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ABOUT COVER Editorial Board Member of *World Journal of Hepatology*, Reiji Sugita, MD, Associate Professor, Doctor, Director, Department of Radiology, Sendai City Medical Center, Sendai 983-0824, Japan

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

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REVIEW

Magnetic resonance evaluations of biliary malignancy and condition at high-risk for biliary malignancy: Current status

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Abstract

Tumors of the biliary tree are relatively rare; but their incidence is rising worldwide. There are several known risk factors for bile duct cancers, and these are seem to be associated with chronic inflammation of the biliary epithelium. Herein, 2 risk factors have been discussed, primary sclerosing cholangitis and reflux of pancreatic juice into the bile duct, as seen in such as an abnormal union of the pancreatic-biliary junction because magnetic resonance imaging (MRI) is used widely and effectively in the diagnosis of these diseases. When biliary disease is suspected, MRI can often help differentiate between benignity and malignancy, stage tumors, select surgical candidates and guide surgical planning. MRI has many advantages over other modalities. Therefore, MRI is a reliable noninvasive imaging tool for diagnosis and pre-surgical evaluation of bile duct tumors. Nowadays remarkable technical advances in magnetic resonance technology have expanded the clinical applications of MRI in case of biliary diseases. In this article, it is also discussed how recent developments in MRI contributes to the diagnosis of the bile duct cancer and the evaluation of patients with risk factors affecting bile duct cancer.

Key words: Biliary; Magnetic resonance imaging; Malignancy; Primary sclerosing cholangitis; Pancreas juice; Reflux

Core tip: Tumors of the biliary tree are relatively rare; but their incidence is rising worldwide. When biliary disease is suspected, magnetic resonance imaging (MRI) can often help differentiate between benignity and malignancy, stage tumors, select surgical candidates and guide surgical planning. Nowadays remarkable technical advances in magnetic resonance technology have expanded the clinical applications of MRI in case of biliary diseases. In this article, it is also discussed how recent developments in MRI contributes to the diagnosis of the bile duct cancer and the evaluation of patients with risk factors affecting bile duct cancer.

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INTRODUCTION

Bile duct malignancies are relatively rare, estimated at 2% of all cancers with an incidence of 0.01%-0.04% in autopsy series[1]; however their incidence is rising worldwide[2,3]. The several known risk factors account for bile duct cancers, and these seem to be associated with chronic inflammation of the biliary epithelium[4-7]. The exact mechanism of tumor development is not completely understood and various possible pathways have been proposed, including chronic inflammatory process in the bile duct, mutation, and parasite-induced DNA damage[4,7-11]. When biliary disease is suspected, optimal imaging studies provide the required information for differentiating between benign and malignant tumors, tumor staging, selection of surgical candidate, and surgical

planning of bile duct cancer. Various imaging modalities, invasive and noninvasive, are employed in diagnosis and staging of bile duct tumors[1,12]. The invasive methods include endoscopic retrograde cholangiopancreatography (ERCP), endoscopic ultrasonography (EUS), intraductal ultrasonography (IDUS), percutaneous transhepatic cholangiography (PTC), and optical coherence tomography. Noninvasive imaging methods include ultrasonography (US), multidetector computed tomography (MDCT), magnetic resonance imaging (MRI), and positron emission tomography-computed tomography (PET-CT). ERCP and PTC are not used as diagnostic tools alone owing to invasive nature. Nowadays ERCP is used for interventions such as biopsy, drainage and EUS/IDUS. US, EUS and IDUS are useful technique for screening biliary diseases particularly gallbladder disease; however their efficacy depends on operator skill and experience. MDCT are accurate and useful imaging techniques for the evaluation of biliary diseases. MDCT offers detailed information about the biliary tree and surrounding structures; however, it has some demerits such as ionized radiation and adverse reaction of intravenous contrast materials. MRI is a reliable noninvasive common imaging tool for the diagnosis and pre-surgical evaluation of bile duct tumors. MRI has many advantages over other modalities: (1) it is completely noninvasive, does not require exposure to ionizing radiation, and does not cause patient discomfort; (2) it does not require expert technicians with sophisticated technical skills. Therefore MRI has become an important diagnostic tool for bile duct diseases.

Moreover nowadays remarkable technical advances in magnetic resonance (MR) technology have increased the clinical applications of MRI for diagnosing biliary diseases[12-15]. In this article, it is discussed how developments in MRI have improved the evaluation of patients with risk factor affecting bile duct cancers and the diagnosis of bile duct cancers.

MRI TECHNIQUE

A pre-procedural fasting is recommended for gallbladder distension and gastric emptying. When fluid is present in the stomach and duodenum, visualization of the bile duct may be obscured by interposition of bowel loop. Therefore administration of oral contrast agent (iron oxide particles, blueberry juice or pineapple juice) is recommended.

Most institutes may perform MR examinations at 1.5 T with a torso coil. Although imaging at 3 T can improve the signal-to-noise ratio and spatial resolution, it may be hampered by dielectric effects, banding, and other pulse sequence-related effects[16-18]. The pulse sequences used for MRI of the bile duct are usually axial T1- and T2-weighted imaging, MR cholangiopancreatography (MRCP), and axial diffusion-weighted imaging (DWI). T1-weighted image may be used under an intravenous contrast material. Most gadolinium contrast agents produce an enhancement pattern similar to that observed with iodine-based CT contrast. The advent of the hepatocyte-specific contrast agents (Gd-EOB-DTPA, Gd-BOPTA, *etc.*) allows the usual early-phase imaging of the arterial, portal, and venous phases, plus delayed-phase hepatic parenchymal and biliary imaging, taking advantage of the fact that about 50% of injected dose of these contrast agents are excreted via the biliary system[19,20].

MRCP use 2 varieties of T2-weighted sequences. One is obtained with a single-shot turbo spin-echo T2-weighted sequence by using a long echo time to selectively display the fluid filled bile ducts. The other is obtained by using a navigator-based respiratory-triggered three-dimensional acquisition sequence with a longer acquisition time[21]. The differences of both are small, and thus either or both are used for MRCP accordingly.

DWI can obtain additional information derived from the microscopic motion of proton in water, which is not possible by using conventional MRI. DWI is a sensitive sequence for the detection of tumors and inflammation of the bile ducts. It has the advantage of quantitative data analysis through the generation of apparent diffusion coefficient (ADC) maps, which can contribute to objective disease assessment and monitoring of response to therapy[22-25].

MRI can allow us to evaluate the analysis of bile and pancreatic juice flow, which may have relate to carcinogenesis of the bile duct tumors. Although by now the flow analysis of the bile duct based on MRI was held by a continuous MRCP examination after secretin injection, a new method [time-spatial labeling inversion pulse (SLIP) imaging] become to evaluate the flow analysis easier and faster than before[26].

CLINICAL INDICATION

Benign biliary diseases and condition at a high-risk for malignancy

Risk factors for bile duct carcinoma include (1) primary sclerosing cholangitis (PSC), (2) reflux of pancreatic juice into the common bile duct, such as in an abnormal arrangement of the pancreato-biliary ductal system (AAPB), (3) exposure to chemicals, and (4) medication such as oral contraceptives and methyldopa[4-7]. In this chapter, MRI applications for benign biliary diseases and condition at a high-risk for malignancy are discussed about PSC and reflux of pancreatic juice into the bile duct because MRI is used widely and effectively for these entities (Table 1).

PSC: PSC is a chronic cholestatic liver disease of possible autoimmune origin, characterized by intra- and extrahepatic bile duct inflammation and fibrosis[4,27-31]. PSC is the most common risk factor for cholangiocarcinoma in Western countries, with a prevalence of cholangiocarcinoma ranging from 8% to 25%[27]. Diagnostic criteria for PSC include (1) typical cholangiographic abnormalities; (2) clinical, biochemical, and hepatic histologic finding; and (3) the exclusion of secondary cause of sclerosing cholangitis.

The diagnosis of PSC was based on characteristic

Table 1 Characteristics of magnetic resonance of each diseaseson

	MR characteristics	Differential diagnosis	Comparison to other modalities	Sensitivity and specificity	Pitfall of MRI
PSC	Diffuse stricture and/or beaded appearance of the bile duct on MRCP	Cholangitis, Cholangiocarcinoma	ERCP is considered the standard method. MRCP is considered being sufficient for diagnosis of PSC	High sensitivity and very high specificity	It is often difficult to differentiate malignant tumors from PSC
Cholangiocarcinoma			MRI with MRCP is usually considered the modality of choice in the diagnosis of cholangiocarcinoma	Diagnosis of biliary stenosis by MRCP is high sensitivity and specificity. The ability of differentiation between benign obstruction and malignant is low	Minimal invasion along the mucosa and in the perineural space is difficult to diagnose
Intrahepatic cholangiocarcinoma	The tumor shows an irregular shaped solid mass with peripheral rim enhancement and incomplete concentric pooling of contrast material on dynamic study	Metastasis, Mixed HCC, cholangiocellular carcinoma			
Extrahepatic cholangiocarcinoma	The most common pattern of the tumor growth is focal infiltration of the ductal wall or the periductal-infiltrating type, resulting in focal strictures	PSC, cholangitis (IgG4, infection, AIDS), sarcoidosis			
Gallbladder carcinoma	In the diffusely infiltrative type, the tumor appears as a large solid mass in the gallbladder fossa In the polypoid and mural thickening types, lesion more than 10 mm in diameter or which enhance after intravenous contrast material, are usually malignant	Polyp, adenomyomatosis, xanthogranulomatous cholecystitis, chronic cholecystitis	Usually, US is used as an initial diagnostic modality As a second step, CT, MRI with MRCP, and /or traditional cholangiography is often used for obtaining additional information	Conventional MRI showed 74% of sensitivity and 68%-83% specificity, while DWI set added to conventional MRI showed high sensitivity and specificity	It is often difficult malignant from benign tumors
Ampullary carcinoma	It is difficult to diagnose because of the small tumor on MRI. DWI has the potential for differentiating malignant from benign ampullary tumors	Cholangiocarcinoma, Pancreas cancer, adenoma, inflammatory diseases, carcinoid	MRI with MRCP is more accurate than CT in differentiating between malignant and benign lesions	High sensitivity (100%) and low specificity (59.1%-63.6%). Adding of DWI to conventional MRI improve specificity	It is often difficult to diagnose because of the small tumor

PSC: Primary sclerosing cholangitis; MRCP: Magnetic resonance cholangiopancreatography; ERCP: Endoscopic retrograde cholangiopancreatography; HCC: Hepatocellular carcinoma; MRI: Magnetic resonance imaging; US: Ultrasonography; CT: Computed tomography.

cholagiographic finding in combination with clinical, biochemical, and histologic features. Therefore ERCP was considered the standard method for diagnosis of PSC. However, owing to developments in MR technology, MRCP has become another important modality[32-41]. The result of a meta-analysis showed that MRCP had high sensitivity and very high specificity for the diagnosis of PSC[33] (Figure 1). The radiological characteristics of PSC mimic those of cholangiocarcinoma[42]. Both make differential diagnosis quite difficult even with current diagnostic modalities including MRI.

