type C	Severe (or moderate)	Any	> 1 sector	Absent
Type D	Severe (or moderate)	Any	< 1 sector	Present

analysis review^[71] of cystic puncture and sclerotherapy including a total of 526 patients in 16 studies, 76%-100% of cases had partial cyst volume remission, while 72%-100% of cases had partial symptom remission, and 56%-100% of cases reported disappearance of symptoms. However, some researchers reported that the recurrence rate of cysts was as high as 80% undergone cyst aspiration and sclerosis, and the recurrence rate of symptoms was as high as 50%^[72]. Nevertheless, PLD patients are often diagnosed with multiple cysts, thus this procedure actually is seldom used in PLD patients.

Transcatheter arterial embolization: Transcatheter arterial embolization (TAE) is using embolic agents to selectively embolize the branches of the arteries that supply blood to the cysts, thereby destroying the cells of the cystic wall, cutting off the source of the cystic fluid, and controlling the disease progression^[73]. The application of this treatment is mainly due to the recent study showed that the cysts in PLD were mainly supplied by the hepatic artery^[74]. A retrospective study with a small sample by Zhang *et al*^[75] found that liver volume of PLD patients after TAE decreased by 32%, 31%, and 33% at 1 year, 2 years, and 3 years, respectively, while liver cyst volume reduced by 36%, 37%, 38%. Hoshino *et al*^[76] collected 244 PLD cases undergone liver TAE, and the liver volume decreased by 94.7% (95% CI: 93.5%-95.8%) at 6 mo and 90.8% (95% CI: 88.7%-92.9%) at 1 year after TAE, respectively. A recent preliminary study^[77] also showed positive effects on improvement of symptoms and shrinkage of cyst volume in PLD patients. Meanwhile, a study^[78] showed its failure rate is as high as 69.6%, including uncontrolled symptoms, postoperative liver failure and death. Nevertheless, there is still a need for more well-designed large-scale studies to investigate their safety and efficacy before widespread adoption. And efforts should be made to investigate its potential as an adjuvant therapy.

Surgical therapy

Fenestration: Being different with cyst puncture and sclerotherapy, fenestration is often used for the treatment of multiple cysts, as Gigot type I-II or Schnelldorfer type B patients. In addition, the procedure can also be applied to cases when cyst puncture and sclerotherapy failed^[79]. With the development of laparoscopic techniques, fenestration is now usually performed using laparoscope, but sometimes it has to be completed under the ordinary surgery due to uncontrollable bleeding, blind spots or technique, *etc*^[1]. Symptoms are greatly relieved in 92% of cases undergone fenestration^[80], however 33.7% of patients suffer symptomatic recurrence and 26.4% need reintervention^[81]. Patients with multiple cysts larger than 5 cm in diameter have a higher recurrence rate than patients with smaller volume cysts^[82]. Common complications of this procedure include ascites, pleural effusion, hemorrhage, and bile leakage. A meta-analysis^[83] showed that the recurrence rate through open surgery was slightly lower, however without statistical significance, than through laparoscopic approach (5% *vs* 6%), and most recurrent cysts do not require second surgery. However, we believe the incidence of complication of laparoscopic fenestration will reduce, which is related to the continuous updating of surgical instruments and the increasing experience of surgeons. Besides, considering the convenience and small trauma, we still recommend laparoscope as first priority.

Hepatic resection: Hepatectomy is often used in severe Gigot II PLD patients with at least one liver segment that is not affected by the cysts^[35]. The extent of resection depends on the size and distribution of the cysts. However, due to the compression of the cyst, the distorted Glisson system and hepatic venous outflow obstruction increase the difficulty of resection. Thus, for cysts that cannot be removed, fenestration is often performed with hepatic resection^[3]. Meanwhile, hepatic venous outflow obstruction is frequent in PLD and has major consequences on intraoperative bleeding and

Table 4 Qian classification

	Number of cysts	Symptomatic hepatomegaly
Grade 0	0	No
Grade 1	1-10	No
Grade 2	11-20	No
Grade 3	> 20	No
Grade 4	> 20	Yes

postoperative ascites and liver failure^[84]. In a retrospective study by Chebib *et al*^[85] including 186 PLD patients undergone hepatectomy, postoperative liver volume is reduced by an average of 61% comparing to preoperative, with a high complication rate of 21% and a mortality rate of 2.7%. On the other hand, some studies^[83,86] claimed that hepatectomy could greatly alleviate symptoms and prolong the needs of liver transplantation with an acceptable safety profile. In addition, application of somatostatin analogues after hepatectomy can inhibit the growth of residual cysts and prevent the occurrence of new cysts^[86]. Despite the compromising relief of symptom and reduction of liver volume, it is presently not recommended as a first-line treatment plan, because of the high complication and mortality rate and the potential difficulties for the future liver transplantation due to abdominal adhesion^[1,87]. Nevertheless, we consider the most important issues may be when and how to perform this procedure, or in other words, which group of patients benefit from hepatectomy, which would maximize the value of the surgery.

Liver transplantation: Liver transplantation is currently the only cure for PLD, which is mainly applied in Gigot type III patients with severe symptoms that seriously affect the quality of life of patients, as well as untreated complications such as portal hypertension and malnutrition^[1]. In the PLD classification designed by Schnelldorfer *et al*^[35], liver transplantation is suitable for patients with type D. Compared with hepatocellular carcinoma and chronic liver failure, the survival rate of PLD is significantly higher than that of the former two, respectively^[88,89]. It has been reported that the 5-year survival rate was 92.3%^[90], while a more recent research found it as 85.1%^[88]. Meanwhile, most of patients have a significant improvement on health-related quality of life assessment after transplantation^[91]. Even liver transplantation performs comparatively well in PLD, however, due to the lack of liver donors, relatively low urgency and low mortality rate, it is difficult to extensive perform and necessary to carefully evaluate the indications of liver transplantation.

MEDICAL TREATMENT PROCESS

Although some institutions have attempted to make guidance in treating PLD^[92], there is currently no widely accepted international guideline for treatment of PLD. Various therapies were used to treat different types of PLD in different medical centers. Schnelldorfer *et al*^[35] gave the corresponding preferred treatment based on Schnelldorfer classification. Base on this, we modified the process according to the clinical experience of our medical center in treating PLD, which is being used in our medical center, and here we summarize it as **Figure 1**.

Ultrasound should be the first choice for first visit of suspicious PLD patients. Enhanced computer tomography is used for patients diagnosed with PLD to determine Schnelldorfer classification. The treatment plan is determined based on the classification, liver function and computerized three-dimensional imaging which is used to calculate liver volume and estimate resident liver volume after resection for ensuring safety of hepatectomy. For patients with asymptomatic or mild symptoms, Schnelldorfer type A, simple observation or long-acting somatostatin analogue can be applied without any surgical interventions. Schnelldorfer B patients with large cysts but limited number should be treated with cyst aspiration and sclerosis or fenestration depending on situation of cysts, to reduce cyst volume and relieve symptoms. For Schnelldorfer type C patients with excessive cysts, normal liver function and sufficient liver parenchyma volume, only fenestration cannot achieve long-term relief of symptoms. Under the premise of ensuring the pre-retained inflow of the hepatic lobe and the safety of the outflow tract, it is more appropriate to choose hepatectomy with fenestration to remove the liver segment occupied by the cyst, which would minimize the liver volume for controlling symptoms for a longer time.

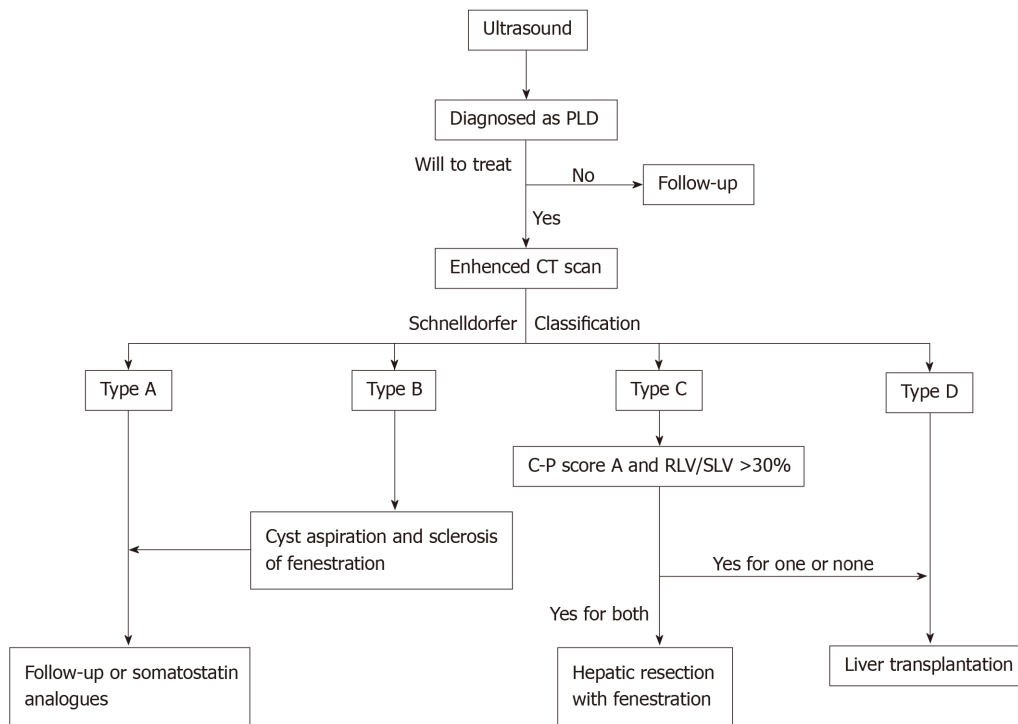


Figure 1 Polycystic liver disease diagnosis and treatment process in our medical center based on Schnelldorfer classification. PLD: Polycystic liver disease; LV/SLV: Remaining liver volume / standard liver volume; CT: Computer tomography.

However, if Schnelldorfer is classified as type C with impaired liver function (child-pugh score B or C) or insufficient residual liver volume (remaining liver volume / standard liver volume < 30%), liver transplantation is recommended. In addition, somatostatin analogue can be considered after fenestration or hepatectomy. Hepatectomy is no longer suit for Schnelldorfer type D patients, and liver transplantation is most appropriate regardless of the condition of liver function.

CONCLUSION

PLD is a hereditary genetic disease. Although PLD progresses with age, only a small number of patients have symptoms that require treatment. At present, the treatment of PLD is mainly drugs intervention, cyst puncture and sclerotherapy, fenestration, transcatheter arterial embolization, liver resection, liver transplantation. Clinical drug therapy for PLD is currently focused on somatostatin analogues, while many other drug targets are being developed as more and more clinical trials validating their effectiveness. Liver transplantation is now the only cure for PLD, but it cannot be carried out in large quantities due to complicated reasons. Except liver transplantation, the other four surgical and interventional treatments can be widely used for PLD patients with different conditions. But considering the high recurrence rate, serious complications and mortality, it is necessary to carefully consider the indications. Besides, various combination therapies should be investigated in future researches for better effectiveness. In addition, for reference, we provide the diagnosis and treatment process being applied in our medical center, which is based on Schnelldorfer classification and the experience of the medical center.

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Basic Study

High omega arachidonic acid/docosahexaenoic acid ratio induces mitochondrial dysfunction and altered lipid metabolism in human hepatoma cells

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Abstract

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a common cause of liver disease worldwide and is a growing epidemic. A high ratio of omega-6 fatty acids to omega-3 fatty acids in the diet has been implicated in the development of NAFLD. However, the inflicted cellular pathology remains unknown. A high ratio may promote lipogenic pathways and contribute to reactive oxygen species (ROS)-mediated damage, perhaps leading to mitochondrial dysfunction. Therefore, these parameters were investigated to understand their contribution to NAFLD development.

AIM

To examine the effect of increasing ratios of omega-6:3 fatty acids on mitochondrial function and lipid metabolism mediators.

METHODS

HepG2-derived VL-17A cells were treated with normal (1:1, 4:1) and high (15:1, 25:1) ratios of omega-6: omega-3 fatty acids [arachidonic acid (AA): docosahexaenoic acid (DHA)] at various time points. Mitochondrial activity and function were examined *via* MTT assay and Seahorse XF24 analyzer, respectively.

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Triglyceride accumulation was determined by using EnzyChrom™ and levels of ROS were measured by fluorescence intensity. Protein expression of the mediators of lipogenic, lipolytic and endocannabinoid pathways was assessed by Western blotting.

RESULTS

High AA:DHA ratio decreased mitochondrial activity ($P < 0.01$; up to 80%) and promoted intracellular triglyceride accumulation ($P < 0.05$; 40%-70%). Mechanistically, it altered the mediators of lipid metabolism; increased the expression of stearoyl-CoA desaturase ($P < 0.05$; 22%-35%), increased the expression of peroxisome proliferator-activated receptor- α ($P < 0.05$; 30%-40%) and increased the expression of cannabinoid receptor 1 ($P < 0.05$; 31%). Furthermore, the high ratio increased ROS production ($P < 0.01$; 74%-115%) and reduced mitochondrial respiratory functions such as basal and maximal respiration, ATP production, spare respiratory capacity and proton leak ($P < 0.01$; 35%-68%).

CONCLUSION

High AA:DHA ratio induced triglyceride accumulation, increased oxidative stress and disrupted mitochondrial functions. Stimulation of lipogenic and steroidal transcription factors may partly mediate these effects and contribute to NAFLD development.

Key words: Non-alcoholic fatty liver disease; Lipogenesis; Omega fatty acids; Mitochondrial dysfunction; Reactive oxygen species; Oxidative stress

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Core tip: A high ratio of omega 6:3 fatty acids in the diet has been implicated in the development of non-alcoholic fatty liver disease, a growing epidemic of major concern. The cellular pathology induced by such high ratios remains unknown. Here, we observed that in human hepatoma HepG2 (VL-17A) cells, high omega-6:omega-3 ratio reduced mitochondrial activity, increased triglyceride accumulation, elevated reactive oxygen species levels and interrupted several mitochondrial functions. Moreover, the increased expression of stearoyl-CoA desaturase, decreased expression of peroxisome proliferator-activated receptor α and elevation in cannabinoid receptor-1 expression collectively lead to lipogenesis and lipotoxicity, which are key features of non-alcoholic fatty liver disease development.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) overarches all chronic liver conditions resulting from high liver fat content that are not caused by alcohol consumption, steatogenic medication or monogenic disorders. It encompasses a broad spectrum of pathological states ranging from the initial steatosis (fatty liver), which may progress to non-alcoholic steatohepatitis (NASH), followed by fibrosis and cirrhosis, and may result in liver failure or hepatocellular carcinoma^[1]. NAFLD is believed to arise in a multi/repeated hit stage process. In the first stage, lipids (triglycerides) accumulate in the hepatocytes due to factors such as increased consumption of lipids and/or carbohydrates, insulin resistance-induced release of free fatty acids from the adipose tissue, elevated lipogenesis, reduced fatty acid oxidation, and reduced lipoprotein secretion from the liver. Subsequent stages are triggered by inflammation, oxidative stress, gut dysbiosis and mitochondrial dysfunction, which causes cellular injury eventually leading to liver fibrosis^[2,3].

The current global prevalence of NAFLD is approximately 25%^[4] and a common

cause of liver cancer in United States^[1]. With the growing epidemic of obesity in the Western world and the Middle East, NAFLD is predicted to be the most frequent indication for liver transplantation by 2030^[5]. It is also associated with the metabolic syndrome, increasing the risk for cardiovascular disease, insulin resistance and type 2 diabetes^[6]. Thus, effective preventive and prophylactic measures are urgently required.

A high omega 6: omega-3 fatty acid dietary ratio has been correlated with NAFLD pathogenesis^[7]. A normal omega 6:3 ratio is around 1-4:1, where omega-3 fatty acids regulate hepatic lipogenesis and are anti-inflammatory and anti-thrombotic^[8,9]. However, Western diets contain higher omega 6:3 ratios ranging from 15-20:1^[8,10] that disrupt the regulation of intrahepatic lipids, and promote a prothrombotic and proinflammatory environment^[10]. In NAFLD/NASH patients, omega-3 levels are significantly low, which correlates with hepatic steatosis and increased proinflammatory arachidonic acid (AA) metabolic products^[11,12]. The therapeutic effects of low dietary omega 6:3 ratio have been observed in animal models and in clinical trials with NAFLD/NASH patients^[13-15]. In this scenario, it is pivotal to understand the yet unknown underlying cellular mechanisms that prevail under states of high omega-6: omega-3 ratio. This may identify cellular and molecular targets that may help in formulating therapeutic interventions to effectively prevent and treat NAFLD.

Accordingly, this study examined the effects of high ratio of omega-6: omega-3 fatty acids on mitochondrial functions, and several parameters and regulators of lipid metabolism to understand the underlying mechanisms and extrapolate their contribution to fatty liver development and NAFLD pathogenesis. Here, the effect of high AA (omega-6): docosahexaenoic acid (DHA) (omega-3) ratio was studied on intracellular triglyceride accumulation, mitochondrial activity, oxidative stress and mitochondrial functions. Moreover, to understand the responses of the mechanistic mediators, we examined the expressions of stearoyl-CoA desaturase (SCD1) - the lipogenic enzyme involved in fatty acid synthesis, peroxisome proliferator-activated receptor alpha (PPAR- α) - a transcription factor that mediates fatty acid oxidation, sterol regulatory element-binding protein 1 (SREBP1c) - a transcription factor that induces genes involved in fatty acid synthesis, and cannabinoid receptors 1 and 2 (CB1, CB2), as their activation has been implicated in fatty liver development^[16,17].

MATERIALS AND METHODS

Cell culture

VL-17A, human hepatoma cell line (overexpress *ADH* and *CYP2E1*)^[18] were maintained at 37 °C in 5% CO₂ in a high-glucose (25 mmol) Dulbecco's Modified Eagle Medium (DMEM) (Lonza Ltd, United Kingdom) supplemented with L-Glutamine (2 mmol) (Lonza Ltd, United Kingdom), penicillin (100 U/mL) (Lonza Ltd, United Kingdom), streptomycin (100 mg/mL) (Lonza Ltd, United Kingdom), sodium pyruvate (1 mmol) and 10% foetal calf serum (FCS) (Biosera, United Kingdom)^[19,20]. Prior to maintenance, cells were grown in Plasmocin Prophylactic (5 µg/mL in DMEM) for four weeks.

Treatments

Cells were treated with different ratios of omega-6 AA: omega-3 DHA (1:1, 4:1, 15:1 and 25:1) for the required period. AA and DHA were of > 98% purity and acquired from Sigma-Aldrich (Gillingham, United Kingdom). Fatty acids were prepared as 8 mmol stock in DMSO and then diluted in 1% FCS low-glucose (1 g/L) DMEM to obtain the required treatment concentrations^[20]. The fatty acids were in free form with a concentration of 0.02% bovine serum albumin (BSA) in the FCS^[20].

Following the treatments, various parameters were examined such as mitochondrial activity, triglyceride accumulation, reactive oxygen species (ROS) levels and mitochondrial functions (basal and maximal respiration, ATP production, protein leak and spare respiratory capacity). Also, protein expression of the mediators of lipogenic, lipolytic and endocannabinoid pathways, SCD1, PPAR- α , SREBP1c, CB1 and CB2 were studied. Data were expressed relative to the experimental control *i.e.*, cells not treated with omega fatty acids but treated with 0.5 % DMSO in maintenance medium (referred to as untreated control). Comparisons were drawn between the three groups: (1) Untreated control; (2) Moderate omega-6: omega-3 ratios of 1:1 and 4:1; and (3) High ratios of 15:1 and 25:1.

Assessment of lipotoxicity

Mitochondrial activity, and thereby lipotoxicity, was assessed by using the MTT

assay, adapted from previous studies^[21,22]. Briefly, cells (2.5×10^4 cells/200 μ L DMEM/well) were incubated overnight in 96-well plates and then treated with different ratios of omega fatty acids for 24, 48 and 72 h. After the treatment, MTT (5 mg/mL) (Sigma-Aldrich, United Kingdom) was added and the cells were incubated for 2 h at 37 °C. Cells were then washed with PBS, treated with DMSO (100 μ L/well) and incubated for 15 min at room temperature. Absorbance was measured at 550 nm using a VersaMax microplate reader (Molecular Devices, United Kingdom).

Measurement of cellular triglyceride content

Cells (1×10^5 cell/mL) were incubated overnight in a 24-well plate and then treated with different ratios of omega fatty acids for 24, 48 and 72 h. Following the treatments, intracellular triglyceride content was determined by using EnzyChrom™ triglyceride assay kit (BioAssay Systems, United States), as per manufacturer's instructions. Triglyceride content was normalised to protein content, as determined by using Bio-Rad protein assay kit according to manufacturer's instructions. Data were expressed as mg triglyceride/mg protein.

Measurement of intracellular ROS levels

Cells (1×10^4 cells/ 200- μ L DMEM) were incubated overnight in a 96-well plate. Then, the cells were treated with different ratios of omega fatty acids for 30 min, 1 h, 2 h, 3 h, 6 h and 24 h. Following this, the cells were treated with 1 μ mol 2',7'-dichlorofluorescein diacetate (Sigma-Aldrich, United Kingdom) (in PBS) and incubated at 37 °C for 45 min. Fluorescence intensity was measured at an excitation of 485 nm and an emission of 535 nm by using FLUOstar OPTIMA (Jencons-PLS, United Kingdom). Fluorescence levels were expressed as percentage of the control.

Examination of mitochondrial respiratory function

The mitochondrial oxygen consumption rate (OCR) was evaluated using the XF Cell Mito Stress Test Kit as per manufacturer's instructions (Agilent Technologies, Craven Arms, United Kingdom). Plates were analysed using the Seahorse XF24 (Agilent Technologies, United Kingdom). Essentially, cells (1.5×10^4 cells/100 μ L DMEM) were seeded in a SeaHorse 24-well culture microplate. After incubation for 2 h at 37 °C and 5% CO₂, 150 μ L of DMEM (10% FCS) was added to the cells. The cells were re-incubated overnight at 37 °C and 5% CO₂. The following day, cells were treated with various AA:DHA ratios in 10% FCS DMEM and plates were incubated at 37 °C and 5% CO₂ for 24 h. The next day, Seahorse XF Assay Medium without glucose was supplemented with sodium pyruvate (1 mmol) and glucose (25 mmol) and the pH was adjusted to 7.4. Cells were washed twice with this Seahorse medium and incubated at 37 °C and 0% CO₂ for 45 min. To monitor the OCR of the cells, SeaHorse XF analyser was calibrated with the sensor cartridge containing Mito stress drugs FCCP (1 μ mol), an uncoupling agent; oligomycin (1 μ mol) for ATP synthase inhibition and an antimycin/Rotenone mixture (0.5 μ mol) for inhibition of oxidative phosphorylation and electron transfer, respectively. Following the measurement of OCR, 1% Triton X-100 were used to lyse the cells and the protein content was determined by using Bio-Rad protein assay kit. Seahorse cell Mito stress parameters were calculated following manufacture's protocol, normalized to protein content and the data were expressed relative to control.

Western Blotting determination of protein expression

Cells were treated with different ratios of omega fatty acids for 24 h. Then, total cellular protein was extracted using 1% Triton X-100 in PBS containing a protease inhibitor cocktail tablet and the protein concentration was determined by using Bio-Rad protein assay kit, as per manufacturer's instructions (Bio-Rad Laboratories, United Kingdom). Protein (20-40 μ g) was separated on 10% SDS-PAGE gels (Thermo Scientific Pierce, United Kingdom) by electrophoresis for 90 min at 120 V and transferred onto a nitrocellulose membrane (0.45 μ m) for 1 h at 350 A. Membranes were blocked with 1% BSA, except when confirming β -actin where 5% BSA was used. The proteins examined were SCD1 (1:4000) (Abcam, United Kingdom), PPAR- α (1:2000) (Abcam, United Kingdom), CB1, (1:4000) (Santa Cruz Biotechnology, United Kingdom) CB2 (1:2000) (Abcam, United Kingdom), and β -actin (1:4000) (Abcam, United Kingdom). West PICO Chemiluminescent Substrate (ThermoFisher, United Kingdom) was used for detection and the X-ray films were scanned on a Bio-Rad GS-800 Calibrated Densitometer (Bio-Rad, United Kingdom)^[23,24]. During post-densitometry analysis, data were expressed relative to corresponding β -actin levels and compared to the experimental control.

Statistical analysis

The statistical methods of this study were reviewed by Dr Claire Robertson, Senior

Lecturer in Nutritional Epidemiology, University of Westminster.

As variances were equal between groups, a one-way ANOVA followed by post-hoc analysis by Tukey's test was used to assess differences between omega fatty acid ratios and control treated groups. Data analysis was conducted using SPSS version 23.0. Data were expressed as the mean \pm SEM, $n = 3-5$, and where $P \leq 0.05$ was considered significant.

RESULTS

High omega-6:3 (AA:DHA) ratio induced lipotoxicity

After 24 h, high AA:DHA ratios reduced mitochondrial activity, when compared to the untreated control [15:1 by 17% ($P < 0.05$) and 25:1 by 82% ($P < 0.01$)] (Figure 1A). Furthermore, high AA:DHA ratio (15:1) induced reduction in activity when compared to the moderate ratios of 1:1 (by 22%, $P < 0.05$) and 4:1 (by 20%, $P < 0.01$) (Figure 1A). A similar pattern was induced by the high 25:1 ratio, which showed reduced activity in comparison to 1:1 (by 83%, $P < 0.05$) and 4:1 (by 83%, $P < 0.01$) ratios (Figure 1A).

After 48 h, only the 25:1 ratio caused a significant reduction in activity compared to the control, 1:1, and 4:1 by 51%, 54%, 56%, respectively ($P < 0.01$) (Figure 1B). At 72 h, both the high ratios (15:1 and 25:1) reduced mitochondrial activity. The 15:1 ratio markedly reduced mitochondrial activity in comparison to the control (by 91%, $P < 0.01$), 1:1 (by 92%, $P < 0.01$) and 4:1 (by 91%, $P < 0.01$) ratios (Figure 1C). Similarly, the 25:1 ratio reduced mitochondrial activity in comparison to the control (by 90%, $P < 0.01$), 1:1 (by 91%, $P < 0.01$) and 4:1 (by 89%, $P < 0.01$) (Figure 1C).

High omega-6:3 (AA:DHA) ratio- increased lipid accumulation

Significant changes in cellular lipid were observed after 24 h. Interestingly, in comparison to the untreated control, the 1:1 ratio reduced triglyceride accumulation (by 43%, $P = 0.01$), whereas a high 25:1 ratio elevated intracellular triglyceride levels (by 42%, $P < 0.01$) (Figure 2A). Importantly, in comparison to treatment with 1:1 ratio, lipid concentration was elevated with 4:1 ratio (by 75%, $P < 0.05$), 15:1 ratio (by 53%, $P < 0.05$), and with 25:1 ratio (by 41%, $P < 0.01$) (Figure 2A).

Similarly, after 48 h, treatments with 4:1, 15:1 and 25:1 ratios increased triglyceride accumulation in comparison to the control (by 117%, $P = 0.01$; 177%, $P < 0.05$; and 91%, $P < 0.05$, respectively), and also when compared to treatment with 1:1 ratio (by 91%, $P < 0.01$; 143%, $P < 0.01$ and 68%, $P < 0.05$, respectively) (Figure 2B). No significant alterations in triglyceride accumulation were observed after 72 h (Figure 2C).

High omega-6:3 (AA:DHA) ratio reduced PPAR- α and increased SCD1

Molecular mediators of lipid metabolism were examined. When compared to the untreated control, SCD1 expression increased significantly following treatments with 15:1 and 25:1 ratios [by 22% and 33%, ($P < 0.05$), respectively] (Figure 3A). Furthermore, treatment with 25:1 ratio increased its expression compared to the moderate 1:1 ratio treatment (by 35%, $P < 0.05$) (Figure 3A).

PPAR- α expression significantly decreased with 15:1 ratio when compared with the control and 1:1 treatments (by 29% and 35%; $P < 0.05$, respectively) (Figure 3B). This trend of decreasing expression was more evident following treatment with the 25:1 ratio, when compared to control and 1:1 treatments (by 35%, $P < 0.05$ and 40%, $P < 0.01$, respectively) (Figure 3B). No changes in SREBP1-c expression was observed at all ratios (Figure 3C).

High omega-6:3 (AA:DHA) ratio altered CB expression

Following treatment with 1:1 ratio, CB1 expression increased only subtly compared to the control (by 17%, $P < 0.05$), but the increment was significantly greater at 25:1 ratio (by 31%, $P < 0.01$) (Figure 3D). CB2 expression showed no significant alterations, except with 15:1 ratio, which reduced CB2 expression compared to the control ($P < 0.01$) (Figure 3E), although the 25:1 ratio which showed a general increased expression when compared to all ratios.