AAPB: AAPB is a congenital anomaly defined as the junction of the pancreatic and bile ducts being located outside the duodenal wall. As the contraction of the sphincter of Oddi within the duodenal wall does not functionally affect the junction in patients with this congenital abnormality, continuous pancreaticobiliary reflux occurs, resulting in a high incidence of biliary cancer. AAPB can be divided into (1) AAPB with biliary dilatation (choledochal cyst) and (2) AAPB without biliary dilatation.

AAPB with choledochal cyst: Choledochal cysts are rare congenital biliary tract anomalies characterized by biliary tree dilatation. Although the incidence in the Western population is 1 in 100000 to 150000 live births, it is much higher in Asian countries, particularly Japan, where they can be found in up to 1 in 1000 live birth[43-45]. Choledochal cysts are usually classified into several types, based on anatomical findings. According to Todani's classification system, choledochal cysts include five main types.

In Todani's classification system, almost all patients with choledochal cyst are classified into 3 types (type I a, I c and IV-A), and that associated with AAPB. Biliary tract malignancies were seen in 10%-30% of patients with choledochal cyst and it increases with age[45]. A prompt and accurate diagnosis of choledochal cyst, follow by surgical is therefore essential.

In diagnostic imaging, researchers have shown that MRCP can offer diagnostic information equivalent to that of ERCP for assessment of choledochal cysts in adults[46,47] (Figure 2). Although MRCP should not replace ERCP totally in pediatric patients, MRCP should be considered the first-choice imaging technique for evaluation of choledochal cysts. MRCP can provide preoperative information about minute structure of AAPB

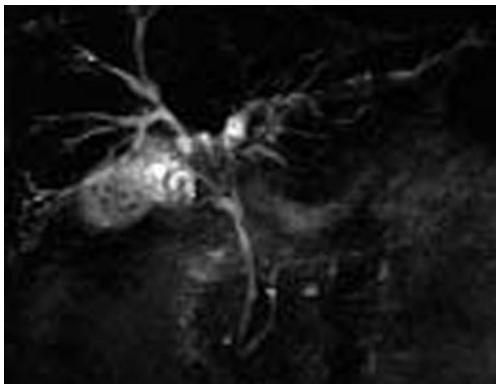

Figure 1 Primary sclerosing cholangitis in a 54-year-old man. Magnetic resonance imaging shows multifocal strictures and beading of the bile duct.

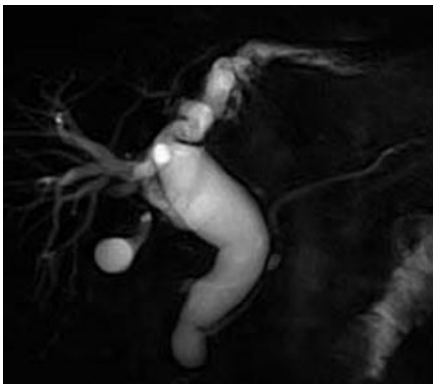

Figure 2 Choledochal cyst Todani Ⅳ-A type in a 58-year-old man. Magnetic resonance imaging shows dilatation both intrahepatic and extrahepatic bile ducts with abnormal arrangement of the pancreato-biliary ductal system.

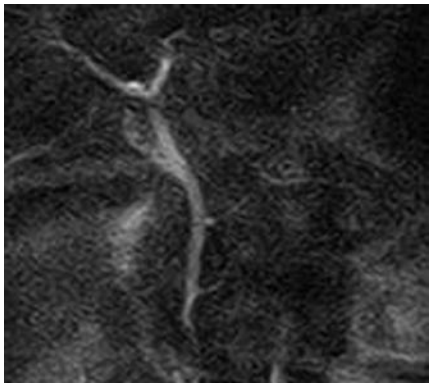

Figure 3 Magnetic resonance image in a 48-year-old woman with abnormal arrangement of the pancreato-biliary ductal system without a choledochal cyst.

in children with choledochal cysts[48].

AAPB without choledochal cyst: AAPB patients without choledochal cyst, similar to those with choledochal cyst, experience continuous reciprocal reflux between pancreatic juice and bile[49]. Because the hydro pressure within the pancreatic duct is usually greater than that within the bile duct, pancreatic juice frequently refluxes into the bile duct in these patients, which results in a high incidence of cancer of the biliary tract.

Although AAPB patients with and without choledochal cyst have a risk of biliary malignancy, the usual sites of malignancy differ. To the contrast bile duct and gallbladder cancers were seen in 34% and 65% of AAPB with choledochal cysts, only gallbladder cancer was found in almost all of 38% of AAPB without biliary dilatation[50]. Once AAPB is diagnosed, prophylactic flow-diversion surgery (bile duct resection and biloenteric anastomosis) is performed for patients with choledochal cyst.

Treatment of patients with AAPB without biliary dilatation is controversial. Prophylactic cholecystectomy is performed in many institutions. However, some surgeons propose excision of the extrahepatic bile duct, together with gallbladder.

The diagnostic criteria for AAPB have been established on the basis of ERCP. Although Kamisawa *et al*[50] have shown that MRCP can be used to detect AAPB (Figure 3), they have reported that some atypical cases with relative short common channel cannot be diagnosed by MRCP, and should be confirmed by ERCP.

AAPB cases with choledochal cysts have clinical symptoms due to cholangitis or pancreatitis in childhood, and thus they tend to be diagnosed in childhood. Patients without choledochal cysts are usually not diagnosed until adulthood, when they have already progressed to advanced stage gallbladder carcinoma, which has a poor prognosis. An appropriate strategy is necessary to detect and manage these cases. Takuma *et al*[51] have suggested that MRCP should be performed in patients who are found to have gallbladder wall thickening by US.

Pancreatic juice reflux without AAPB

Recently, several case series have been published on the reflux of pancreatic juice into the bile duct without a morphologically AAPB, and the correlation of such cases with biliary diseases, especially biliary malignancies, is drawing attention[52-57]. These cases could not detected by existing imaging modalities based on morphological change.

Several reports have shown that high amylase levels in bile samples on ERCP, which indicate reflux of pancreatic juice, or reflux of contrast medium into the pancreatic duct during intraoperative cholangiography, were found in 26%-87% of patients with normal pancreaticobiliary duct anatomy[58,59].

Several reports have revealed that MRCP can be used to detect pancreatic juice reflux in those patients[53,55]. In patients without AAPB, reflux of pancreatic juice into the common bile duct can be indirectly observed by using secretin-stimulating MRCP. The cause of such reflux may be dysfunction of the sphincter of Oddi.

The new method of time-SLIP technique, used in vascular studies, has the potential to visualize pancreatic juice flow directly[26] (Figure 4). Researchers have shown that this method can be used to detect pancreatic juice flow reflux in the normal patients (Figure 5). The new

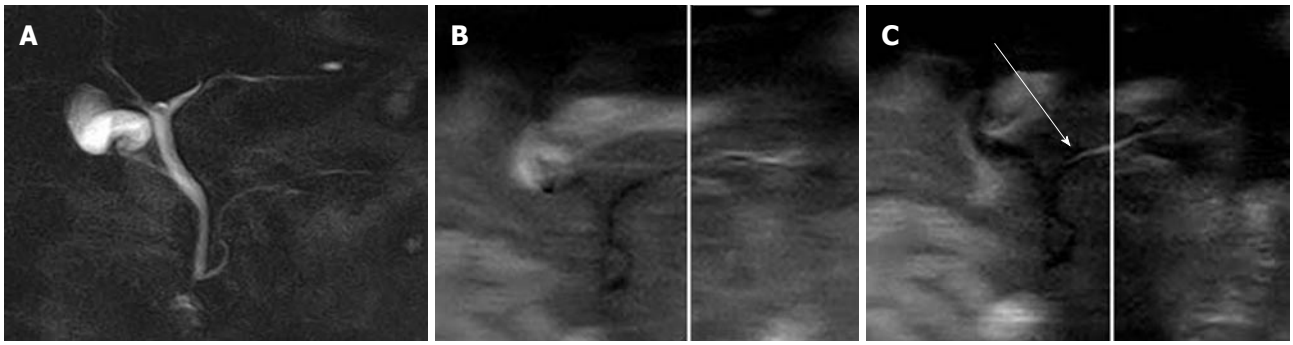

Figure 4 Flow of pancreatic juice by time-spatial labeling inversion pulse imaging. A: Magnetic resonance cholangiopancreatography image; B: Time- spatial labeling inversion pulse image obtained by applying labeling pulse box surrounded by lines to the body and tail portions of the main pancreatic duct, not showing movement; C: Flow of pancreatic juice in duct from body into the head of pancreas is identified by high signal intensity (arrow).

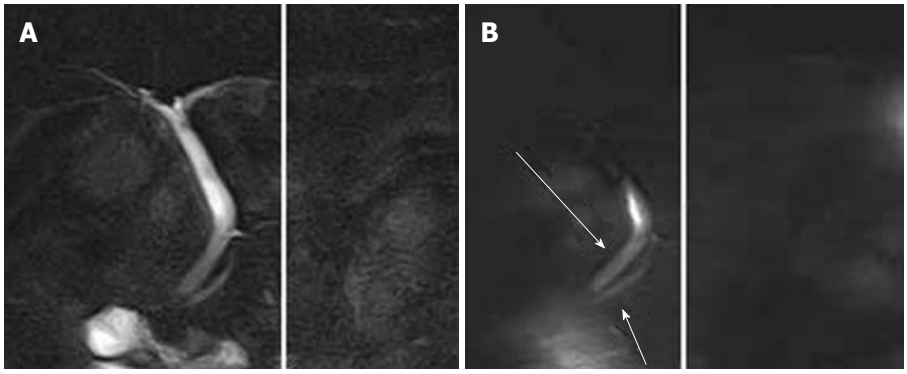

Figure 5 Pancreatic juice reflux into the biliary tree by time-spatial labeling inversion pulse imaging. A 56-year-old female patient underwent magnetic resonance imaging after abnormal laboratory findings. Magnetic resonance cholangiopancreatography revealed normal morphology, but time- spatial labeling inversion pulse imaging showed pancreatic juice reflux into the biliary tree. A: Magnetic resonance cholangiopancreatography image; B: Flow of pancreatic juice from body of the pancreas into the head of the pancreas is identified by high signal intensity (arrows).

technique may reveal more information on the rate of pancreaticobiliary reflux in the population with normal biliary anatomy and help determine whether is associated with an increased incidence of biliary malignancy.

BILIARY MALIGNANCIES

In general, the diagnosis of biliary tumors, particularly early detection and differential diagnosis, is still challenging, although many sensitive direct and indirect techniques have been adopted.

Cholangiocarcinoma

Cholangiocarcinoma arise from the epithelial cells lining the biliary tree. Intrahepatic cholangiocarcinoma arise within the intrahepatic ducts and extrahepatic cholangiocarcinoma originate in the bile duct along the hepatoduodenal ligament. Extrahepatic biliary carcinomas are further divided into hilar, also called Klatskin tumors, and distal tumors. Hilar tumors represent approximately 60%-70% of cholangiocarcinoma, distal tumors represent 20%-30%, and intrahepatic cholangiocarcinomas represent 5%-10%[1,4,5].

The tumors are rare, estimated at 3% of all gastrointestinal cancers. They are the second most common type of primary hepatic tumors[4,7,8]. This ratio includes intrahepatic and extrahepatic tumors. The patients present mostly in the 6th and 7th decades of life.

The pathologic classification of cholangiocarcinoma categorize into 3 types: mass-forming, periductal infiltrating, and intraductal growing[60]. The intraductal growing type is currently thought to be the counterpart of intraductal papillary mucinous neoplasm of the pancreas[13,61-67].

MRI with MRCP is usually considered the modality of choice for the diagnosis of cholangiocarcinomas. Several studies have shown that MRI has sensitivity and specificity > 90%. However, its ability to differentiate between benign and malignant obstruction is low and variable, according to the authors[68].

Intrahepatic cholangiocarcinoma: Intrahepatic cholangiocarcinoma is the second most common primary hepatic malignant tumors after hepatocellular carcinoma[13,68,69]. The important prognostic factors of intrahepatic cholangiocarcinoma are tumor size, lymph node metastasis, and vascular invasion.

The mass-forming type makes up a large percentage of intrahepatic cholangiocarcinoma, and shows an irregular shaped solid mass with peripheral rim enhancement

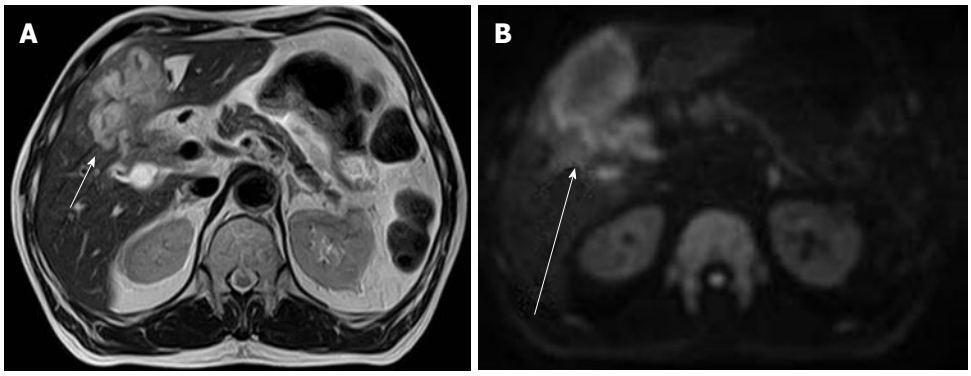

Figure 6 Intrahepatic cholangiocarcinoma in a 70-year-old man. A: Axial T2-weighted image shows high signal intensity liver mass (arrow); B: Diffusion-weighted imaging shows high signal intensity in the lesion (arrow).

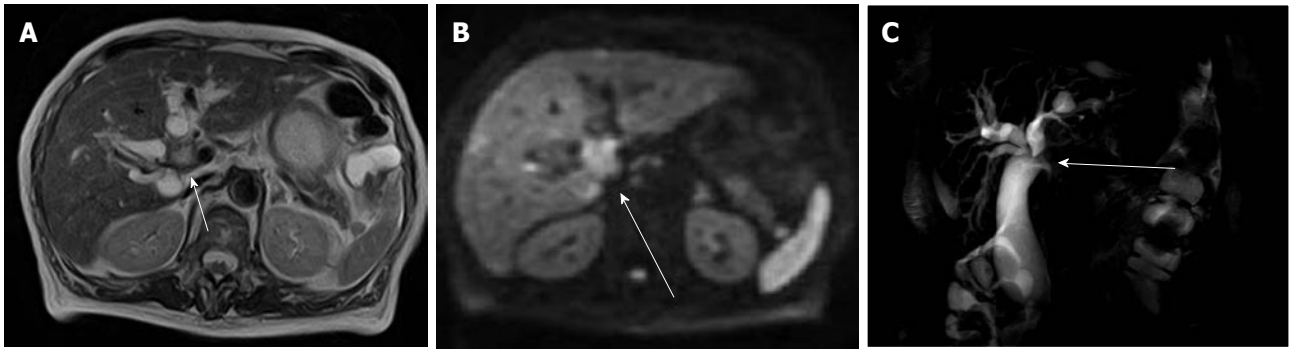

Figure 7 Hilar bile duct cancer in an 84-year-old woman. A: Axial T2-weighted image shows wall thickening and high signal intensity of hilar bile duct (arrow); B: Diffusion-weighted imaging shows high signal intensity in the lesion (arrow); C: Magnetic resonance cholangiopancreatography shows occlusion of the hilar bile duct (arrow).

and incomplete concentric pooling of contrast material on dynamic studies[13,70-72]. The MRI appearances depend on the degree of fibrosis, coagulative necrosis, cell debris, and mucin production. Capsular retraction, bile duct dilatation distal to the tumor, vascular encasement, and central scar have been also reported.

Several researchers have reported that the use of hepatocyte-specific contrast agent (Gd-EOB-DTPA) may aid in the diagnosis of intrahepatic cholangiocarcinoma[73-76]. They have shown that Gd-EOB-DTPA enhanced images displayed increased lesion conspicuity and better delineation of daughter nodules and intrahepatic metastases. Other researchers have reported that DWI may be also useful for detection of bile duct cancers[77,78] (Figure 6).

Extrahepatic biliary cancer: Extrahepatic biliary carcinomas are divided into hilar, also called Klatskin tumors, and distal tumors. Hilar tumors represent approximately 60%-70% and distal tumors 20%-30%[4,5]. The most common pattern of tumor growth is focal infiltration of the ductal wall or the periductal-infiltrating type, resulting in focal strictures. The mass-forming and intraductal-growing types are less common[13].

The role of MRI is to detect and characterize the tumor, and determine respectability. On cross-sectional MRI, the lesion appears ill-defined, and moderately hypo- to isointense on T1-weighted images and mildly iso- to hyperintense on T2-weighted images as compared to adjacent liver parenchyma.

Hilar bile duct cancers are most commonly of the infiltrative type and less frequently exophytic or polypoid lesions[13,14]. Many studies have reported that MRI, including MRCP, is useful in the staging of perihilar bile duct cancers[79-84] (Figure 7). MRI cannot assess tumor in stented ducts[81,82]. Minimal invasion along the mucosa and in the perineural space may escape detection if it is below the limit of resolution[82,83].

Distal extrahepatic cholangiocarcinomas are most commonly of the infiltrative type and grow intramurally, beneath the bile duct epithelium. The accuracy of MRCP is reported to be comparable to that of ERCP for differentiating extrahepatic bile duct carcinoma from benign stricture[60,85-92]. Although some overlap exists, in general the presence of a long segment of extrahepatic bile duct stricture with irregular margins and asymmetric narrowing is suggestive of cholangiocarcinoma, whereas a short segment with regular margins and symmetric narrowing indicates a benign cause[87]. The addition of a contrast-enhanced dynamic study to evaluate the longitudinal tumor extent of bile duct cancers is controversial. One report has shown favorable results, but another report showed no improvement in diagnostic accuracy[93,94].

Several researchers have reported on the utility of

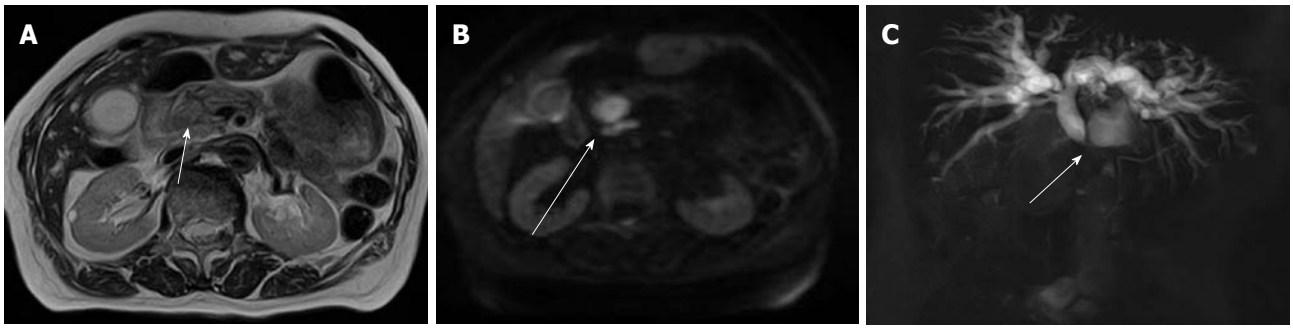

Figure 8 Distal extrahepatic cholangiocarcinoma in an 83-year-old woman. A: Axial T2-weighted image shows wall thickening and slight high mass of the distal common bile duct (arrow); B: Diffusion-weighted imaging shows high signal intensity in the lesion (arrow); C: Magnetic resonance cholangiopancreatography shows occlusion of the distal common bile duct (arrow).

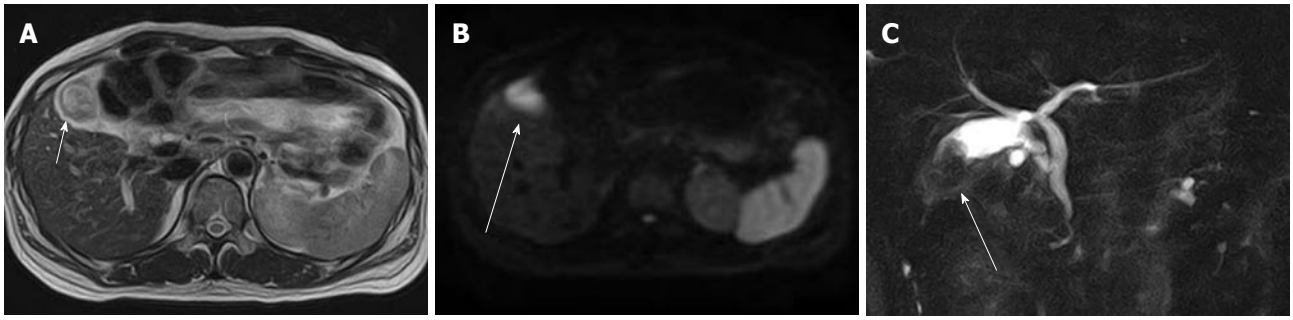

Figure 9 Gallbladder carcinoma in a 56-year-old woman. A: Axial T2-weighted image shows focal wall thickening (arrow); B: Diffusion-weighted imaging shows high signal intensity in the lesion (arrow); C: Magnetic resonance cholangiopancreatography shows a filling defect in the gallbladder (arrow). Abnormal arrangement of the pancreato-biliary ductal system is identified.

DWI in these lesions, and it may play an important role in the diagnosis of extrahepatic tumors[95,96] (Figure 8).

Gallbladder cancer

Primary carcinoma of the gallbladder is the most common malignancy of the biliary tract. Spread of gallbladder carcinoma to the liver is common due to the thinness of the gallbladder's smooth muscular layer and the proximity to the liver, allowing spread to lymphatic channels[97-101]. Gallbladder carcinomas exhibit 3 typical patterns: polypoid, mural thickening, and diffusely infiltrative[102]. Nearly 70% of gallbladder carcinoma present as diffusely infiltrative lesions[97].

Usually, US is used as an initial diagnostic modality. As a second step, CT, MRI with MRCP, and/or traditional cholangiography is often used for obtaining additional information. Comparative studies of CT and MRI with MRCP are desirable.