High omega-6:3 (AA:DHA) ratio increased ROS production

In comparison to the untreated control, ROS levels significantly increased with all ratios after 30 min (1:1 and 15:1 by 74%, $P < 0.05$; 4:1 and 25:1 by 88% and 104%, respectively, $P < 0.01$) (Figure 4A). No major differences were observed in ROS levels between the moderate ratios (1:1 and 4:1) and high ratios (15:1 and 25:1).

Similarly, after 1 h, while ROS levels increased following treatments with 15:1 ratio (by 117%; $P < 0.01$) and 25:1 ratio (by 96%; $P < 0.01$), treatments with 1:1 and 4:1 ratios did not cause any significant change compared to the control (Figure 4B).

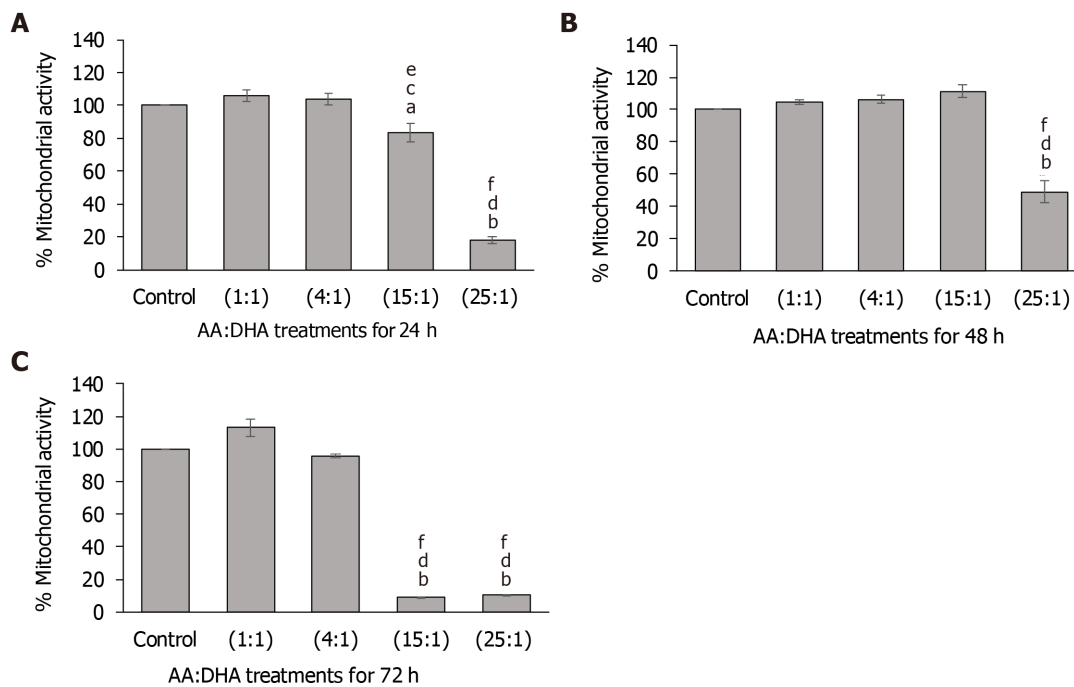


Figure 1 Effect of arachidonic acid: docosahexaenoic acid ratios on mitochondrial activity. A-C: VL17-A cells were treated with different ratios of arachidonic acid: docosahexaenoic acid and mitochondrial activity was assessed after 24 h (A), 48 h (B) and 72 h (C). ^a $P < 0.05$ and ^b $P < 0.01$ compared to control; ^c $P < 0.05$ and ^d $P < 0.01$ compared to 1:1 ratio; ^e $P < 0.05$ and ^f $P < 0.01$ compared to 4:1 ratio. Data is presented as mean \pm SE ($n = 3$). AA: Arachidonic acid; DHA: Docosahexaenoic acid.

Interestingly, treatment with high ratios (15:1 and 25:1) significantly elevated ROS levels compared to the moderate ratios of 1:1 and 4:1. Specifically, when compared with the 1:1 and 4:1 ratio, the 15:1 ratio increased ROS levels by 123% ($P < 0.01$) and 75% ($P < 0.01$) respectively, and 25:1 ratio increased ROS levels by 102% ($P < 0.01$) and 58% ($P < 0.01$), respectively (Figure 4B).

After 2 h, in comparison to 1:1 ratio, ROS levels decreased with the 4:1 ratio (by 17% $P < 0.01$) (Figure 4C). However, in comparison to the control and 4:1 ratio, ROS levels were significantly elevated with the high 15:1 ratio (by 31%, $P < 0.05$ and 58% $P < 0.01$, respectively). These were also high with the 25:1 treatment when compared with the 4:1 treatment (by 30% $P < 0.05$) (Figure 4C). No effect was observed after 3 h, 6 h or 24 h for all ratios (data not shown for brevity).

High omega-6:3 (AA:DHA) ratio disrupted mitochondrial functions

Elevation in AA:DHA ratios caused a gradual and significant decrease in basal respiration when compared to control [1:1 (by 20%, $P < 0.05$); 4:1 (by 25%, $P < 0.01$); 15:1 (by 38%, $P < 0.01$); and 25:1 (by 40%, $P < 0.01$)] (Figure 5A). Similar results were observed when compared to treatment with 1:1 ratio, whereby basal respiration significantly reduced with 15:1 and 25:1 ratios (by 19% and 21%, respectively, $P < 0.05$) (Figure 5A).

Maximal respiration decreased gradually with 1:1, 4:1, 15:1 and 25:1 ratios (by 35%, 44%, 54%, and 56% ($P < 0.01$), respectively) (Figure 5B). Spare respiratory capacity showed a similar pattern of significant reductions (by 52%, 56%, 67%, and 68% ($P < 0.01$) over the increasing AA/DHA ratios (Figure 5C). ATP production significantly reduced with elevation in AA:DHA 1:1, 4:1, 15:1, 25:1 ratios (by 25%, 33%, 44% and 41%, respectively, ($P < 0.01$)) (Figure 5D). Proton leak decreased significantly with high omega ratio of 15:1 (by 41% $P < 0.01$) compared to control, and with 25:1 by 50% and 31% ($P < 0.01$), when compared to control and 1:1 ratio, respectively (Figure 5E).

DISCUSSION

NAFLD is recognized as a growing global epidemic affecting 10%-35% of adult population in the world^[25], with highest prevalence in the Middle East and South America^[26]. In the US, it affects at least 18% of the adult population and 90% of obese people with an overall prevalence of 35%^[26]. Moreover, a recent epidemiological study demonstrated an exponential increase in NAFLD burden in the United States^[27].

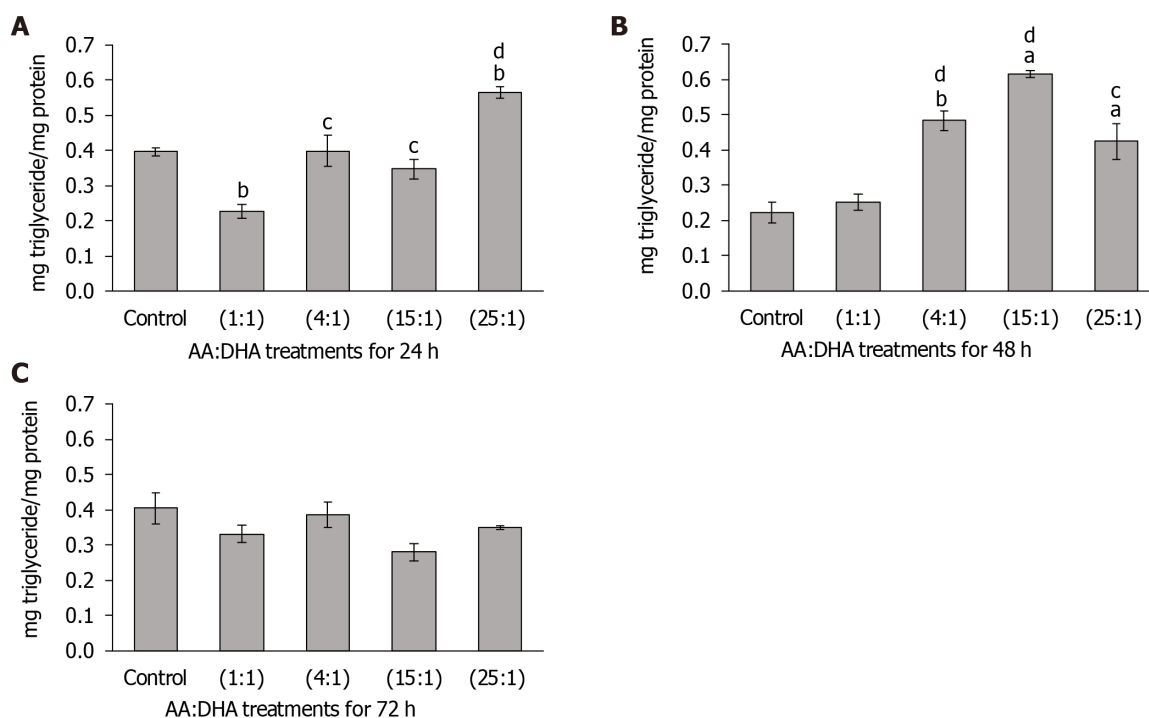


Figure 2 Effect of arachidonic acid: docosahexaenoic acid ratios on intracellular triglyceride accumulation. A-C: VL17-A cells were treated with different ratios of arachidonic acid: docosahexaenoic acid for 24 h (A), 48 h (B) and 72 h (C). Intracellular triglyceride levels were measured and normalised to cellular protein content. ^a $P < 0.05$ and ^b $P \leq 0.01$ compared to control; ^c $P < 0.05$ and ^d $P \leq 0.01$ compared to 1:1 ratio. Data is presented as mean \pm SE ($n = 5$). AA: Arachidonic acid; DHA: Docosahexaenoic acid.

Predicted to “emerge as the leading cause of end-stage liver disease”^[4], NAFLD calls for a thorough understanding of the underlying mechanisms.

High dietary intake of omega-6 fatty acids (mainly in processed foods) and/or lower intake of omega-3 fatty acids has been implicated in the development of fatty liver^[7,28,29]. *In vivo* and *in vitro* studies have demonstrated the significance of omega-3 fatty acids in regulating lipid accumulation, fatty acid oxidation and reversing steatosis-induced mitochondrial dysfunction^[30,31]. The significance of balanced omega-6:3 ratio was more evident in recent trials where treatment of NAFLD patients with omega-3 fatty acids such as eicosapentaenoic acid (EPA) or DHA reduced steatosis and liver enzymes^[32,33]. However, the underlying pathological mechanisms under high omega-6: omega-3 states remain unclear. It is pivotal to understand these mechanisms to identify biochemical pathways and molecular targets that may help in formulating preventative and therapeutic interventions to halt, decelerate or reverse NAFLD progression.

Accordingly, here, we investigated the effect of increasing ratios of omega-6 (AA): omega-3 (DHA) fatty acids on various cellular parameters in VL-17A cells. We compared the effects of high AA: DHA ratios with untreated control and with the effects induced by healthy ratios of 1:1 and 4:1. The aim was to understand these altered mechanisms that prevail during high omega-6: omega-3 states and relate these to NAFLD pathogenesis.

High omega-6: omega-3 ratio (AA:DHA) caused lipotoxicity and promoted steatosis

High AA:DHA ratios significantly reduced mitochondrial activity and thus cell viability (Figure 1). This indicates lipotoxicity due to high omega-6 (AA) content, as its immediate metabolites are strongly proinflammatory^[7]. The majority of studies with fatty acid treatment using *in vitro* models of NAFLD are based on a single 12-48 h time point to enable lipid accumulation and its subsequent detection. Our data showed a marked increase in triglyceride accumulation after 24 h and also at 48 h, in comparison to the untreated control and healthy ratios (1:1 and 4:1) (Figure 2), which is similar to other models of NAFLD where steatosis was reported in primary hepatocytes after 16-24 h treatment with oleic acid^[34], palmitic acid^[35] or stearic acid^[36].

However, after 72 h lipid concentration was normal. This suggests a biological response to high AA:DHA ratios in the short-term. Repeating the treatment again or a sustained period of omega fatty acids in the growth media, would provide further support that the response is biological rather than an acute transient effect. Our

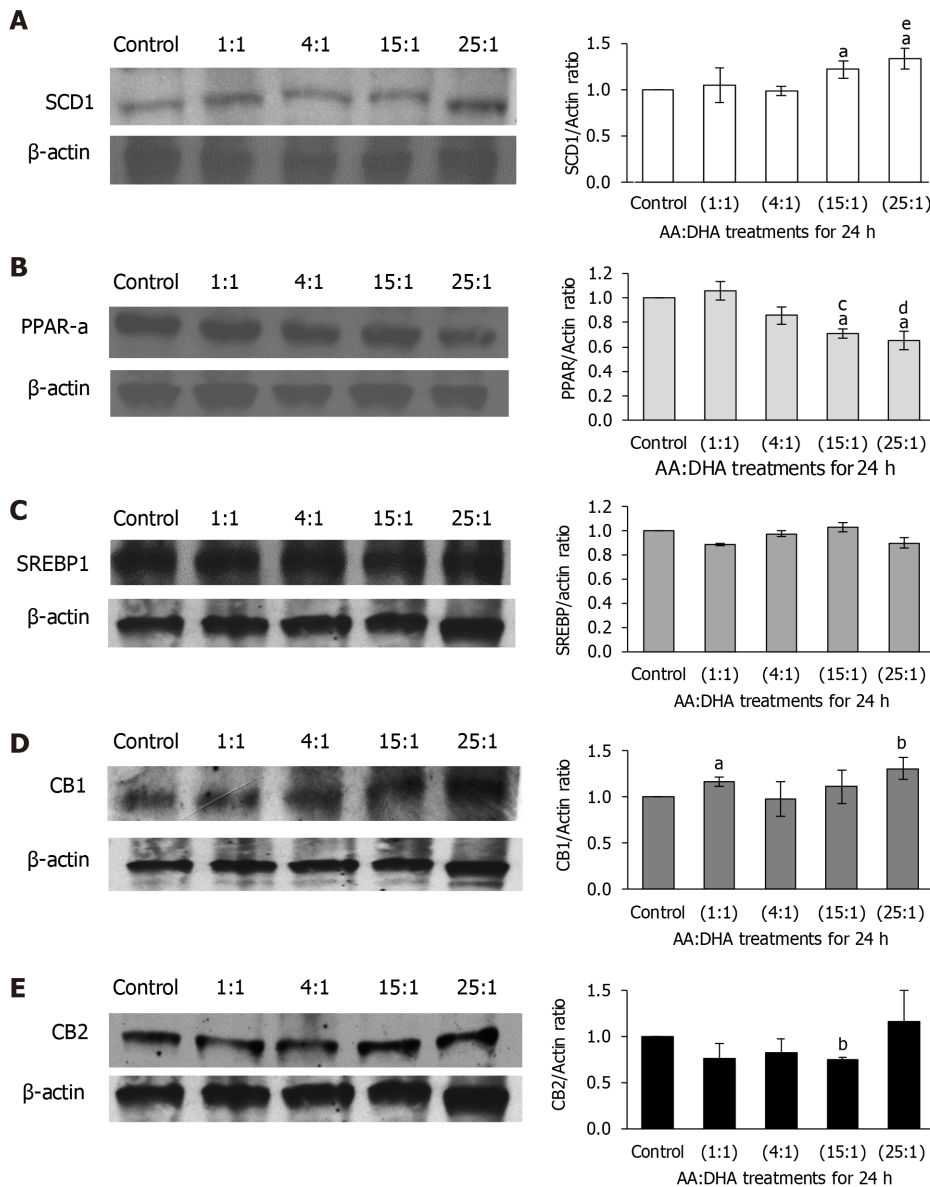


Figure 3 Effect of arachidonic acid: docosahexaenoic acid ratios on mechanistic mediators of lipid metabolism. A-E: VL17-A cells were treated with different ratios of arachidonic acid: docosahexaenoic acid for 24 h. Following this, the expression of several proteins that modulate lipid metabolism were examined, namely, SCD1 (A), PPAR- α (B), SREBP1c (C), CB1 (D) and CB2 (E). ^a $P \leq 0.05$ and ^b $P \leq 0.01$ compared to control; ^c $P < 0.05$ and ^d $P < 0.01$ compared to 1:1 ratio; ^e $P < 0.05$ compared to 4:1 ratio. Data is presented as mean \pm SE ($n = 3-4$). AA: Arachidonic acid; DHA: Docosahexaenoic acid.

findings are generally in line with previous studies where high-fat diet with various AA:EPA and AA:DHA ratios (1:1, 5:1, 10:1 and 20:1) increased hepatic phospholipid AA:eicosapentaenoic acid and AA:DHA in a dose dependent manner, mildly influenced inflammatory signaling, as well as key lipogenic regulators^[37], though lowering the ratio did not prevent lipid accumulation^[37]. Despite the latter study there is substantial evidence that the omega 6:3 ratio is an important contributory factor in NAFLD development. Recently NAFLD co-twin studies showed that the hepatic omega 6:3 ratio is significantly greater when liver fat > 5% suggesting the impact of diet independent of genetics plays a role in NAFLD occurrence^[38]. In other models of NASH where animals were fed a Western diet, omega 6 lipid concentrations were increased in hepatic membranes, whereas omega 3 lipid concentrations were reduced; inflammatory markers were also increased, and this effect was reversed when animals were given DHA^[39]. Whereas with other models of fatty liver, lowering the omega 6:3 ratio attenuated gut and liver injury suggesting that a normal ratio is important in fatty liver reversal^[40].

Under physiological conditions, free fatty acids fuel oxidative stress and produce inflammatory cytokines that induce cell injury. Therefore, their esterification and deposition in the liver as triglycerides provide a protective mechanism to prevent additional liver damage^[2]. Thus, it is possible that the high AA:DHA-ratio-induced

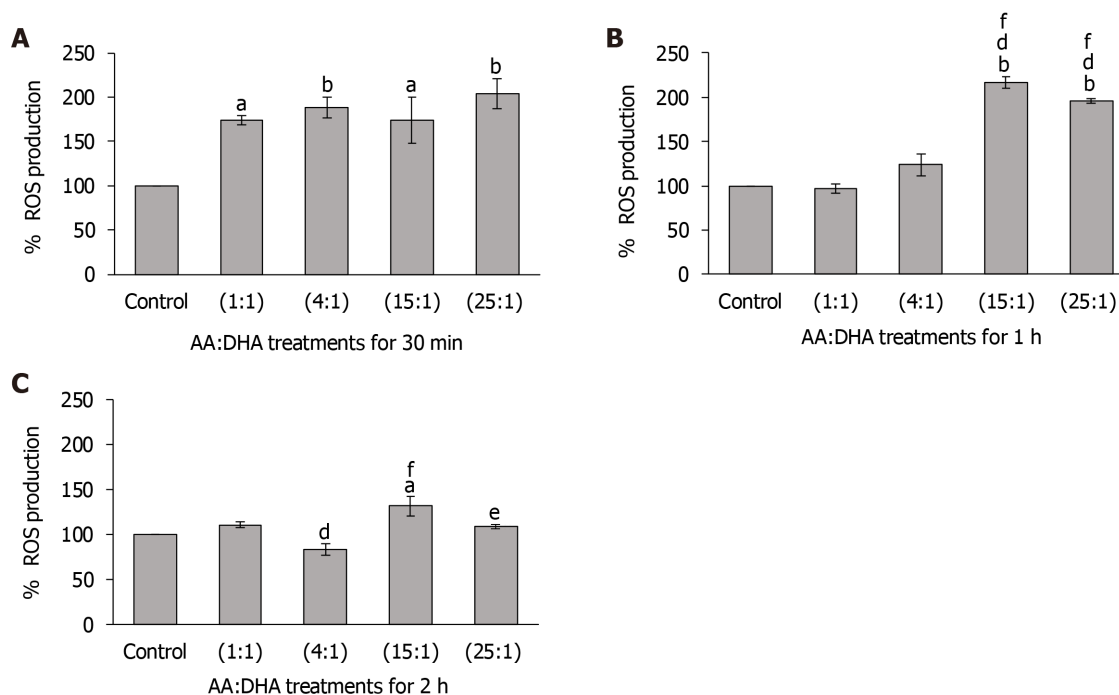


Figure 4 Effect of arachidonic acid: docosahexaenoic acid ratios on ROS production. A-C: VL17-A cells were treated with different ratios of arachidonic acid: docosahexaenoic acid for 30 min (A), 1 h (B) and 2 h (C), and ROS levels were measured. ^a $P < 0.05$ and ^b $P < 0.01$ compared to control; ^d $P < 0.01$ compared to 1:1; ^e $P < 0.05$ and ^f $P < 0.01$ compared to 4:1 ratio. Data is presented as mean \pm SE ($n = 3$). AA: Arachidonic acid; DHA: Docosahexaenoic acid.

elevated triglyceride concentration observed here (Figure 2) are a manifestation of a counter-protective response against the high omega-6: omega-3 ratio. However, it is also possible that this triglyceride accumulation may contribute to fatty liver pathogenesis because excessively increased cellular triglycerides may dysregulate lipid metabolism, promote steatosis and insulin resistance, elevate gluconeogenesis and decrease glycogenesis^[41].

High omega-6: omega-3 (AA:DHA) ratio altered mediators of lipid metabolism: mechanistic evidence for contribution to NAFLD development

Following the confirmation of high AA:DHA-induced cellular triglyceride accumulation (Figure 2), molecular mediators of lipid metabolism were examined. High dietary fat can increase the synthesis of endocannabinoids, which upregulate the activity of CB1 and CB2^[42-44]. This activation has been implicated in the development of NAFLD, partly via upregulation of lipogenic enzymes like SCD1 that promote fatty acid synthesis, and downregulation of PPAR- α expression, which stimulates hepatic oxidation of fatty acids^[7]. Whether high AA:DHA ratio alters these mediators of lipid metabolism remain unknown. Therefore, we studied the effect of high AA:DHA ratio on the expression of SCD1 to understand its lipogenic capacity and PPAR α to examine its potential for fatty acid oxidation.

Here, high AA:DHA ratio increased SCD1 expression (Figure 3A), indicating elevation in lipogenic capacity. This is consistent with a previous study where mice fed a diet with high omega-6: omega-3 ratio demonstrated increased expression of SCD1^[45]. The increase in SCD1 expression could be due to direct binding of liver X receptor (it's activator) or due to activation of its transcription factor SREBP1c^[30,46,47]. Conversely other studies have reported a decrease in lipid droplet levels combined with a downregulation in SCD1 & SREBP levels following DHA treatment for 12 h in primary hepatocytes^[48], although here no significant alterations were observed in SREBP1c expression (Figure 3C). Finally, high AA:DHA ratio reduced PPAR α expression (Figure 3B), which indicated reduced capacity of fatty acid oxidation under these conditions. Elevation in SCD1 in combination with a reduction in PPAR α expression may partly explain the mechanisms underlying fatty liver development under high omega-6:3 environment.

We also examined the expression of CB1 and CB2 under conditions of high omega-6:3 ratio to evaluate its stimulatory potential for fatty acid synthesis. As hypothesized, CB1 expression significantly increased following treatment with a high 25:1 ratio (Figure 3D). Although CB1 activation via LXR is thought to upregulate SREBP1c^[49], our data suggested no direct correlation. This is similar to studies in HepG2 cells

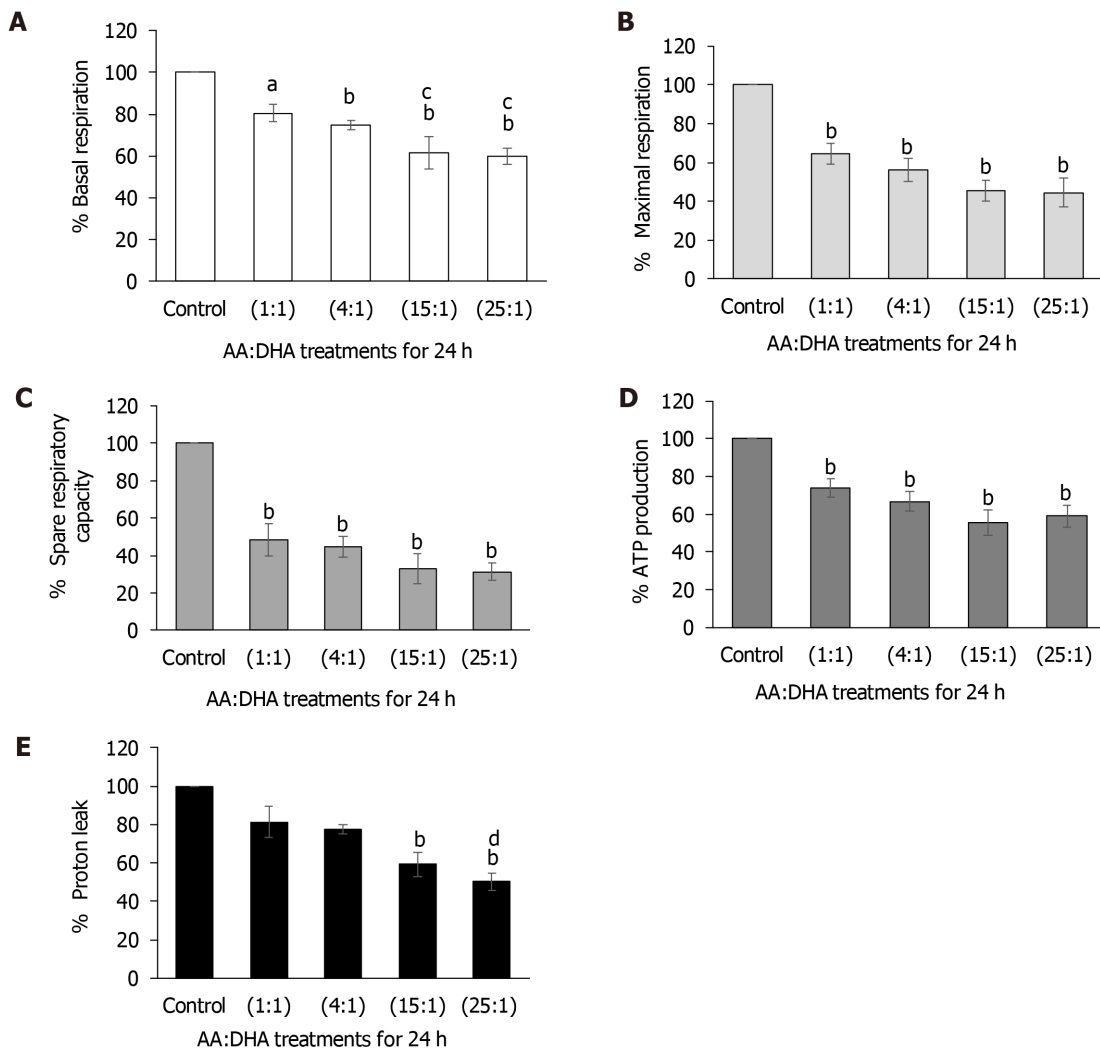


Figure 5 Effect of arachidonic acid: docosahexaenoic acid ratios on mitochondrial respiratory functions. VL17-A cells were treated with different ratios of arachidonic acid: docosahexaenoic acid for 24 h and various parameters that determine the mitochondrial respiratory functions were examined. A: Elevation in AA:DHA ratios caused a gradual and significant decrease in basal respiration when compared to control. Similar results were observed when compared to treatment with 1:1 ratio, whereby basal respiration significantly reduced with 15:1 and 25:1 ratios; B: Maximal respiration decreased gradually with 1:1, 4:1, 15:1 and 25:1 ratios (by 35%, 44%, 54%, and 56%, respectively); C: Spare respiratory capacity showed a similar pattern of significant reductions (by 52%, 56%, 67%, and 68% over the increasing AA/DHA ratios); D: ATP production significantly reduced with elevation in AA:DHA 1:1, 4:1, 15:1, 25:1 ratios (by 25%, 33%, 44% and 41%, respectively); E: Proton leak decreased significantly with high omega ratio of 15:1 compared to control, and with 25:1 by 50% and 31%, when compared to control and 1:1 ratio, respectively. ^a $P < 0.05$ and ^b $P < 0.01$ compared to control; ^c $P < 0.05$ and ^d $P < 0.01$ compared to 1:1 ratio. Data is presented as mean \pm SE ($n = 4-5$). AA: Arachidonic acid; DHA: Docosahexaenoic acid.

where CB1 agonist did not significantly alter the expression of SREBP-1c^[50]. Furthermore, liver CB1 mRNA expression negatively correlated with hepatic PPAR α expression, but not SREBP1c in patients with NASH^[51], which is similar to the pattern observed in this study. Furthermore, inhibition of CB1 receptors has shown to improve lipogenesis *in vitro*^[52]. Since high omega-6:3 ratio promotes obesity via both, AA-derived eicosanoid metabolites and over-activation of the cannabinoid system^[40], it is possible that cannabinoid-independent pathways may sufficiently operate for high omega-6-induced fatty acid synthesis or other regulators may predominate. For example, CB1 receptors could be regulated by SREBP-1c, ChREBP and LXRs^[52]. Overall, these findings suggest a role for CB1 activation under high omega 6:3 ratios leading to enhanced steatosis.