The role of MRI is to characterize the tumor, and determine respectability[103,104]. Gallbladder carcinoma usually exhibits low to intermediate signal intensity on T1-weighted sequences and heterogenous hyperintensity on T2-weighted sequences with a characteristically ill-defined contour[105]. In the polypoid and mural thickening types, lesion more than 10 mm in diameter or which enhance after intravenous contrast material, are usually malignant. The diffusely infiltrative type, the tumor appears as a large solid mass in the gallbladder fossa, obscuring the gallbladder. The presence of gallstones within the mass may be helpful in making the diagnosis. In tumor staging, differentiation between stage T1 (lesions confined to the muscular layer) and stage T2 (lesions confined to subserosal or perimuscular connective tissue) is important, because vastly different operative procedures used depending on the stage. Yoshimitsu *et al*[101] have reported that submucosal enhancement on a delayed phase dynamic MRI study is a useful sign for differentiating between the stages.

Several researchers have showed that DWI may be useful in the diagnosis of gallbladder carcinoma[106-109] (Figure 9). The sensitivity and specificity of conventional MRI alone was 74% and 68%-83%, respectively; these values increased when DWI was used along with conventional MRI[24].

Ampullary cancer

Ampullary carcinoma tends to appear as small mass that causes biliary obstruction. Although CT and MRI are used to evaluate ampullary carcinoma, it is difficult to diagnose because of the small tumors and difficulty of differentiating between the tumors and surrounding normal structure. MRI, including MRCP, has been reported to be more accurate than CT[110,111]. MRI in ampullary carcinoma has a high sensitivity and low specificity[112]. EUS and ERCP are usually used to identify ampullary carcinoma.

Histologically, most ampullary carcinoma develop

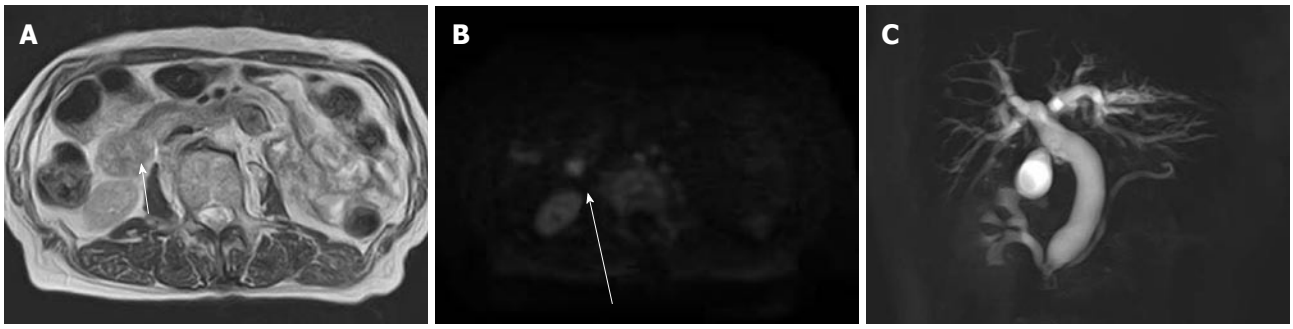

Figure 10 Ampullary cancer in an 84-year-old woman. A: T2-weighted axial image shows a focal mass in the ampullary region (arrow); B: Diffusion-weighted imaging shows high signal intensity within an ampullary cancer (arrow); C: Magnetic resonance cholangiopancreatography shows marked dilatation of the bile duct and slight dilatation of the main pancreatic duct.

from 1 of 2 types of epithelium, resulting in an intestinal-type adenocarcinoma arising from the intestinal epithelium lining the duodenal papilla and pancreaticobiliary-type adenocarcinoma developing from the biliary epithelium of the ampullary portion. The subtypes of ampullary tumors have different prognoses. Chung *et al*[113] have shown MRI may be helpful in determining the subtypes of ampullary tumors.

Several studies have reported that DWI has the potential for differentiating malignant ampullary tumors from benign ampullary tumors[114,115]. Researchers have reported that malignant tumors have a low ADC value compared to that of benign tumors (Figure 10).

CONCLUSION

MRI is a promising non-invasive imaging technique for evaluating biliary lesions. MRI can be used for diagnosis, tumor characterization, preoperative planning, and follow-up of malignant biliary lesions.

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REVIEW

Life cycle and pathogenesis of hepatitis D virus: A review

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Abstract

Hepatitis D virus (HDV) is a defective RNA virus which requires the help of hepatitis B virus (HBV) virus for its replication and assembly of new virions. HDV genome contains only one actively transcribed open reading frame which encodes for two isoforms of hepatitis delta antigen. Post-translational modifications of small and large delta antigens (S-HDAg and L-HDAg) involving phosphorylation and isoprenylation respectively confer these antigens their specific properties. S-HDAg is required for the initiation of the viral genome replication, whereas L-HDAg serves as a principal inhibitor of replication and is essential for the assembly of new virion particles. Immune mediation has usually been implicated in HDV-associated liver damage. The pathogenesis of HDV mainly involves interferon-α signaling inhibition, HDV-specific T-lymphocyte activation and cytokine responses, and tumor necrosis factor-alpha and nuclear factor kappa B signaling. Due to limited protein coding capacity, HDV makes use of host cellular proteins to accomplish their life cycle processes, including transcription, replication, post-transcriptional and translational modifications. This intimate host-pathogen interaction significantly alters cell proteome and is associated with an augmented expression of pro-inflammatory, growth and anti-apoptotic factors which explains severe necroinflammation and increased cell survival and an early progression to hepatocellular carcinoma in HDV patients. The understanding of the process of viral replication, HBV-HDV interactions, and etio-pathogenesis of the severe course of HDV infection is helpful in identifying the potential therapeutic targets in the virus life cycle for the prophylaxis and treatment of HDV infection and complications.

Key words: Hepatitis B virus; Hepatitis D virus; Hepatitis delta antigens; Virus replication; Host-pathogen interactions; Hepatitis D virus pathogenicity; Interferon-alpha; Hepatocellular carcinoma

Core tip: A scarcity of literature about the hepatitis D virus (HDV) life cycle and pathogenesis is clearly evident. Severity of HDV associated liver disease and lack of an efficient treatment regime therefore warrant urgent research into HDV biology. Virus entry inhibitors and isoprenylation inhibitors may play a role in preventing HDV superinfection and liver cirrhosis. As inhibition of interferon-α signaling by HDV plays a pivotal role in failure to clear the virus, testing interferon-free treatment regimens should undergo clinical trials. A low degree of heterogeneity in hepatitis delta antigen encourages the development of a vaccine using its immunogenic sequences.

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INTRODUCTION

The smallest virus known to infect humans, hepatitis delta virus (HDV), is increasingly again becoming a cause of

fulminant hepatitis or a more rapid progression of liver disease in the setting of chronic hepatitis B virus (HBV) infection[1]. HDV is a defective satellite RNA virus which requires the helper function of HBV for its replication and assembly of new virions[2]. An estimated 15-20 million individuals with HBV worldwide are found infected with HDV[2], highlighting a need to exactly understand the pathogenesis and molecular biology of the virus.

GENOMIC STRUCTURE AND TAXONOMIC CLASSIFICATION

HDV is a small, spherical virus with a diameter of about 36 nm[1,3]. The viral genome is a circular, single-stranded, negative sense RNA molecule with an internal core delta antigen surrounded by an envelope derived from HBV surface proteins[4,5]. The genomic RNA of HDV is composed of approximately 1700 nucleotides, packaged with about 200 molecules of hepatitis delta antigen (HDAg)[6] to form a single viral particle. Due to extensive base-pairing within the RNA molecule, the genome appears as a double-stranded rod like structure and thus it remarkably resembles viroids (plant pathogens)[1,7]. However, unlike viroids, the HDV has more nucleotides in its genome compared to 200-400 nucleotides in a viroid RNA. Furthermore, viroids lack protein coding ability whereas HDV codes for its own HDAg and requires HBV for its propagation[7]. The envelope surrounds the genome and HDAg composed of all three HBV envelope proteins, namely small hepatitis B surface antigen (S-HBsAg), medium HBsAg and large HBsAg. The HDV genome also exhibits a self-cleavage activity by encoding a ribozyme domain about 80-100 nucleotides long. HDV seems to be a unique animal virus because of its high GC nucleotide content and circle rolling mechanism of replication[8-10]. The virus is currently assigned the separate genus deltavirus and yet awaits a complete taxonomic classification, including order and family.

Unlike most RNA viruses, HDV does not encode its own replicase or RNA-dependant RNA polymerase to replicate its genome. Rather, it makes use of cellular RNA polymerases which are DNA-dependant RNA polymerases. Since HDV genomic RNA has negative or anti-messenger polarity, during replication three different forms of RNA are being made: circular genomic RNA, circular complementary anti-genomic RNA and a linear anti-genomic RNA through a circle rolling mechanism. The circle rolling mechanism involves unidirectional replication of nucleic acids to form multiple copies of circular genome using cellular RNA polymerases[11]. Linear polyadenylated anti-genomic RNA serves as messenger RNA (mRNA) to produce HDAg.

Since HDV is presented with a limited protein coding capacity encoding only one HDAg with two isoforms, it thus makes use of host cellular machinery (cellular proteins) to accomplish the processes which are essential for its life cycle, such as transcription, replication, post-transcriptional and translational modifications[9,12].

HDV ANTIGEN

HDV genome has been found to contain several open reading frames (ORFs)[13]. Out of all the open reading frames, only one appears to be actively transcribed and encodes for antigen (HDAg)[5], the function of the rest of the ORFs is still unknown. There are two isoforms of HDAg, small HDAg 24 kDa (S-HDAg) composed of 195 amino acids and large HDAg 27 kDa comprising of 214 amino acids (L-HDAg). The open reading frame transcribes into a mRNA using host cell RNA polymerase Ⅱ which translates to produce S-HDAg. A post-transcriptional modification by the cellular enzyme adenosine deaminase-1 (ADAR 1) replaces the stop codon (UAG at position 196) on the mRNA by a tryptophan (codon UGG), extending the reading frame by an additional 19 amino acids leading to the production of L-HDAg[5,11,14]. The nineteen extra amino acids added at the carboxyl terminal of L-HDAg confer it functional properties that are different from S-HDAg. S-HDAg is required for the initiation of the viral genome replication, whereas L-HDAg which is synthesized in the late stage of viral replication serves as a principal inhibitor of replication and is essential for the assembly of new virion particles[8,9]. L-HDAg not only regulates HDV genome replication but also its own synthesis by inhibiting viral replication which prevents editing of amber/W site necessary for the expression of L-HDAg[15].

The HDAg contains different functional domains, such as RNA-binding domain, coiled-coil sequence and nuclear localization sequence. The L-HDAg in addition contains a few more domains which include virus assembly signal (VAS) and nuclear export signal[8,9]. VAS in the L-HDAg renders it obligate for virion assembly[16]. HDAg may directly activate transcription of the viral genome by binding to RNA. HDAg may also facilitate transcription elongation by replacing transcription repressor bound to RNA polymerase II[8,9,16]. In the absence of HBsAg, both the S-HDAg and L-HDAg tend to localize in the nucleus as they carry nuclear localization signals. Thus, HBsAg is essential for cytoplasmic translocation of L-HDAg as only L-HDAg bears nuclear export signal for the purpose of its established role in virion assembly[17].