Except for 25:1 ratio, CB2 receptors showed moderately reduced expression when compared to control (Figure 3E), which was significantly lower following treatment with 15:1 ratio (Figure 3E). While some studies suggest that CB2 activation promotes NAFLD, other studies indicate a preventative role^[42,53,54]. However, other studies have also reported a strong downregulation of CB2 gene expression in human hepatocytes following fatty acid treatment^[50]. Further studies using agonists/antagonists of the CB1 and 2 receptors are required to determine their exact role in NAFLD development.

High AA:DHA ratio altered redox biology

Increased oxidative stress is one of the key characteristics of NAFLD, which promotes tissue injury^[55]. There is no data yet on the effect of high AA:DHA ratios on ROS production. Here, we observed that ROS levels increased with all AA:DHA ratios within 30 min and generally beyond this time when compared to the untreated control (Figure 4). Moreover, high AA:DHA ratios elevated ROS levels in comparison to the healthy ratios of 1:1 and 4:1 at 1 h and 2 h (Figure 4B, 4C), clearly indicating the impact of high AA:DHA ratio on ROS production. After 3, 6 or 24 h no increase in ROS production occurred, supporting the point that incubation time is an important aspect in this model. This is similar to other models of NAFLD, where ROS production was significantly increased after 30 min of palmitic acid treatment^[56]. Alternatively, after 30 min incubation with DHA ROS levels were lower than steatotic HepG2 cells^[31]. However, other studies have shown an increase in ROS levels after a single measurement at 24 h of palmitic acid treatment^[57,58]. This highlights the variance in time dependent ROS effects depending on the model used, although the net effect is increased oxidative stress.

The high ROS effect could be attributed to the high AA content in the AA:DHA ratio, which acts as a strong inhibitor of complexes I and III in the respiratory chain^[59]. This would hinder electron flow through the respiratory chain causing electron leakage from these complexes and reduce mitochondrial membrane potential, thereby promoting ROS production^[60]. However, other sources of ROS could be via CYP2E1 metabolism of AA^[61]. Interestingly the high 15:1 ratio maintained the high levels of ROS after 1 h and 2 h, whereas the lower ratios of 1:1 and 4:1 either restored ROS levels to that of control or decreased ROS production (Figure 4B, 4C). This restoration could be due to fatty acid oxidation and the capability of the respiratory chain to handle the excess electrons released when the ratio was in the recommend healthy range.

High AA:DHA ratio disrupted mitochondrial functions

Disturbed mitochondrial function has been implicated in the pathogenesis of NAFLD^[55]. A 30%-40% decrease in the respiratory rate along with mitochondrial uncoupling has been reported in obese patients with NASH^[62]. Therefore, we investigated the impact of high AA:DHA ratio on several mitochondrial functions. We observed that high AA:DHA ratios (15:1 and 25:1) decreased basal and maximal respiration, spare respiratory capacity, proton leak and ATP production (Figure 5). The mechanisms responsible for this could relate to destabilization of cytochrome C affecting electron flow and ultimately ATP synthesis^[63], the direct uncoupling protonophoric ability of fatty acids which impairs ATP synthesis^[63] or the opening of the mitochondrial permeability transition pores causing mitochondrial membrane potential loss leading to mitochondrial swelling and cell death^[64]. These findings are similar to animal studies with fatty liver, where a reduction in the respiratory rate, increased oxidative stress, and reduction in complex I activity was observed^[65-67].

Further work is required to elucidate the exact damaging effect of omega 6 fatty acids, however, it is important to state that not all omega 6 fatty acids are harmful/proinflammatory or omega 3 fatty acids are protective, and thus it is important not to generalize fatty acids, but to focus on specific fatty acids.

Limitations of the study

In terms of study limitations, there are a number of caveats. Firstly, measurement of hepatic phospholipid AA: eicosapentaenoic acid and AA:DHA, the SCD activity which measures the conversion of fatty acids to lipids, and mRNA levels of fatty acid synthase and acetyl-CoA carboxylase would confirm the lipid changes following high omega 6:3 ratio supplementation in HepG2 cells. As we only observed ROS induced injury over a short period of time, other markers of oxidative stress such as lipid peroxides and protein carbonyls, combined with markers of endoplasmic reticulum stress, such as c-Jun N-terminal kinase, and measurement of AA metabolites such as leukotrienes would provide further weighting behind the observations. The precise role of CB1 and CB2 receptor activation requires further elucidation; application of receptor agonists/antagonists would provide insight into lipogenesis under high omega ratios. Finally, it will be important to examine the effect of adding DHA directly to the cells after omega treatment to ascertain whether lipid accumulation can be reversed. Nutritional interventions that focus on particular omega 3 fatty acids is of interest due to the growing body of evidence showing reversal of NAFLD upon treatment with omega fatty acids.

In conclusion, this is the first study using high to normal omega 6:3 ratios in VL17A (HepG2 cells). These cells overexpress CYP2E1 which can metabolize AA, thus they resemble metabolic changes occurring in hepatocytes. In addition, the majority of *in vitro* NAFLD models of fatty acid treatment are based on a single 12-48 h time point to

enable lipid accumulation and its subsequent detection. As far as we are aware no studies have reported a time course effect of omega fatty acids from 24 to 72 h in HepG2 cells studying lipid accumulation or ROS production, combined with lipogenic markers.

In summary, high omega 6:3 ratios of AA:DHA stimulated lipid synthesis by reducing fatty acid oxidation (decrease in PPAR alpha expression), increased CB1 expression, and also promoting the conversion of unsaturated fatty acids to saturated fatty acids *via* SCD1 with the net effect of lipid accumulation in human hepatoma (VL17A) cells. High ratios also led to increased oxidative stress, which is an important feature in the inflammatory state. Lipids synthesised in the liver are thus susceptible to oxidative attack by ROS. The loss of mitochondrial function possibly due to ROS or direct uncoupling effect of AA also promotes cell death, thus compounding the inflammatory effects. These results suggest that high omega 6:3 ratios can possibly lead to key steps in the progression from fatty liver to NASH.

ARTICLE HIGHLIGHTS

Research background

Fatty liver disease is due to the consumption of excess dietary calories as well as a disruption in lipid metabolism and leads to the condition non-alcoholic fatty liver disease (NAFLD). The prevalence of NAFLD in most Western societies ranges from 20%-50%. Whilst the mechanisms for NAFLD development are complex, NAFLD patients show higher levels of omega-6 fatty acids than omega 3 fatty acids. Omega 6 fatty acids are known to be damaging to the liver causing toxicity, but their precise role in the pathology of NAFLD is not understood. This was an important question since NAFLD is a major public health problem and alternative interventions are required.

Research motivation

The main treatment options for NAFLD are dietary and lifestyle changes. We therefore addressed the question of how diet increases the risk of NAFLD, specifically the role of omega 6 fatty acids in relation to omega 3 fatty acids. Since this is a modifiable risk factor it is important to understand how high levels of omega 6 fatty acids can damage the liver and whether omega 3 fatty acids can minimise/reduce this damage. This will lead to improved understanding of NAFLD development and new therapeutic treatment options.

Research objectives

This research study aimed to understand the role of high omega 6:omega 3 ratios in a hepatic cell line model of NAFLD, thus allowing its contribution to lipotoxicity, oxidative stress and mitochondrial function to be investigated. These findings may help explain the progression from fatty liver to the inflammatory state, non-alcoholic steatohepatitis.

Research methods

Human hepatoma cells, named VL-17A were treated with a range of high and normal ratios of omega-6: omega-3 fatty acids [arachidonic acid (AA): docosahexaenoic acid (DHA)]. These novel experiments examined the effect of these ratios on mitochondrial function, oxidative stress and viability. Changes in lipid accumulation combined with the expression of relevant lipogenic proteins was also assessed.

Research results

High omega-6:omega-3 (AA:DHA) ratio altered several processes in VL-17A cells. It reduced mitochondrial activity indicating lipotoxicity, increased triglyceride accumulation, elevated reactive oxygen species levels and interrupted several mitochondrial functions. Moreover, our study provided mechanistic data as to which of these detrimental effects may be mediated under high omega-6: omega-3 conditions and contribute to NAFLD development. Specifically, these include increased expression of stearoyl-CoA desaturase and decreased expression of peroxisome proliferator-activated receptor alpha. Also, elevation in cannabinoid receptor CB1 expression was observed that has been positively associated with fatty liver development. The present study clearly demonstrates the potential long-term consequences of high omega-6:3 ratios in NAFLD development.

Research conclusions

The conclusions from this study strongly suggest that high omega 6:omega 3 ratios are detrimental to liver function, promoting an oxidant environment combined with higher amounts of lipid accumulation. These features are the hallmark of NAFLD indicating that altered omega 6:3 fatty acid ratios play an important role in NAFLD development and progression. Therefore, the original hypothesis was confirmed which can form the basis for further mechanistic studies examining other omega 6 fatty acids in NAFLD pathogenesis. The study also supports various studies where clinical interventions using omega 3 fatty acids have been utilised for NAFLD treatment.

Research perspectives

Whilst further work is required, when investigating patients with NAFLD, measurement of

circulating omega fatty acids should be considered. This may lead to the possibility of treating patients with omega 3 fatty acids. Other interventions that ameliorate oxidative stress and which improve mitochondrial function also require future research as this may lead to the reversal of NAFLD.

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Retrospective Study

Platelet-albumin-bilirubin score - a predictor of outcome of acute variceal bleeding in patients with cirrhosis

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Abstract

BACKGROUND

The albumin-bilirubin (ALBI) score was validated as a prognostic indicator in patients with liver disease and hepatocellular carcinoma. Incorporating platelet count in the platelet-albumin-bilirubin (PALBI) score improved validity in predicting outcome of patients undergoing resection and ablation.

AIM

To evaluate the PALBI score in predicting outcome of acute variceal bleeding in patients with cirrhosis.

METHODS

The data of 1517 patients with cirrhosis presenting with variceal bleeding were analyzed. Child Turcotte Pugh (CTP) class, Model of End-stage Liver Disease (MELD), ALBI and PALBI scores were calculated on admission, and were correlated to the outcome of variceal bleeding. Areas under the receiving-operator characteristic curve (AUROC) were calculated for survival and rebleeding.

RESULTS

Mean age was 52.6 years; 1176 were male (77.5%), 69 CTP-A (4.5%), 434 CTP-B (29.2%), 1014 CTP-C (66.8%); 306 PALBI-1 (20.2%), 285 PALBI-2 (18.8%), and 926 PALBI-3 (61.1%). Three hundred and thirty-two patients died during hospitalization (21.9%). Bleeding-related mortality occurred in 11% of CTP-B, 28% of CTP-C, in 21.8% of PALBI-2 and 34.4% of PALBI-3 patients. The AUROC for predicting survival of acute variceal bleeding was 0.668, 0.689, 0.803 and 0.871 for CTP, MELD, ALBI and PALBI scores, respectively. For predicting rebleeding the AUROC was 0.681, 0.74, 0.766 and 0.794 for CTP, MELD, ALBI and PALBI scores, respectively.

CONCLUSION

PALBI score on admission is a good prognostic indicator for patients with acute

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variceal bleeding and predicts early mortality and rebleeding.

Key words: Variceal bleeding; Platelet-albumin-bilirubin score; Albumin-bilirubin score; Rebleeding

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Core tip: This study describes a comparative analysis of the performance of different scoring systems in a large number of patients with acute variceal bleeding. The platelet-albumin-bilirubin score performed better in predicting short-term outcome and the incidence of rebleeding compared with the other 4 scoring systems, the Child Pugh score, albumin-bilirubin score and Model of End-stage Liver Disease and its modification for acute variceal bleeding.

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INTRODUCTION

Acute variceal bleeding is a frequent, ominous complication of liver cirrhosis and portal hypertension and is responsible for high morbidity and mortality^[1].

The Child-Turcotte-Pugh (CTP) and Model of End-stage Liver Disease (MELD) scores are two of the most important models for predicting the survival of upper gastrointestinal bleeding. The CTP score was originally developed to be a prognostic score in patients with cirrhosis and portal hypertension undergoing surgery for variceal bleeding^[2]. The limitations of the CTP score in assessing liver functions in patients with upper gastro-intestinal tract (GIT) bleeding are the subjective assessment of encephalopathy and amount of ascites, together with the interrelated variables such as ascites and serum albumin, besides the parameters that are scored based on arbitrarily defined cutoff points^[3,4].

The MELD score was initially developed to determine prognosis following a trans jugular intra-hepatic porto-systemic shunt procedure, and is widely used in liver transplant settings to prioritize donor liver allocation^[5,6]. Johnson *et al*^[7] developed the albumin-bilirubin (ALBI) score, which depends on two laboratory variables; bilirubin and albumin, omits the subjective CTP variables ascites and hepatic encephalopathy, and results are expressed as three grades with three different cutoff points^[7]. Roayaie *et al*^[8] proposed modifying the ALBI score by including the platelet count as an indicator of portal hypertension.

In this study, we aimed to determine the value of the platelet-albumin-bilirubin (PALBI) score in predicting the outcome of patients with cirrhosis presenting with acute variceal bleeding.

MATERIALS AND METHODS

This retrospective study included 1517 patients with cirrhosis presenting with acute variceal bleeding who were admitted to the National Liver Institute Hospital. The study was approved by the institutional review board (IRB number IRB00003413).

Inclusion criteria

This study included patients with acute variceal bleeding. Acute upper gastrointestinal bleeding was considered in patients with liver cirrhosis presenting with hematemesis, defined as either one or more than one episode of vomiting either fresh blood or a coffee ground-like material, or reported or observed melena, with a drop in hemoglobin, and blood in the nasogastric tube.

All patients were managed in the emergency unit and subjected to the following: (1) Resuscitation to maintain hemodynamic stability (systolic blood pressure above 80-90 mmHg); (2) Blood transfusion using type and cross-matched packed red cells

and plasma as required to increase hematocrit to 25%-30% or hemoglobin to above 9 g/dL; (3) Vaso-active drugs as required and according to requirement (octreotide or terlipressin); and (4) Antibiotic prophylaxis was given in the form of I.V. cefotaxime 1 g/12 h for up to 5 d, unless acute infection, especially spontaneous bacterial peritonitis, was diagnosed, which was treated accordingly.

Urgent endoscopy was performed within the first six hours. The diagnosis of variceal hemorrhage was considered when active bleeding from an esophageal or gastric varix was observed or when a sign of recent bleeding, such as a "white nipple", was observed. Variceal hemorrhage was inferred when varices were the only pathology found, with blood present in the stomach. Patients with other causes of acute upper gastrointestinal bleeding were not considered for this analysis.

Esophageal varices were managed by band ligation or injection sclerotherapy if banding was not feasible. Bleeding from gastric varices was managed by injection of tissue adhesive (histoacryl) injection.

Variceal bleeding was considered to have been controlled if the following criteria were met: Stable blood pressure (no reduction in systolic pressure exceeding 20 mmHg, once the blood pressure had stabilized); a stable hemoglobin concentration (> 9 g/dL), measured twice daily; and a hematocrit above 30% (measured hourly during the first 12 h and every 2 to 6 h thereafter, depending on the patient's hemodynamic status), with a transfusion requirement of no more than two units in a 2-h period and fewer than four units within the first 4 h after endoscopic therapy.

Patients were followed for 5 d after the control of acute bleeding for occurrence of rebleeding. Rebleeding was defined as the occurrence of new hematemesis or melena after a period of 24 h or more from the 24-h point of stable vital signs and hematocrit/hemoglobin following an episode of acute bleeding^[9]. In patients with significant rebleeding, defined as frank hematemesis, new onset of melena, fresh blood in nasogastric tube aspirate, or hemodynamic compromise, with a decrease in hemoglobin level of > 2 g/dL, re-endoscopy was performed.

The CTP class, MELD, ALBI, MELD-Acute variceal bleeding (MELD-AVB) and PALBI scores were calculated from admission labs, and were correlated with control of bleeding, rebleeding, and in-hospital mortality. (1) The CTP score was calculated numerically as previously described including bilirubin, albumin, international normalized ratio (INR), and presence and grade of ascites and encephalopathy. CTP class was A if the score was 5-6, B if the score was 7-9, and C if the score was 10 or higher^[2]; (2) The MELD score was calculated as: $0.957 \times \log_e(\text{creatinine mg/dL}) + 0.378 \times \log_e(\text{bilirubin mg/dL}) + 1.120 \times \log_e(\text{INR}) + 0.643$ ^[5]; (3) The ALBI score was calculated as: $=(\log_{10} \text{bilirubin} \times 0.66) + (\text{albumin} \times -0.085)$ where bilirubin is in $\mu\text{mol/L}$ and albumin in g/L. ALBI was categorized into three grades: ALBI-1 (≤ -2.60), ALBI-2 (> -2.60 to -1.39), ALBI-3 (> -1.39)^[6]; (4) MELD-AVB was adapted from the basic MELD score through this equation $\text{logit}, -5.312 + 0.207 \times \text{MELD}$; bootstrapped R^2 , 0.3295; and (5) PALBI score was calculated as: $(2.02 \times \text{Log}_{10} \text{bilirubin}) + [-0.37 \times (\text{Log}_{10} \text{bilirubin})^2] + (-0.04 \times \text{albumin}) + (-3.48 \times \text{Log}_{10} \text{platelets}) + [1.01 (\text{Log}_{10} \text{platelets})^2]$ where bilirubin is in $\mu\text{mol/L}$ and albumin in g/L, and platelet count in $1000/\mu\text{L}$. PALBI was categorized as: PALBI 1 (score ≤ 2.53), PALBI 2 (score > 2.53 and ≤ 2.09), and PALBI 3 (score > 2.09)^[7].

The areas under the receiving-operator characteristic curve (AUROC) were calculated for survival and rebleeding.

Statistical analysis

Data were collected and entered into the computer using SPSS program version 23 for statistical analysis (IBM Corp., Armonk, NY, United States). Continuous data were expressed as the mean \pm SD and the median with minimum and maximum. Categorical data were expressed as the frequency (percentage). A comparison between the chosen variables and control of bleeding, rebleeding, and mortality was performed. Univariate analysis included the Fisher's exact test or Chi-square test for categorical variables and the analysis of variance for continuous variables. Multivariate logistic regression using the stepwise selection method was performed starting from the variables with $P < 0.01$ in the univariate analysis. ROC curve analyses were performed to determine the value of CTP class, MELD, ALBI, MELD-AVB and PALBI scores in predicting the in-hospital mortality and the control of bleeding. AUROCs with 95% confidence intervals were calculated and compared. Also, the AUROCs were tested for significance using DeLong test. The sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, positive predictive value, and negative predictive value with 95% confidence intervals were reported at the best cutoff value. $P < 0.05$ was considered statistically significant.

RESULTS

This analysis contains the data of 1517 patients who presented with acute variceal bleeding. There were 1176 males (77.5%), and mean patient age was 52.6 ± 10.3 years.

Three hundred and thirty-two (21.9%) patients died during hospitalization, and 364 (23.9%) developed hepatic encephalopathy during admission.

All patients received resuscitation, blood as needed, antibiotics, and vasoactive drugs. Endoscopy was performed as soon as feasible, and the mean time between admission and endoscopy was 5.6 ± 1.9 h (median 4 h) (Table 1). The mean number of blood units transfused was 2.8 ± 1.1 units, and the mean duration of hospitalization was 5 d. The baseline characteristics of the patients on admission are shown in Table 1.

Endoscopy revealed that 1283 patients (84.57%) had esophageal varices as the source of bleeding. Seven hundred and eighty-five patients (51.74%) had band ligation alone; 301 patients (19.84%) had sclerotherapy as a single treatment modality, while 197 (12.9%) had combined treatment modalities. Gastric varices were present in 366 patients, and were the only source of bleeding in 165. Histoacryl injection was conducted in 271 patients, in 165 as the only therapeutic intervention and with band ligation of esophageal varices in 106. Sixty-nine patients (4.54%) received conservative medical treatment (Table 2).

Sixty-nine patients were CTP class A (4.5%), 434 were CTP class B (29.2 %) and 1014 were CTP class C (66.8%). 692 (46%) had ascites and 364 (24%) presented with hepatic encephalopathy. Twenty-six patients were ALBI grade 1 (1.6%); 669 were ALBI grade 2 (44.1%), and 822 were ALBI grade 3 (54.1%). Using the PALBI score to classify patients, 306 patients were PALBI grade 1 (20.2%), 285 patients were PALBI grade 2 (18.8%), and 926 patients were PALBI grade 3 (61%). Table 3 shows the patient classification using the different scores.

The incidence of early rebleeding (within 1 wk) was 3.12% and the incidence of recurrent bleeding (after 1 wk) was 8.9%. The predictors of rebleeding are shown in Table 4.

In-hospital mortality among CTP classes A, B and C were 0%, 11% and 28%, among ALBI grades 1, 2 and 3 were 0%, 7.2% and 34.5%, and among PALBI grade 1, 2, and 3 were 0%, 21.8% and 34.4%, respectively (Table 3).

Table 5 shows the predictors of mortality, where PALBI grade showed the highest odds ratio.

The AUROC for predicting survival following acute variceal bleeding was 0.668, 0.689, 0.803, 0.849 and 0.871 for CTP, MELD, ALBI, MELD-AVB and PALBI scores, respectively (Figure 1). PALBI score showed a significantly higher performance than MELD, ALBI and CTP (Table 6). For predicting rebleeding, the AUROC was 0.681, 0.74, 0.766, 0.769 and 0.794 for CTP, MELD, ALBI, MELD-AVB and PALBI scores, respectively (Figure 2). The performance of PALBI was significantly higher than that of MELD-AVB, MELD, ALBI and CTP (Table 6).

DISCUSSION

The present study found a mortality rate of 21.9% due to variceal bleeding. Randomized, controlled trials have shown that mortality due to variceal bleeding in cirrhosis has decreased over the past 3 decades from about 50% to 20%-30%, but this figure is still remarkably high. Hence, stratifying the risk for mortality is paramount^[10]. The best method to stratify risk is not clear. In this study we report the important predictive value of the PALBI score.

The PALBI score, proposed by Roayaie *et al*^[8] was initially used for the assessment of patients with HCC undergoing resection or ablation. It was later validated in several studies in predicting the outcome of interventions for the management of HCC^[11,12]. This report, however, evaluates the predictive power of the PALBI score in a large cohort of patients with cirrhosis presenting with acute variceal bleeding, in comparison to ALBI, MELD and CTP scores. In the overall analysis, PALBI score performed significantly better than ALBI, MELD score and CTP classification.

The performance of CTP and MELD scores in predicting survival of patients with upper gastrointestinal bleeding is comparable^[13,14]. The better performance of ALBI in the current study was most probably due to omitting the subjective factors (ascites and encephalopathy) in the CTP score. The MELD score also includes creatinine that may be affected temporarily during gastrointestinal tract bleeding. Additionally, the INR results, as noted by Lisman *et al*^[15] showed great variation among seven different European laboratories and Trotter *et al*^[16] also confirmed such a variation in INR levels among 14 different laboratories in the United States. However, the adapted version

Table 1 Characteristics of all studied participants

Variable	Number/mean (%)	Median	SD
Age (yr)	52.6	52	10.3
Male:Female	1176 (77.5):341 (22.5)	-	-
In-hospital mortality	332 (21.9)	-	-
Serum albumin (g/dL)	2.4	2.4	0.6
Serum total bilirubin ($\mu\text{mol/L}$)	64.98	30.78	99.18
Serum creatinine (mg/dL)	1.3	1	0.97
International normalized ratio (INR)	1.7	1.6	0.53
Hemoglobin level (g/dL)	9.5	9.4	2.35
Hematocrit	28.8	29	6.25
Platelet count ($\times 10^3/\text{mm}^3$)	116	104	56
Blood units transfused	2.8	2	1.1
Time to first endoscopy (h)	5.6	4	1.9
Duration of admission (d)	5	4	3.3
MELD score	15.2	14.8	3.2

SD: Standard deviation; MELD: Model of End-stage Liver Disease.

for variceal bleeding showed significantly better performance compared with the basic version.

The performance of PALBI, an updated version of ALBI, in predicting mortality was significantly better than the ALBI score possibly because the PALBI score includes platelet count which reflects the effect of portal hypertension; the main cause of acute variceal bleeding. In other scenarios as testing the prognostic performance after radiofrequency ablation, ALBI has been proved to be superior to PALBI^[17]. Thus, it appears that ALBI has a better prognostic power in patients with minimal liver dysfunction, whereas PALBI appears to be of more value in stratifying risk for portal hypertension.

The present study is the first to evaluate the performance of the PALBI score in predicting in-hospital mortality after variceal bleeding. The performance of the ALBI score in predicting the in-hospital mortality of acute upper gastrointestinal bleeding was previously tested by Zou *et al*^[18]. Their estimated AUROC result for the ALBI score was in agreement with the current study. However, they found the performance of ALBI comparable to the CTP and MELD scores. However, it is worth noting that in their study the cohort was much smaller than the present cohort (631 *vs* 1517) and unlike the current study not all patients underwent endoscopy and hence the source of bleeding was not restricted to variceal bleeding.

The incidence of early rebleeding during hospitalization (within 1 wk) among our patients was 3.12%. Several studies reported overall rebleeding rates of 2.56%, 3.2%, 6.1%, 3.9%^[19-23]. PALBI was significantly better than MELD, CTP and ALBI in predicting rebleeding. If the PALBI score could replace the CTP score and MELD, it would be easier and quicker to identify candidates for an early transjugular intra-hepatic porto-systemic shunt procedure. Furthermore, the use of PALBI score allows for better survival stratification within a CTP class. PALBI is a purely objective score incorporating both liver function and portal hypertension and is not subject to the inconsistencies of CTP resulting from the inclusion of ascites and encephalopathy.

Despite the limitation of being a retrospective single-center study, the large number of patients with all patients undergoing endoscopy and bleeding confirmed to be from varices, provides further validity to our findings.

In conclusion, the PALBI score is a simple, objective score that may be a good option for predicting in-hospital rebleeding and mortality in patients with acute variceal bleeding. We recommend future prospective studies to further validate the PALBI score and its value in predicting long-term prognosis.

Table 2 Treatment modalities

Treatment modality	n (%)
Band ligation	785 (51.8)
Sclerotherapy	301 (19.9)
Fundal varix (histoacryl injection alone)	165 (10.8)
Combined treatment	197 (13)
Conservative treatment	69 (4.5)

Table 3 Patients distribution according to Child Turcotte Pugh, Albumin-bilirubin, Model of End-stage Liver Disease and Platelet-albumin-bilirubin scores

Class	No. of patients (%)	Bleeding mortality (%)
CTP A	69 (4.5)	0
CTP B	434 (29.2)	11
CTP C	1014 (66.8)	28
MELD < 12	186 (12)	0
MELD 12-20	353 (23.5)	16.3
MELD > 20	978 (64.5)	31.9
ALBI grade 1	26 (1.7)	0
ALBI grade 2	669 (44.1)	7.2
ALBI grade 3	822(54.1)	34.5
MELD-AVB < 11	159 (10.5)	0
MELD-AVB 11-19	370 (24.4)	14.3
MELD-AVB > 19	988 (65.1)	36.3
PALBI grade 1	306 (20.2)	0
PALBI grade 2	285(18.8)	21.8
PALBI grade 3	926 (61)	34.4

PALBI: Platelet-albumin-bilirubin score; ALBI: Albumin-bilirubin; MELD: Model of End-stage Liver Disease; CTP: Child Turcotte Pugh; MELD-AVB: MELD-acute variceal bleeding.

Table 4 Predictors of rebleeding

Parameter	OR	95%CI	P value
PALBI	3.987	1.994-7.775	< 0.001
Conservative therapy	2.473	1.237-4.822	< 0.001
Previous beta blockers	0.387	0.194-0.755	< 0.001
ALBI	3.214	1.607-6.267	< 0.01
Time to endoscopy	1.572	1.086-3.065	< 0.01
Ascites	2.319	1.160-4.522	< 0.05
Spleen size	1.961	1.181-3.824	< 0.05
Diabetes	1.631	1.116-3.180	< 0.05
Platelet count	0.445	0.223-0.868	< 0.05
INR	1.618	1.109-3.155	< 0.05
Bilirubin	1.981	1.291-3.863	< 0.05
Albumin	0.613	0.457-0.897	< 0.05

ALBI: Albumin-bilirubin; PALBI: Platelet-albumin-bilirubin score; INR: International normalized ratio.