POST-TRANSLATIONAL MODIFICATIONS OF HDAG

The post-translational modifications of HDAg have been reported[8,7,18] and are of immense importance as these may modulate HDAg function and may lead to progression of the viral cycle[17]. These post-translational modifications include serine and threonine phosphorylation, lysine acetylation, arginine methylation, lysine sumoylation and cysteine farnesylation[9,12]. Since HDV is deficient in enzymes responsible for post-translational modifications, HDV precisely depends upon cellular proteins to accomplish these processes.

Phosphorylation occurs at the serine and threonine residues of S-HDAg, whereas only serine residues are phosphorylated in L-HDAg[9,19]. Post-translational phosphorylation of S-HDAg at serine-177 is crucial for its interaction with the cellular RNA polymerase Ⅱ[20], which is responsible for genomic HDV RNA synthesis (HDV antigenome RNA replication). Phosphorylation of S-HDAg at serine-177 and phosphorylation at serine-2 residue[21] therefore enhances HDV replication. Furthermore, RNA-binding activity of HDAg has found to be mediated by phosphorylational modification and thus seems essential for viral replication[21].

Acetylation of lysine residues of both the S-HDAg and L-HDAg have been reported and are being associated with modulation of HDV replication. Mu *et al*[22] found that a substitution of acetylated lysine-72 of S-HDAg by an alanine re-localized the mutant S-HDAg into the cytoplasm and was associated with the diminished viral RNA accumulation and earlier L-HDAg appearance. Methylation of the arginine residue of S-HDAg has been observed to influence HDV replication[17,18] and thus methylation inhibitors result in an inhibition of HDV replication.

Sumoylation is a newly known post-translational modification which involves the conjugation of S-HDAg with a small ubiquitin-related modifier isoform-1 (small ubiquitin-like protein), results in sumoylation of multiple lysine residues in S-HDAg and an enhanced genomic RNA and mRNA synthesis with no effect on antigenomic transcription[9,23].

Isoprenylation is the most important modification which results in farnesylation of the cysteine-211 which resides in the isoprenylation signal sequence located at the carboxyl terminal of L-HDAg. Farnesylated L-HDAg inhibits viral RNA replication and facilitates virion assembly by mediating the direct binding between L-HDAg and HBV envelope proteins[9]. Farnesyl inhibitors, although in their early phase of development, may improve disease outcome.

CELL ATTACHMENT, ENTRY, UNCOATING AND REPLICATION

The mechanism of entry of the HDV into the hepatocytes is not clearly understood, however, it is thought to be similar to HBV. HDV enters hepatocytes by binding to the carbohydrate side chains of heparin sulphate proteoglycan present on the surface of hepatocytes[24]. The N-terminal aminoacids of the pre-S1 domain of L-HBsAg are thus obligatory to HDV entry into hepatocytes. Mutations/deletions in highly conserved pre-S1 sequence and acetylation or myristoylation of pre-S1 N-terminal amino acids have been found to inhibit HDV entry into hepatocytes[25]. Recently, Yan *et al*[26] have identified a putative receptor for the entry of HBV and HDV into the hepatocytes. The authors proposed that pre-S1 domain of L-HBsAg interacts with sodium-taurocholate cotransporting polypeptide, an integral transmembrane glycoprotein involved in enterohepatic circulation, to facilitate HDV infection.

After HDV enters the cell, the uncoating of viral particle occurs and HDAg translocates the viral genome into the nucleus where RNA polymerases I and Ⅱ are employed to replicate the genome (Figure 1). Polymerase I involves the transcription of antigenome from viral genome in the nucleolus, while polymerase Ⅱ catalyzes genome replication from antigenome and transcription of mRNA in the nucleoplasm[27].

The process of replication starts with the transcription of antigenome using the viral genome through a circle rolling mechanism which produces an antigenomic RNA of more than one unit length. The antigenomic RNA is then self-cleaved by intrinsic ribozyme activity and ligated to form a circular antigenome using cellular ligases. The antigenomic RNA is then used to produce genomic RNA in the nucleoplasm. The mRNA is transcribed using the same genomic transcript and is translated to produce HDAg. It is therefore evident that HBV plays no role in HDV replication and it can proceed even in the absence of the helper virus. It is required only for cell entry, virion assembly and export.

PATHOGENESIS

HDV replicates only in the hepatocytes. The cellular damage associated with HDV infection thus involves mainly the liver. Immune-mediated liver damage is thought to be implicated in HDV infection[28]. However, data from experimental chimpanzees has also suggested a direct cytopathic effect of HDV on hepatocytes, particularly in acute hepatitis setting[29-32]. It is postulated that in acute HDV infection, infected hepatocytes undergo degenerative changes characterized by shrunken eosinophilic cytoplasm and pyknotic nuclei as well as the presence of minimal inflammatory cells in the liver parenchyma, consistent with cytopathic hepatocellular damage. These findings are also evident from *in vitro* (cell culture system)[33] and human studies[34,35]. Small delta antigen expressed by infected hepatocytes is thought to be responsible for this direct cytopathic effect of HDV[33], while large delta antigen per se is non-cytotoxic, promotes persistence of HDV (chronicity) and makes hepatocytes susceptible to immune-mediated damage.

Experimental woodchuck models have proven very helpful in furthering our knowledge of HDV pathogenesis and the chronicity associated with HDV superinfection[36,37], owing to the marked resemblance between the course of disease in woodchuck models and the outcome of HDV superinfection in humans. In addition, these models are also invaluable for testing the efficacy and protective role of new treatments for HDV, including vaccine candidates. Studies on these experimental models have disclosed that both the protein immunization and DNA immunization for HDV are insignificant in protecting against HDV superinfection[38,39], highlighting the need of adopting different approaches to de-

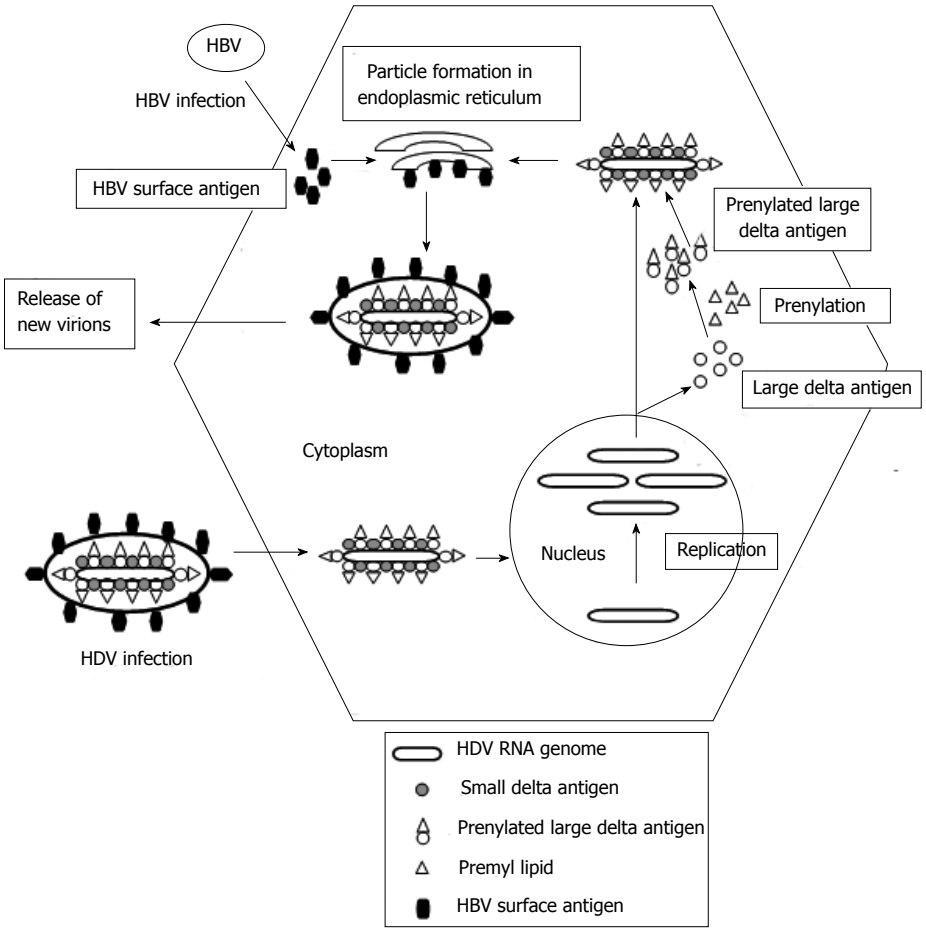

Figure 1 Hepatitis D virus replication cycle. HBV: Hepatitis B virus; HDV: Hepatitis D virus.

velop an HDV vaccine.

Variation in immune-mediated responses during acute and chronic HDV infection has been noticed[37,40], which may explain the persistence and chronicity of HDV superinfection. Cytotoxic T lymphocytes are mainly responsible for clearing the virus by destroying HDV-infected cells. Delayed and insufficient immune response with ability of recognizing only limited viral epitopes has been implicated in failure to clear the infection coupled with establishment of chronic infection. Fulminant hepatic failure has been observed in 1% of HBV/HDV co-infected patients while in 5% of those superinfected with HDV. An exaggerated immune response, particularly a cell-mediated one, is proposed to be involved in causing massive hepatocyte necrosis and liver damage in fulminant hepatic failure[41,42].

The pathogenesis of HDV is also thought to be influenced by the interaction of HDV with the HBV[28,43], which has not yet been clearly elucidated by investigating bodies. HDV infection is known to occur either as a coinfection or a superinfection. A coinfection with HBV/HDV usually eradicates both the organisms and often results in complete recovery, while a superinfection frequently progresses into chronic hepatitis D infection[44]. Patients with chronic hepatitis B who develop superinfection with hepatitis D may also go into acute on chronic liver failure, leading to ascites and hepatic encephalopathy.

HBV-HDV INTERACTION

HBV coinfection with other hepatitis viruses is associated with various patterns of reciprocal inhibition of viral replication. HDV has been frequently shown to suppress HBV replication[45]. L-HDAg up-regulates the myxovirus resistance-A transcription, an interferon-inducible antiviral response mediator which is involved in suppression of HBV replication. It is therefore suggested that the liver disease in HBV/HDV superinfection is mainly due to HDV[8]. Chronic HDV/HBV infection causes a more severe liver disease than HBV monoinfection alone; the disease runs a rapidly progressive course, leading to early cirrhosis, decompensation and hepatocellular carcinoma (HCC), and a shorter 5 year survival[46].

Suppression of HBV replication by HDV is not sustained for an overall period of the disease as the viral response changes over time[47]. One study revealed significant HBV replication in about half of the patients[48]. Taken together, three phases of chronic hepatitis D have been proposed: (1) early active phase with active HDV replication and suppression of HBV; (2) a second moderately active one with decreasing HDV and reactivating HBV; and (3) late phase with the development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or with remission resulting from the marked reduction of both viruses[49].