Table 5 Multivariate analysis: Predictors of in-hospital mortality

Parameter	OR	95%CI	P value
PALBI	4.187	2.093-8.164	< 0.001
MELD-AVB	3.842	2.983-7.431	< 0.01
ALBI	3.153	1.576-6.148	< 0.001
MELD	2.981	1.490-5.812	< 0.001
CTP	2.144	1.072-4.180	< 0.01
Bilirubin	1.981	1.015-3.862	< 0.01
Albumin	0.269	0.134-0.524	< 0.01
Age	1.718	1.034-3.350	< 0.05
Ascites	1.547	1.173-3.016	< 0.05
Encephalopathy	1.869	1.124-3.644	< 0.05
Hematocrit	0.191	0.095-0.372	< 0.05
Platelet count	0.543	0.321-0.853	< 0.05
INR	1.923	1.061-3.749	< 0.05
Creatinine	1.561	1.180-3.043	< 0.05

PALBI: Platelet-albumin-bilirubin score; ALBI: Albumin-bilirubin; MELD: Model of End-stage Liver Disease; CTP: Child Turcotte Pugh; MELD-AVB: MELD-acute variceal bleeding; INR: International normalized ratio.

Table 6 Area under the receiver operating characteristic curve values of different scores to predict the outcome of acute variceal bleeding and the incidence of rebleeding

Predicting outcome of acute variceal bleeding					
Score	CTP	MELD	ALBI	MELD-AVB	PALBI
AUROC	0.668	0.689	0.803	0.849	0.871
P value ¹	< 0.01	< 0.01	< 0.01	0.043	-
Predicting rebleeding					
Score	CTP	MELD	ALBI	MELD-AVB	PALBI
AUROC	0.681	0.74	0.766	0.769	0.794
P value ¹	< 0.01	< 0.05	0.052	0.032	-

¹P values are tested with Delong test between each consecutive test. PALBI: Platelet-albumin-bilirubin score; ALBI: Albumin-bilirubin; MELD: Model of End-stage Liver Disease; CTP: Child Turcotte Pugh; MELD-AVB: MELD-acute variceal bleeding; INR: International normalized ratio.

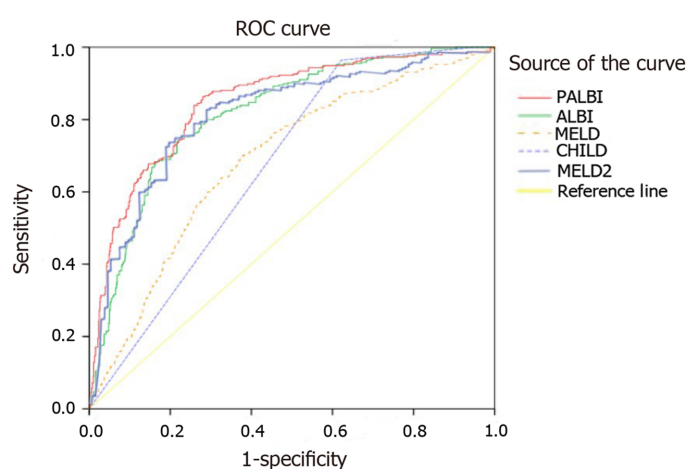


Figure 1 The AUROCs of the platelet-albumin-bilirubin score, albumin-bilirubin, Model of End-stage Liver Disease, Child Turcotte Pugh and Model of End-stage Liver Disease-acute variceal bleeding scores for predicting outcome of acute variceal bleeding. PALBI: Platelet-albumin-bilirubin score; ALBI: Albumin-bilirubin; MELD: Model of End-stage Liver Disease; CTP: Child Turcotte Pugh; MELD-AVB: MELD-acute variceal bleeding.

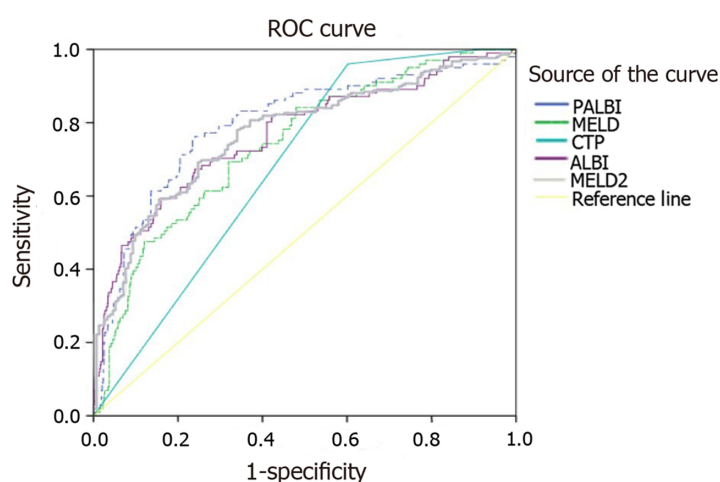


Figure 2 The AUROCs of the platelet-albumin-bilirubin score, albumin-bilirubin, Model of End-stage Liver Disease, Child Turcotte Pugh and Model of End-stage Liver Disease-acute variceal bleeding scores for predicting rebleeding. PALBI: Platelet-albumin-bilirubin score; ALBI: Albumin-bilirubin; MELD: Model of End-stage Liver Disease; CTP: Child Turcotte Pugh; MELD-AVB: MELD-acute variceal bleeding.

ARTICLE HIGHLIGHTS

Research background

In 1964, the CTP score was proposed to assess patients with portal hypertension related to gastrointestinal tract bleeding and was used later to assess patients with cirrhosis in general. Many doubts have been raised recently regarding the performance of the CTP score as a subjective scoring system with abrupt points and overlapping parameters. Recently, new scoring systems such as the ALBI and PALBI scores were proposed which use linear predictive equations to overcome the disadvantages of the CTP score.

Research motivation

To identify a reliable prognostic score to predict the short-term outcome of patients with acute variceal bleeding.

Research objectives

Scoring systems with more specific parameters and using linear predictive equations showed better performance than subjective point-based scoring systems.

Research methods

We retrospectively analyzed the data of a large number of patients with acute variceal bleeding and their short-term outcome.

Research results

The PALBI score is a simple, objective score that is considered a good option for predicting in-hospital rebleeding and mortality in patients with acute variceal bleeding in comparison to other scoring systems. However, we still recommend performing a prospective study to better analyze the performance of these scoring systems.

Research conclusions

The PALBI score could be used to predict the short-term outcome and the incidence of rebleeding in patients with acute variceal bleeding.

Research perspectives

Performing a prospective large-scale multicenter study to test the performance of these scores in different management settings.

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Successful liver transplantation for acute sickle cell intrahepatic cholestasis: A case report and review of the literature

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Abstract

BACKGROUND

Sickle cell hepatopathy (SCH) is an inclusive term referring to any liver dysfunction among patients with sickle cell disease. Acute sickle cell intrahepatic cholestasis is one of the rarest and most fatal presentations of SCH. We present the 23rd reported case of liver transplantation (LT) for SCH; a rare case of acute sickle cell intrahepatic cholestasis managed with LT from a hepatitis C virus (HCV) nucleic acid amplification test positive donor.

CASE SUMMARY

A 29-year-old male with a past medical history of sickle cell disease presented with vaso-occlusive pain crisis. On examination, he had jaundice and a soft, non-tender abdomen. Initially he was alert and fully oriented; within 24 h he developed new-onset confusion. Laboratory evaluation was notable for hyperbilirubinemia, leukocytosis, anemia, thrombocytopenia, acute kidney injury and elevated international normalized ratio (INR). Imaging by ultrasound and computed tomography scan suggested a cirrhotic liver morphology with no evidence of biliary ductal dilatation. The patient was diagnosed with acute sickle cell intrahepatic cholestasis after excluding competing etiologies of acute liver injury. He underwent LT from an HCV nucleic acid amplification test positive donor 9 d after initial presentation. The liver explant was notable for widespread sinusoidal dilatation with innumerable clusters of sickled red blood cells and cholestasis. On postoperative day 3, HCV RNA was detectable in the patient's peripheral blood and anti-HCV therapy with glecaprevir/pibrentasvir was initiated on postoperative day 23. He subsequently achieved sustained virologic

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response after completing 3 mo of therapy and has been followed clinically for 12 mo post-transplant.

CONCLUSION

This case highlights the utility of LT as a viable treatment option for acute sickle cell intrahepatic cholestasis.

Key words: Case report; Sickle cell hepatopathy; Acute intrahepatic cholestasis; Liver transplant; Hepatitis C virus; Post-operative surveillance

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Core tip: Acute sickle cell intrahepatic cholestasis is a rare and life-threatening form of sickle cell hepatopathy, with a mortality rate approaching 40%. Patients typically present with fever, abdominal pain and jaundice. Rarely, it may progress to an acute liver failure phenotype, commonly associated with multi-system organ failure. Diagnosis is made after excluding other causes of acute liver injury. Treatment options include exchange blood transfusion and liver transplantation.

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INTRODUCTION

Sickle cell disease (SCD) is the most common inherited blood disorder affecting up to 100000 Americans. It is an autosomal recessive disorder caused by a point mutation in the beta chain of chromosome 11 with a substitution of glutamic acid to valine. This substitution with a hydrophobic amino acid allows hemoglobin S (HbS) to polymerize on deoxygenation, forming rod-like polymers and resulting in poorly deformable sickled cells. Decreased red blood cell (RBC) pliability precipitates hemolysis and increases RBC membrane adhesiveness with the endothelium, which predisposes to vaso-occlusion of small blood vessels, which is often followed by reperfusion injury^[1].

Involvement of the hepatobiliary system is observed in 10%-40% of sickle cell crises^[1]. Hepatobiliary involvement may be related to conditions that are not unique to SCD but are more commonly seen in this group of patients compared to the general population as a result of chronic hemolysis and the resultant need for frequent blood transfusions. These conditions include cholelithiasis, cholecystitis, choledocholithiasis, acute cholangitis, pancreatitis, viral hepatitis and hemosiderosis. Hepatobiliary manifestations unique to patients with SCD include acute sickle cell hepatic crisis, acute hepatic sequestration and acute sickle cell intrahepatic cholestasis (the result of RBC sickling inside the hepatic sinusoids) (Table 1).

CASE PRESENTATION

Chief complaint and history of present illness

A 29-year-old African American male with a past medical history of SCD (Hb SS), maintained with exchange transfusions every 4-6 wk, with resultant hemosiderosis and cirrhosis presented with vaso-occlusive pain crisis in his lower extremities and uncontrolled epistaxis. His outpatient medications included deferasirox, folic acid and oxycodone. He denied tobacco, alcohol or drug use.

Physical exam

On initial examination, his vital signs were within normal limits. He was markedly jaundiced and was alert and fully oriented. His abdomen was soft without tenderness or organomegaly and with normal bowel sounds. Within 24 h of presentation, he developed new-onset confusion attributed to hepatic encephalopathy.

Table 1 Summary of unique manifestations of sickle cell hepatopathy

SCD manifestation	Pathophyiology of the disease	Histopathology	Clinical presentation	Amino-transferases	ALP	Bilirubin	Management
Acute sickle cell hepatic crises	Sickled RBCs obstruct liver sinusoids causing ischemic infarction	- Presence of sickle cell aggregates in the liver sinusoids - Kupffer cell hypertrophy and centrilobular necrosis	Fever, abdominal pain, jaundice and tender hepatomegaly	Elevated up to 3 fold the upper limit of normal followed by rapid resolution	Normal to slightly elevated	Conjugated hyperbilirubinemia up to 15 mg/dL, usually normalizes within 2 weeks	Supportive; hydration, oxygenation, pain control and blood exchange as needed
Acute hepatic sequestration	Kupffer cell erythrophagocytosis traps sickled RBCs resulting in blood pooling within liver sinusoids	- Presence of dilated blood-filled liver sinusoids	Sudden severe RUQ pain and rapidly worsening anemia with appropriate reticulocytosis; severe cases can present with shock and hepatomegaly	Normal	Elevated; up to 650 U/L	Conjugated hyperbilirubinemia up to 24 mg/dL	Cautious blood transfusion or exchange transfusion; excessive transfusion can result in rapid rise of Hb during resolution phase precipitating stroke and heart failure
Acute intrahepatic cholestasis	Diffuse sickling in liver sinusoids leading to widespread ischemia as well as Kupffer cell hypertrophy and extramedullary hematopoiesis which contribute to cholestasis	- Presence of massively dilated blood sinusoids with clusters of sickled RBCs - Presence of intracanalicular and intrahepatic cholestasis - Ballooning of hepatocytes, necrosis, inflammation	Fever, RUQ pain, acute liver failure and multi-system organ failure	Elevated; typically > 1000 U/L	Normal or elevated up to >1000 U/L	Conjugated hyperbilirubinemia up to > 30 mg/dL	Supportive with exchange transfusion and LT
Sickle cell cholangiopathy	Incomplete occlusion of the peribiliary vascular plexus results in hypoxia and dilatation of the bile ducts; recurrent insults can result in ischemic stricture	- Presence of ischemic necrosis and fibrosis of the bile ducts	Jaundice and biliary stone complications, imaging can reveal non-obstructive bile duct dilatation and/or obstructive biliary strictures	Normal or elevated	Elevated	Elevated	ERCP stenting and balloon dilatation, LT

SCD: Sickle cell disease; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; RBC: Red blood cell; RUQ: Right upper quadrant; Hb: Hemoglobin; LT: Liver transplant; ERCP: Endoscopic retrograde cholangiopancreatography.

Laboratory examinations

Laboratory evaluation was notable for conjugated hyperbilirubinemia with a total serum bilirubin 57 mg/dL and direct serum bilirubin 30 mg/dL, alkaline phosphatase 306 U/L, aspartate transaminase 227 U/L, and alanine transaminase 54 U/L. White blood cell count was 38.6 k/ μ L, hemoglobin was 6.3 g/dL and platelet count was 39 k/ μ L. Coomb's testing was negative, fibrinogen was 412 mg/dL, and INR was 2.3. His model for end-stage liver disease-sodium score was 40.