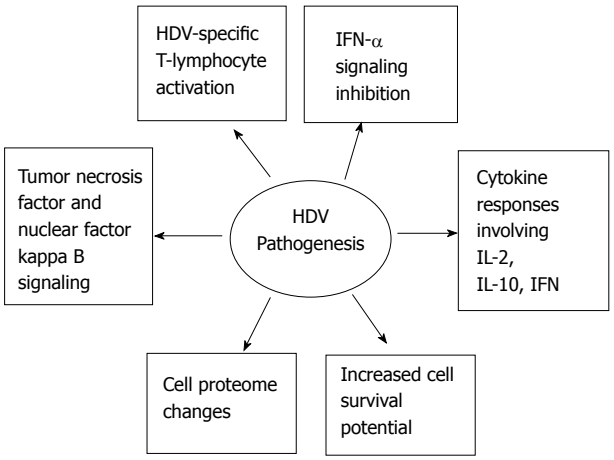

Figure 2 Depicts hepatitis D virus pathogenesis. HDV: Hepatitis D virus; IL: Interleukin; IFN: Involves interferon.

INTERFERON-ALPHA SIGNALING INHIBITION

The pathogenesis of HDV mainly involves interferon-α (IFN-α) signaling inhibition[50], HDV-specific T-lymphocyte activation and cytokine responses[51,52,53], tumor necrosis factor-alpha (TNF-α) and nuclear factor kappa B signaling[54,55], together with modifications in cell proteome and an associated increased cell survival potential[56] (Figure 2).

IFN-α signaling by the virus-infected cells to warn their neighboring cells of a viral presence is a first line of defense of the host to eradicate viruses. IFN-α is induced by the double-stranded RNA presented during viral replication[57]. The IFNs thus produced exert their effect by binding to α and β-IFN receptors on the cell surface, resulting in activation of the tyrosine kinases of the janus kinase (JAK) family which in turn phosphorylate tyrosine residues of the cytoplasmic transcription factors acting as signal transducer and activator of transcription (STAT). Activation of JAK/STAT signaling pathway stimulates the expression of IFN-induced genes. The IFN-α-stimulated genes then code for the antiviral proteins, namely myxovirus resistance A, double-stranded RNA (dsRNA)-activated protein kinase and 2',5'-oligoadenylate synthetase which, in turn, mount an antiviral response[58]. The resultant activated non-specific innate and specific acquired immune responses help combat the viral infection, but this is not the case in the setting of HDV infection.

Since HDV is composed of a single-stranded RNA molecule, it is not expected that it would stimulate IFN-α release[59]. On the other hand, interferon-alpha signaling activation has shown to be inhibited in HDV infection[50]. HDV averts tyrosine kinase-2 (tyr-2) activation preventing phosphorylation of STAT-1 and STAT-2 and their intra-nuclear translocation for the expression of IFN-induced genes. HDV thus interferes with the activation of JAK/STAT signaling pathway by IFN-α signaling inhibition[50] which may be implicated in viral persistence and treatment failure (Figure 3).

IFN-α is also known to stimulate the cellular enzyme ADAR 1 and thus L-HDAg expression by increased editing of mRNA[60]. IFN-α signaling failure in HDV infection may prevent an early production of L-HDAg and cessation of viral replication. From the above description, it could be concluded that inhibition of IFN-α signaling in an HDV infected individual plays a pivotal role in failure to clear the virus and also confers resistance to IFN-α treatment. Babiker *et al*[61] tested an interferon-sparing antiviral treatment regime consisting of tenofovir disoproxil fumarate and lamivudine in a patient with severe acute HDV infection. Successful suppression of HDV RNA was achieved after 16 mo of treatment, along with significant reductions in HBV DNA and HBsAg levels. The interferon-sparing regimens should undergo more clinical trials to establish their efficacy for the treatment of hepatitis D.

T-CELL RESPONSES

It is already established that the pathogenesis of liver injury in HDV infection is not directly cytopathic but immune-mediated mechanisms are known to be involved. After the entry of virus into hepatocytes, its antigen is processed in endoplasmic reticulum of cell cytoplasm and presented on the cell surface in association with major histocompatibility complex- I (MHC- I) protein. $CD8^+$ cytotoxic T lymphocytes recognize endogenously synthesized antigen presented in association with class- I MHC proteins and kill the virus infected cell by two mechanisms: release of perforins and granzymes and an interaction between FAS receptor and FAS ligand. Both of these mechanisms lead to DNA fragmentation and apoptosis of the target cells[62].

Secondly, exogenous antigens and noninfectious viral particles are endocytosed by the surrounding antigen-presenting cells (APCs) (which include macrophages, B lymphocytes and dendritic cells) which present the antigens on their surface in association with class Ⅱ MHC proteins. $CD4^+$ helper T-cells recognize antigens when they are presented in association with class Ⅱ MHC proteins. A virus-specific clone of helper T-cells is thus activated and starts proliferating to bring about clonal expansion in order to clear the viral infection. Proliferating helper T-cells are categorized into three subtypes: type 0 (Th-0), Th-1 and Th-2 T-cells on the basis of their functions and cytokines they produce. Thus, an HDV-specific activated clone of helper T-cell is the key component around which the pathogenesis of HDV revolves[52].

CYTOKINES

The pathogenesis of HDV involves activation of a clone of HDV-specific helper T-cell which, in turn, expresses cytokines, mainly interleukin-2 (IL-2), IL-2 receptor, IL-10 and IFN-γ[51,52,63]. IL-2, being a T-cell growth and differentiation factor, stimulates both HDV-specific help-

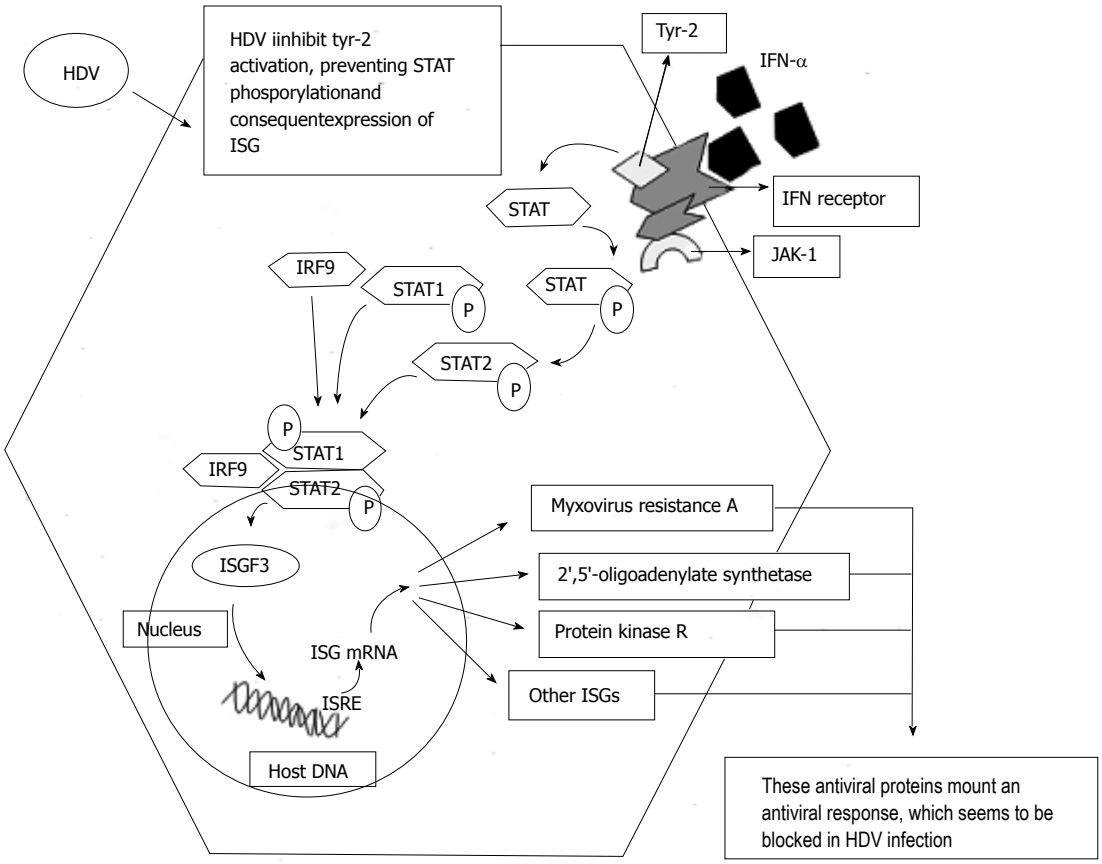

Figure 3 Interferon-α signaling and inhibition by hepatitis D virus. HDV: Hepatitis D virus; INF: Interferon; STAT: Signal transducer and activator of transcription; JAK: Janus kinase; IRF9: Interferon regulatory factor 9; ISGF3: INF-stimulated gene factor 3; ISG: INF stimulated genes; ISRE: INF-stimulated response elements; P: Phosphorylated residues.

er T-cell and $CD8^+$ cytotoxic T-cell to proliferate and undergo clonal expansion. Cytotoxic T-cells destroy virus-infected cells while the activated helper T-cells under the influence of IL-12 develop into subtype Th-1 cells and specifically secrete IFN-γ.

In HDV infection, HDV-specific Th-1 and cytotoxic T-cells have been shown to produce a large amount of IFN-γ[52] which stimulates phagocytosis and killing by macrophages and up-regulates the expression of class-Ⅰ and class-Ⅱ MHC proteins on cell surfaces. IFN-γ may inhibit viral replication directly or from its immune-modulatory and immune-stimulatory effects[51]. IFN-γ also stimulates the secretion of IFN-γ induced protein-10 (CXCL-10), a chemoattractant which recruits natural killer cells (NK cells), T-cells, macrophages/monocytes and dendritic cells to destroy HDV-infected cells with the consequent viral clearance at the cost of inflammatory infiltrates causing liver damage[61].

It is also postulated that in chronic hepatitis, production of IFN-γ by the Th-1 helper T-cells induces hepatocytes to express class-Ⅱ MHC protein in addition to class-Ⅰ MHC protein in an attempt to increase their capacity to clear the infection[53]. This may be associated with severe liver necrosis and increased severity of hepatitis in HDV- infected patients as more uninfected hepatocytes expressing MHC-Ⅱ protein will also be recognized and killed by helper T and cytotoxic T-cells.

Since HDV-specific $CD4^+$ T-cell response has been identified in HDV infected patients in response to HDAg, there are specific sequences on HDAg identified which are being recognized and processed by APCs[52] and presented on the cell surface in association with MHC-Ⅱ protein to mount an HDV-specific antiviral response by $CD4^+$ helper T-cells. Nisini *et al*[52] thus employed synthetic peptides spanning the entire HDAg sequence in an attempt to determine HDAg-specific helper T-cell recognition of specific antigenic determinants. They studied their fine specificity to identify immunogenic epitopes of HDAg that could be used to generate a vaccine for the prophylaxis and treatment of HDV infection[52]. Furthermore, two studies have identified a low degree of heterogeneity in HDAg which encourage the development of a vaccine using these immunogenic sequences of HDAg[64,65].

The study results of Nisini *et al*[52] also suggested that HDAg-specific T-cell response in peripheral blood of HDV-infected individuals is associated with reduced HDV replication and anti-HD IgM disappearance with the consequent reduced activity of HDV-induced liver disease[52].

A vigorous immune response involving HDAg-specific T-cell response and cytotoxic killing of HDV-infected cells following HDV infection thus results in both viral clearance and augmented liver damage.