An extensive workup for acute liver injury was undertaken. Acute and chronic viral hepatitis testing was negative. Serologic testing for autoimmune hepatitis and genetic causes of liver disease, including hereditary hemochromatosis, alpha-1 antitrypsin deficiency and Wilson disease was negative. His ferritin was elevated at 1399 ng/mL. His arterial ammonia level was 72 μ mol/L. He was diagnosed with an acute kidney injury with a serum creatinine of 3.48 mg/dL (baseline creatinine 0.6-0.7 mg/dL).

Imaging examinations

A liver vascular ultrasound revealed a cirrhotic liver morphology with patent hepatic vasculature and appropriate flow. An abdominal computed tomography scan showed changes consistent with cirrhosis without biliary ductal dilatation.

FINAL DIAGNOSIS

The patient was diagnosed with acute-on-chronic liver failure with multi-system organ failure secondary to acute sickle cell intrahepatic cholestasis based on the presence of new-onset acute liver injury, encephalopathy, coagulopathy, and acute kidney injury.

TREATMENT

He was admitted to the medical intensive liver unit for further management. He required intubation and mechanical ventilation for acute hypoxic respiratory failure secondary to acute chest syndrome; for this he was treated with empirical vancomycin and meropenem. An exchange transfusion was initiated with subsequent decrease of HbS level from 56.3 to 8.2 g/dL. Despite the exchange transfusion, his hepatic synthetic function did not improve; he was rapidly evaluated and subsequently listed with a model for end-stage liver disease-sodium score of 40 for urgent liver transplantation (LT) for acute sickle cell intrahepatic cholestasis.

The pre-operative evaluation was coordinated by a multidisciplinary team involving hepatology, infectious disease, dermatology, dentistry, anesthesiology, transplant surgery, critical care and apheresis specialists. Nine days after initial presentation, the patient underwent LT from a brain dead, hepatitis C virus (HCV) nucleic acid test (NAT) positive donor utilizing standard piggyback technique and a duct-to-duct biliary anastomosis. The explanted liver was found to be enlarged, measuring 25.4 cm x 16.6 cm x 10.5 cm and weighing 2125.6 g. The capsule was focally micronodular predominantly on the posterior surface. On cut section, the parenchyma was red-brown and appeared congested with focal micronodular areas. The hepatic vasculature and bile ducts were normal.

On microscopic examination, the liver parenchyma showed widespread sinusoidal dilatation with innumerable clusters of sickled red blood cells, scattered pigmented histiocytes and cholestasis. The trichrome stain highlighted extensive sinusoidal fibrosis and established portal-portal bridging fibrosis with evolution toward cirrhosis (stage 3-4, scale 0-4, Batts-Ludwig methodology). The iron stain was notable for patchy hepatocellular and Kupffer cell siderosis (2+) (Figures 1 and 2).

OUTCOME AND FOLLOW UP

Immediately following LT, exchange transfusion was resumed and the patient was started on immunosuppression induction therapy with antithymocyte globulin. He was also initiated on mycophenolate mofetil, tacrolimus and a steroid taper for maintenance of immunosuppression. On postoperative day 3, HCV ribonucleic acid was found to be > 100000000 IU/mL. Genotyping revealed HCV genotype 1A; anti-HCV therapy with glecaprevir/pibrentasvir was started on post-operative day 23 after insurance approval. The patient progressed routinely and was discharged. He achieved sustained virologic response after completing 3 mo of anti-HCV therapy. At 12 mo post-operatively, his liver synthetic function is preserved on chronic tacrolimus immune suppressive therapy; he undergoes monthly exchange transfusions with a target HbS < 20%.

DISCUSSION

Only 22 cases of LT for SCH have been reported in the literature. The majority of transplants have been performed for acute liver failure (ALF) secondary to acute sickle cell intrahepatic cholestasis. We report the 23rd LT for SCH; the first reported case of an HCV NAT positive donor that facilitated urgent LT for an HCV NAT negative patient with acute sickle cell intrahepatic cholestasis. Our patient subsequently achieved sustained virologic response after being treated with 3 mo of glecaprevir/pibrentasvir.

Sickle cell hepatopathy (SCH) is an inclusive term referring to any liver dysfunction among patients with SCD. SCH can present with both acute or chronic liver dysfunction, but has primarily been used in the literature to describe the acute hepatic manifestations of SCD. At times, SCH has been used to denote acute intrahepatic cholestasis specifically^[1-6].

Acute intrahepatic cholestasis related to SCD is the most severe, and often fatal, form of SCH, associated with a mortality rate approaching 40%. Patients may present

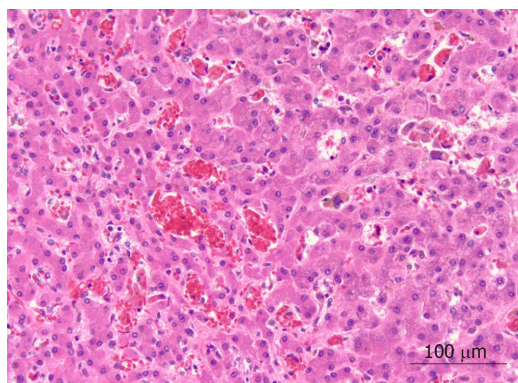


Figure 1 Liver parenchyma showing widespread sinusoidal dilatation with innumerable clusters of sickled red blood cells, scattered pigmented histiocytes and cholestasis ($\times 20$ magnification).

with severe acute hepatic crisis with fever, right upper quadrant pain and leukocytosis; however, this condition is characteristically accompanied by significant jaundice and can rapidly progress into ALF. Patients typically experience a dramatic increase in conjugated bilirubin with reported levels ranging between 30 and 273 mg/dL. Hemolysis and acute kidney injury also contribute to hyperbilirubinemia. Liver biochemistries including the aspartate transaminase, alanine transaminase and alkaline phosphatase levels may reach values exceeding 1000 mg/dL; however, normal to only slightly elevated values are possible. Coagulopathy, evidenced by elevated prothrombin time, partial thromboplastin time, INR and hypofibrinogenemia, is typically seen. Multi-factorial acute kidney injury is commonly observed as well. To date, no cohesive list of diagnostic criteria has been proposed; we propose that multi-system organ failure with extreme hyperbilirubinemia and accompanied by altered mental status in patients with SCD should raise the clinical index of suspicion for acute intrahepatic cholestasis with an ALF phenotype^[1,2].

Liver biopsy will typically demonstrate dilated blood sinusoids with clusters of sickled RBCs, associated with fibrosis and as intracanalicular and intraductal cholestasis^[1,3,7]. Findings of extramedullary hematopoiesis and Kupffer cell hypertrophy with intracellular engulfed sickled RBCs have also been described in the literature^[3]. According to the degree of hypoxic injury, ballooning of hepatocytes, and in more severe cases, widespread anoxic necrosis with areas of acute and chronic inflammation have also been reported^[1,8]. Based on the described histopathologic characteristics, it is believed that this condition results from diffuse sickling in the blood sinusoids leading to widespread ischemia, hepatocyte injury and fibrosis^[1]. Kupffer cell hypertrophy and extramedullary hematopoiesis may also contribute to liver sinusoidal obstruction with compression of the adjacent bile ducts, contributing to cholestasis.

A recent report in the literature of acute sickle cell intrahepatic cholestasis by Kwun Lui *et al*^[3] described a more complex histological pattern of injury, including a combination of centrilobular fibrosis, occasional occlusion and constriction of the central veins, and sinusoidal fibrosis. This pattern more closely resembles the histological findings of chronic sinusoidal obstruction syndrome and veno-occlusive disease. This finding prompts the suggestion that intrahepatic cholestasis in SCD may result from RBC-mediated damage of small vessel endothelium, resulting in endothelial cell death and subsequent fibrosis of the small hepatic veins and sinusoids. Progression of this process would result in obstruction and distention of the sinusoids. It has been therefore hypothesized that endothelial dysfunction is the direct consequence of ischemic RBC-mediated injury in the small outflow veins and sinusoids.

In patients with SCD and intra-hepatic cholestasis, cause of death is typically related to multi-system organ failure. Therapy is aimed at aggressive supportive measures, including exchange transfusions to replace HbS and correct anemia. Coagulopathy is usually treated with blood product and factor transfusions. Temporary renal replacement therapy may be required for acute kidney injury; renal function should correct with improvement of liver function^[1]. If supportive measures fail, LT remains the only viable therapeutic option. Outcomes for patients with intrahepatic cholestasis undergoing LT are summarized in Table 2. Recurrence of this disease process has been reported after LT; the role of hydroxyurea to prevent sickle cell intrahepatic cholestasis is uncertain^[9,10].

In patients with SCD, an acute presentation of acute-on-chronic liver failure with an

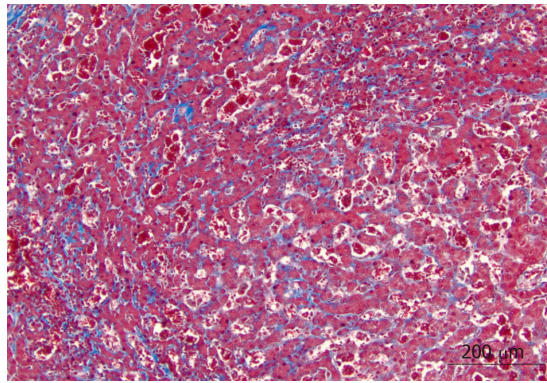


Figure 2 Trichrome stain highlighted extensive sinusoidal fibrosis and established portal-portal bridging fibrosis with evolution toward cirrhosis ($\times 10$ magnification).

ALF phenotype has been reported in patients with underlying chronic liver disease related to chronic viral hepatitis and/or iron overload^[3]. Vaso-occlusive events may also precipitate acute-on-chronic liver failure in patients with advanced chronic liver disease. Patients are at elevated risk of hepatic decompensation despite exchange transfusion given their low hepatic synthetic reserve; LT should accordingly be considered as a viable rescue therapy in selected patients^[6].

Two case series describing LT for SCD patients are present in the literature. The first series described 6 patients with 1-, 5- and 10-year survival rates of 83.3%, 44.4% and 44.4% respectively^[6]. The second series described 3 pediatric patients, followed for a mean of 4.3 years, with a reported survival rate of 66%^[9].

The risk of vaso-occlusive crisis is increased peri-operatively, and crises may involve the transplanted liver. It has been recommended to initiate exchange transfusion before surgery with an HbS target of $< 20\%$ - 30% and for the HbS level to be maintained between 8 and 10 g/dL post-operatively in the long term^[6,9].

Iron overload is a risk factor for progression of hepatic fibrosis related to chronic HCV infection among transplanted liver organs. Hemosiderosis should be managed by minimizing simple blood transfusions as able, by considering exchange blood transfusion as an alternative, and with iron chelators as indicated. Total body iron stores should be monitored serologically on a regular basis. Liver biopsy remains the gold standard for determination of hepatic iron concentration, however; magnetic resonance imaging in combination with serum ferritin level has been proposed as a non-invasive alternative^[11,12]. However, serum ferritin levels may vary over time in the setting of infectious or inflammatory processes, vaso-occlusive crises and liver dysfunction^[13]. Chelation therapy is indicated in adults after receiving 20-30 blood units, in patients with a serum ferritin > 3000 ng/mL with a hepatic iron index of > 7 - 9 mg/g dry weight^[14].

The evaluation of abnormal liver biochemistries after LT may be challenging in patients with SCD; it can be challenging to non-invasively differentiate acute and/or chronic rejection and infectious processes from the normal hepatic pathophysiology of SCD. As part of normal SCD physiology, kupffer cells will continue to engulf sickled RBCs, which can lead to congestion of the liver allograft and mild elevation of aminotransferases and bilirubin levels^[15]. Furthermore, low grade fever and leukocytosis are commonly seen in patients with SCD during vaso-occlusive crises^[16]. Liver biopsy remains the gold standard for evaluation of possible rejection following LT^[17].

CONCLUSION

In conclusion, we present a rare case of acute sickle intrahepatic cholestasis managed with successful LT. This case represents the first report of an HCV NAT positive allograft being transplanted into an HCV negative SCD patient, and is the 23rd reported case of LT in SCD patients overall. Unfortunately, LT will not reverse the underlying pathophysiology of SCD; diligent post-transplant hematologic and immunosuppressive management care is needed in these cases. As our understanding of SCH evolves, paralleling technical advances in LT, post-LT management should be aimed at improving quality of life and optimizing survival.

Table 2 Reports of patients with intrahepatic sickle cell cholestasis who underwent liver transplantation

Ref.	Year	Number of cases	Age of the patient	Outcomes
Emre <i>et al</i> ^[15]	2000	1	6	First transplant was complicated by graft failure from veno-occlusive disease, required re-LT. Second transplant was complicated by graft failure from hepatic artery thrombosis, required re-LT. The patient died 6 mo after third LT from sepsis.
Ross <i>et al</i> ^[18]	2002	1	49	The patient died 22 mo after LT due to pulmonary embolism.
Gilli <i>et al</i> ^[19]	2002	1	22	The patient was alive 3 mo after LT.
Baichi <i>et al</i> ^[7]	2005	1	27	Post-LT course was complicated by sepsis, multiorgan failure, perihepatic hematoma and hemorrhagic ascites; the patient died 35 d after LT.
Mekeel <i>et al</i> ^[9]	2007	2	8,17	Patients were followed up over a mean period of 4.2 yr. Patient 1 was alive at end of follow-up with mild recurrent HCV. Patient 2 had recurrent sickle cell hepatopathy post-transplant and died of cerebral complications 6 yr following LT.
Hurtova <i>et al</i> ^[6]	2011	5	32-47	Patient 1 died of recurrent HCV-induced decompensated cirrhosis and sepsis 11 yr after LT. Patient 2 had recurrent HCV with moderate fibrosis; died of ischemic cholangitis and sepsis after 4 yr after LT. Patient 3 had recurrent HCV infection. He was alive 8 yr after LT. Patient 4's post-operative course was complicated by posterior leukoencephalopathy; the patient died from sepsis 16 mo after LT. Patient 5 developed biliary strictures requiring stenting. The patient was alive 42 mo after LT.
Blinder <i>et al</i> ^[20]	2013	1	37	Immediate post-LT course was complicated with seizure and respiratory failure. The patient had no post-operative SCD-related complications in the 12 mo after transplant and was maintained on hydroxyurea without need for exchange transfusion.
Lui <i>et al</i> ^[3]	2018	1	29	The patient was alive 7 mo after LT with no reported complications.

LT: Liver transplant; HCV: Hepatitis C virus; SCD: Sickle cell disease.

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