CELL PROTEOME MODIFICATIONS AND ROLE OF CELLULAR PROTEINS

Since HDV lacks essential enzymes to carry out its own replication and cell cycle, host cell proteins and components are thus intimately involved in the HDV pathogenesis [9,12].

There are a number of cellular proteins identified to date which interact with HDV RNA and HDAg to accomplish HDV life cycle processes. The interaction of cellular proteins with HDV RNA and HDAg significantly alters cell proteome. Cellular RNA polymerase subunits, helicases, RNA-binding proteins, heterogeneous ribonucleoproteins (hnRNPs) and transcriptional and splicing factors have been known to interact with S-HDAg to facilitate HDV RNA transcription and translation. Host transcription factors interacting with HDAg may remarkably alter cellular gene expression resulting in enhanced cytokines, inflammatory enzymes, growth factors and anti-apoptotic proteins[9], which may suggest severe necroinflammation, amplified liver damage and a concomitent increased cell survival in HDV infected patients.

Cellular proteins involved in pathways, such as regulation of cell metabolism and energy pathways, nucleic acid and protein metabolism, apoptosis and cell growth and maintenance, demonstrate a significantly altered expression profile in the presence of HDV components.

HDV AND CIRRHOSIS

Progression of liver disease in HDV infection has been demonstrated to be influenced by many factors, including mode of infection (*i.e.*, coinfection or superinfection), specific HDAg variants[66] and HDV[67] and HBV[68] genotypes. Superinfection with HDV in chronic HBV is associated with a more severe form of liver disease owing to its exacerbation of pre-existing liver damage due to HBV[69]. HBV genotypes have much less convincing evidence of directly influencing liver pathology, rather it regulates HDV viral loads which adversely affect disease outcome[70].

Since HDV infection causes fulminant hepatitis and liver cirrhosis, L-HDAg has been shown to stimulate transforming growth factor-β (TGF-β) and c-Jun-induced signaling cascades which in turn may induce epithelial-mesenchymal transition and fibrogenesis[70,71]. Chronic HDV may thus induce liver cirrhosis by up-regulating the expression of TGF-β. This process is specifically accomplished by the isoprenylation (farnesylationn) of L-HDAg[72]. Isoprenylation inhibitors, which are still in their early phase of development, may play a key role in preventing these undesirable outcomes following HDV infection.

HDV AND HEPATOCELLULAR CARCINOMA

It has already been mentioned that cells infected with HDV appear to have altered gene expression and cellular responses, which is also evident from augmented expression of pro-inflammatory, growth and anti-apoptotic factors. It is thus explanatory that severe liver damage and a concomitant increased hepatic cell survival in HDV-infected patients may lead to HCC.

It is well known that nuclear factor kappa B (NF-κB) dysregulation is associated with inflammation and cancer[73]. L-HDAg has been shown to activate nicotinamide adenosine denucleotide hydro-phosphoric acid oxidase-4 which in turn induces oxidative stress. L-HDAg is therefore able to activate the signal transducer and activator of transcription-3 and the NF-κB through the oxidative stress pathway[54]. L-HDAg may also stimulate TNF-α induced NF-κB, probably *via* TNF receptor-associated factor 2 (TRAF2), a protein involved in the early signal transduction events[56]. This may underscore a possible underlying cause of severe necroinflammation in HDV infection and its progression to HCC. A clinical study has also suggested that HCC in HDV infection may be a secondary effect of the necroinflammation and cirrhosis[74]. In this study, decreased liver size was noticed more in cases of HDV HCC compared to HBV monoinfection group where the liver size was normal or increased. HDV patients had lower platelets and larger varices on endoscopy.

Both L-HDAg and S-HDAg have been shown to enhance clusterin gene expression[56] which is found up-regulated in tumor cells and evidently implicated in tumorigenesis[75]. HDAg amplifies the expression of clusterin gene by enhanced acetylation of histone H3 in clusterin gene promoter region. The similar modification has also been observed in several oncogenic viruses associated with the expression of specific proteins, such as the adenovirus protein E1A[76], the simian virus 40T antigen[77] and the E7 protein of the human papilloma virus[72]. An increased histone acetylation and clusterin protein production are associated with increased survival of HDV-infected cells[56]. This may reliably be implicated in the development of HCC in HDV-infected patients.

RELEVANCE OF GENOTYPES

To date, there are eight genotypes of HDV which have been reported with unexplained variations in their pathogenecity. Furthermore, HDV genotypes have a distinct geographical distribution, apart from HDV genotype 1 which has been observed universally[47].

The available literature is apparently unable to outline a specific HDV genotype associated with the disease severity in HDV infection as different genotypes are prevalent in different parts of the world and none could be correlated with more severe disease outcome with certainty. Any geographical area which reports HDV associated disease severity primarily belongs to the genotypes prevalent in that area. This entity thus requires further research to establish an association between disease severity and different HDV genotype infections. However, Su *et al*[78] have reported genotype I HDV and older age

associated with adverse outcomes in HDV/HBV infection. There are patterns of disease suggesting cytopathic viral illness, as was indicated by outbreaks of severe hepatitis in the northern part of South America[79]. These cases were mostly caused by HDV genotype 3.

CONCLUSION

Although a global decline in HDV prevalence has now been observed due to better prevention practices, mass awareness and expanded HBV vaccination in industrialized countries, it is still a major health concern in the Asia-Pacific region where circumstances favor the spread of hepatitis B and related infections. A scarcity of literature about the HDV life cycle and pathogenesis is clearly evident. Furthermore, severity of HDV-associated liver disease with its adverse outcomes and lack of an efficient treatment regime encourage research into HDV biology. A better understanding of the viral pathogenesis will certainly help the investigating bodies to develop new treatment approaches which would be able to cope with both disease severity and outcome. An innovative approach towards generating a vaccine against HDV is also needed.

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MINIREVIEWS

New compounds able to control hepatic cholesterol metabolism: Is it possible to avoid statin treatment in aged people?

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Abstract

Aging is characterized by the loss of homeostasis that leads to changes in the biochemical composition of tissues, reduced ability to respond adaptively to environmental stimuli, and increased susceptibility and vulnerability to diseases including coronary artery diseases, carotid artery disease and brain vessel disease. Hypercholesterolemia is one of the primary risk factors for these pathologies, whose incidence is highly related to aging. Almost 25% of men and 42% of women older than 65 years have a serum total cholesterol level greater than 240 mg/dL. The mechanisms behind this age-related increase in plasma cholesterol are still incompletely understood, thus, the control of plasma cholesterol content in aged people is more challenging than in adults. In this review the different pharmacological approaches to reduce plasma cholesterol levels, particularly in aged people, will be discussed. In brief, current therapies are mostly based on the prescription of statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) that are pretty effective but that exert several side effects. More attention should be given to potential drug interactions, potential age-related changes in drug pharmacokinetics, adverse effects such as myopathy and competing risks when statins are prescribed to old patients. In combination or in alternative to statin therapy, other agents might be required to reduce low density lipoprotein (LDL) cholesterol levels. Among the available drugs, the most commonly prescribed are those addressed to reduce cholesterol absorption, to modulate lipoprotein lipase activity and bile acid sequestrants: even these pharmacological interventions are not exempt from side effects. The use of antioxidants or organoselenium compounds and the discovery of new proteins able to modulate exclusively LDL receptor recycling such as Proprotein convertase subtilisin kexin 9 and SEC24 offer new pharmacological approaches to selectively reduce the main causes of dyslipidemia.

Key words: Aging; Cholesterol; Hypercholesterolemia

Core tip: The strategies used to reduce plasma cholesterol levels in elderly people are mainly addressed to the inhibition of the rate limiting enzyme of cholesterol biosynthetic pathway, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), in order to increase low density lipoprotein (LDL) receptor membrane exposure and LDL clearance from the circulation. Indeed current therapies are mostly based on the prescription of statins (HMGR inhibitors) that are pretty effective but that exert side effects. More attention should be given to potential drug interactions, potential age-related changes in drug pharmacokinetics, adverse effects such as myopathy and competing risks when statins are prescribed to elderly. Thus, new therapeutic agents should be taken into account.

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INTRODUCTION

Aging is characterized by the loss of homeostasis that leads to changes in the biochemical composition of tissues, reduced ability to respond adaptively to environmental stimuli, and increased susceptibility and vulnerability to diseases including coronary artery, carotid artery, and brain vessel diseases[1-3].

Hypercholesterolemia is one of the primary risk factors for these pathologies, whose incidence is highly related to aging. Almost 25% of men and 42% of women older than 65 years have a serum total cholesterol level greater than 240 mg/dL[4]. The mechanisms behind this age-related increase in plasma cholesterol are still incompletely understood. Of particular interest are the findings that indicate a gradual decline in the fractional clearance of low density lipoprotein (LDL) from the circulation[5-7] and a reduced expression of hepatic LDL receptor (LDLr) with increasing age in some species[8,9]. Experimental data show that aging significantly rises secretion rates of biliary lipids (bile salt, cholesterol and phospholipid), and bile cholesterol content as well as sizes and hydrophobicity indices of the bile salt pool[10]. The age-related disruption of cholesterol metabolism could be caused by the progressive decline and perturbation in homeostasis maintenance that occur in aging[11].

Cholesterol biosynthesis is a tightly regulated metabolic pathway that employs multiple feedback mechanisms to maintain homeostasis. Over the past several decades, much work has focused on the regulation of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), which catalyzes the conversion of HMG-CoA to mevalonate through a four-electron oxidoreduction. This reaction is the rate-limiting step in the synthesis of cholesterol and other isoprenoids such as dolichol, ubiquinone, and prenyls[12].

HMGR is highly regulated. Short-term regulation is achieved by phosphorylation/dephosphorylation reactions exerted by adenosine monophosphate (AMP)-activated kinase (AMPK) and protein phosphatase 2A (PP2A), respectively. Long-term regulation concerns the modulation of HMGR protein levels by several factors, among others Sterol Regulatory Element Binding Protein (SREBP) and Insulin- induced genes (Insigs), which can affect enzyme transcription, degradation and cholesterol uptake by LDLr as a function of an intracellular sterol amount[13].

CHOLESTEROL HOMEOSTASIS REGULATION DURING AGING

Experimental studies on aged rats revealed an increased plasma cholesterol and a sustained hepatic cholesterol biosynthesis, with a full activation of HMGR which results to be completely dephosphorylated[14]. The mechanisms underlying this dysregulation appear to be gender-dependent.

In aged-male rats, the full activation of HMGR observed in aging has been associated with a rise in reactive oxygen species (ROS)[15]. The proposed model is that high ROS levels induce both p38 and AMPK activation. In turn, p38 causes an increased association between PP2A and HMGR, leading to HMGR dephosphorylation and full activation. AMPK phosphorylating activity on HMGR is impaired by the enhanced association of PP2A and HMGR. H_2O_2-treatment confirms that the effect induced by ROS on HMGR dephosphorylation is mediated by the activation of the p38/MAPK pathway in HepG2 cell line[16].

On the contrary, studies performed on estropausal rats, in which estrogen levels are decreased, suggest that the menopause-related increase in HMGR activity is caused by the decreased activation of AMPK observed upon estrogen deficiency[17,18].

HMGR is an attractive target for the treatment of hypercholesterolemia. Indeed a decrease of intracellular cholesterol synthesis leads to a homeostatic response which induces the up-regulation of cell-surface receptors that bind atherogenic lipoproteins such as LDL and VLDL. The reduction of plasma lipoproteins accounts for the clinical utility of HMGR inhibitors, such as statins. Despite their worldwide use and their beneficial effects, statins can cause myopathy characterized by weakness, pain, elevated serum creatine phosphokinase (CK) and, to a lesser extent, rhabdomyolysis, which is a life-threatening condition[19] Elderly patients are especially vulnerable to side effects of statins: this issue as well as the lack of certainty about their efficacy in aging, can affect the prescription of these medications.

Indeed the Heart Protection Study, PROSPER, and SAGE illustrate the benefit of statin treatment on coronary or surrogate end points in higher-risk elderly patients, although the data is still somewhat controversial, particularly for individuals $\geq$ 80 years of age. An observational study of acute-care hospitals in the United States identified no association between statin use at discharge and improved survival rate in acute myocardial infarction patients $\geq$ 80 years of age, although it did find benefit in patients < 80 years of age[20].

Thus an optimal management of cholesterol should take into account the mechanisms at the root of cholesterol metabolism disruption in elderly people, the risk-benefit ratio of statin use and the effects exerted by alternative medications prescribed to reduce hypercholesterolemia. These different pharmacological approaches will be examined in the review.

INHIBITORS OF CHOLESTEROL SYNTHESIS:

Statins

Statins inhibit competitively HMGR activity impairing

HMG-CoA binding. The bulky hydrophobic statins occupy the HMG-binding pocket and part of the CoA cleft[21]. On HMGR inhibition, statins reduce intracellular hepatic cholesterol biosynthesis and decrease intracellular cholesterol accumulation. The reduction of the intracellular cholesterol levels stimulates, *via* SREBP activation, the synthesis of LDLr and their expression on the cell surface. These receptors are responsible for the uptake of LDL and of their precursors, the very LDLs (VLDL) and VLDL remnants, whose hydrolysis produces LDL. The effect of statins on VLDL also explains their role in reducing triglyceride levels[22]. Moreover, hyperlipidemic patients show an increase of Na^+/Li^+ countertransport activity which is rescued by statin treatment[23-25]. As mentioned above, although the statin therapy is generally well tolerated, the most frequent adverse effect is represented by myopathy. To avoid statin side effects, studies have been performed on genetic polymorphisms that can change statin tolerance and efficacy[25-28].

Statin-associated myopathy is characterized by a broad spectrum of symptoms, ranging from benign myalgia up to life-threatening rhabdomyolysis[22], a syndrome characterized by massive muscle necrosis, with the subsequent release of potassium and other ions into the plasma compartment, and severe myoglobinuria, which may cause damages to kidneys and other organs. Rhabdomyolysis may be accompanied by arrhythmias, acute renal failure, and cardiac arrest. These side effects could be ascribable to statins themselves or to the inhibition of some HMGR end products such as prenyls and ubiquinone[29,30].

In order to avoid statin side effects that older people are more likely to develop, new compounds have been tested to reduce cholesterol synthesis through the inhibition of enzymes (squalene synthase, squalene epoxidase, and oxidosqualene cyclase) downstream the farnesyl pyrophosphate branch point of cholesterol biosynthetic pathway[19]. Nevertheless no compounds have yet entered clinical trials.

ANTIOXIDANTS

ω-3 fatty acids

Martini and coworkers demonstrated that a diet supplemented with ω-3 fatty acids completely prevents the age-related hypercholesterolemia in 24-mo old rats by exerting a powerful antioxidant activity: the reduction of intracellular ROS content due to the supplementation of ω-3 fatty acids totally prevented the activation of p38/MAPK responsible for PP2A association with HMGR and the consequent activation of the enzyme. The proper HMGR activation state promotes plasma cholesterol maintenance at physiological levels by completely preventing age-related hypercholesterolemia[16,31].

Resveratrol

Resveratrol (*trans*-3,5,4'-trihydroxystilbene) is a naturally occurring polyphenol present in red wine and berries[32]. *In vivo* studies have revealed that red wine polyphenols are able to inhibit atherosclerotic progression. Cho *et al*[33] demonstrated that resveratrol reduces cholesterol synthesis inhibiting both the activity of hepatic HMGR similarly to atorvastatin, and the expression of HMGR mRNA[33].

Flavonoids

Flavonoids are ubiquitous compounds, occurring in various plants and their derivatives such as tea, herbs, citrus fruits and red wine; many of them have been shown to be strong free radical scavengers and antioxidants. Several epidemiological studies have supported the hypothesis that the antioxidant actions of flavonoids may reduce the risk of developing cardiovascular diseases[34].

Punithavati and Prince reported a significant increase in the activity of HMGR in plasma and liver of isoproterenol treated rats. The increased lipid peroxidation induced by the treatment, enhanced the activity of HMGR, which in turn led to an excessive production and accumulation of cholesterol. Pre-treatment with the flavonoids quercetin and α-tocopherol (vitamin E) normalized the activity of HMGR. Thus, the observed decrease in HMGR after quercetin and α-tocopherol administration in rats might be due to the inhibition of lipid peroxidation[35].

Naringenin is the aglycone of naringin, a naturally occurring flavanone glycoside obtained from citrus fruits and grapefruit. Naringenin hypocholesterolemic effect is due to the reduction of both HMGR and acyl CoA: cholesterol O-acyltransferase activities[36].

Discovery of isoflavones as HMGR inhibitors in soy food is very intriguing. Kinetic studies showed that isoflavones inhibit HMGR by binding the enzyme active site. Therefore isoflavones may exert steric hindrance between HMGR and its substrates through hydrophobic interactions[37].

Tocochromanols

Tocochromanols are a group of amphipathic, lipid-soluble organic molecules composed of a polar moiety derived from tyrosine and a hydrophobic polyprenyl side chain originating from the isoprenoid pathway. Tocochromanols with a phytyl-derived side chain are termed tocopherols whereas those with a geranylgeranyl derived side chain are termed tocotrienols. Some studies clearly demonstrate that tocotrienols are able to inhibit HMGR activity in guinea pigs and chickens. Moreover γ and δ tocotrienols stimulate HMGR ubiquitination and degradation and inhibit SREBP processing[38,39].

Coumarins

Coumarins are an elite class of oxygen heterocycles that show a wide variety of biological effects. They are present in many plants, fungi and bacteria and have found for centuries application in the traditional medicine[40]. Coumarins are extremely variable in structure; their biological activities are influenced by the various types of substitutions in their basic structure which consists of fused benzene and α-pyrone rings. Coumarins and their derivatives

have attracted intense interest in recent years because of their diverse pharmacological properties: many coumarin derivatives have the special ability to scavenge reactive ROS and to influence processes involving free radical injury. In particular both esculetin and a novel synthetic 4-methylcoumarin, 4-methylesculetin (ESC), possess two hydroxyl moieties on their benzene rings, and these were the two most effective radical scavengers among the coumarins selected for a structure-activity relationship study in cell culture[41]. Beyond its antioxidant activity, ESC is able to lower HMGR activity reducing PP2A protein levels leading to HMGR hyperphosphorylation. ESC was demonstrated to lower HMGR protein levels through reduced transcriptional and increased degradational events in HepG2 cells[42].

Organoselenium compounds: Diphenyl diselenide's case

Besides enhancing memory in a menopause model in rats[43,44] and decreasing the depressive-like behavior in ovariectomized mice submitted to subchronic stress[43], diphenyl diselenide [$(PhSe)_2$], an organoselenium compound, has been reported to reduce hypercholesterolemia in cholesterol-fed rabbits[45] and in hyperlipidemic Triton WR-1339-induced mice[46]. Moreover, $(PhSe)_2$ was shown to inhibit human LDL oxidation *in vitro*[45], to reduce foam atherosclerotic lesion in hypercholesterolemic LDL receptor knockout (*LDLr* -/-) mice, to decrease infiltration of inflammatory cells in vessel-wall, and to prevent the upregulation of the proatherogenic monocyte chemoattractant protein-1[47]. Recent results demonstrated that $(PhSe)_2$ is able to increase HMGR phosphorylation/inactivation and LDLr protein levels without directly inhibiting HMGR activity[1].

The lack of any evidence in literature about the potential side effects of antioxidant compounds in elderly people, and the abundance of data highlighting their beneficial effects on ROS production and, as a consequence on lipid profile, make the above mentioned compounds eligible for therapeutical interventions addressed to inhibit the mechanisms underlying hypercholesterolemia in the elderly.

INHIBITORS OF CHOLESTEROL ABSORPTION: FOCUS ON EZETIMIBE

An alternative approach to the inhibition of cholesterol biosynthesis consists on the limitation of cholesterol absorption: to this aim the compound Ezetimide was and is currently used in treatments against hypercholesterolemia.

Ezetimide, or 1-(4-fluorophenyl)-(3R)-[3-(4-fluorophenyl)-(3S)-hydroxyprophyl]-(4S)-(4-hydroxyphenyl)-(2-azetidinone), inhibits intestinal cholesterol absorption by selectively blocking in the jejunal brush border the Niemann-Pick C1-like 1 protein (NPC1L1), the human sterol transport protein that was expressed at the enterocyte/gut lumen (apical) as well as in the hepatobiliary (canalicular) interface[48]. Current evidence points to the NPC1L1 protein working in conjunction with the adaptor protein 2 (AP2) complex and clathrin to facilitate internalization of free cholesterol into the enterocyte. Cholesterol in the gut lumen or bile incorporates into the cell membrane, where it can bind to NPC1L1. The NPC1L1/cholesterol complex is internalized or endocytosed by joining to AP2 clathrin, creating a vesicle complex that then translocates with the help of myosin along microfilaments in the cytosol to a storage endosome called the endocytic recycling compartment. When intracellular cholesterol becomes low, NPC1L1 is released from the endocytic recycling compartment and traffics back along microfilaments to the cell membrane[49].

A meta-analysis of eight randomized placebo controlled trials showed that monotherapy with ezetimibe in hypercholesterolemic subjects was associated with a significant reduction of LDL-cholesterol (LDL-C), of total cholesterol, of triglycerides, and with an increase of high density lipoprotein (HDL) compared to placebo[50].

In terms of elevations in liver function tests, ezetimibe appears to cause similar elevations in transaminases as compared to placebo when given as monotherapy. Also, as combination therapy with statins, ezetimibe does not significantly cause an increase in liver enzymes more than it is observed with statin therapy alone. The addition of ezetimibe to statin therapy does not appear to increase the incidence of elevated creatine kinase levels and was not associated to an increased risk of myositis beyond what is noted intreatment with statin alone[51]. Ezetimibe is well tolerated also by elderly individuals and no substantial differences have been observed between young and old people in the drug effects[52]. Among the inhibitors of cholesterol absorption is worth mentioning Orlistat which is prescribed in obese patients improving plasma lipid profile[53]. No studies have been performed on elderly people treated with orlistat[54].

NIACIN

Nicotinic acid and nicotinamide (collectively termed niacin) serve as precursors of co-enzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate (NAD^+ and $NADP^+$) and are water-soluble vitamins of the vitamin B complex. Niacin was one of the first drugs used to treat hyperlipidemia[55].

Several clinical trials have demonstrated that niacin administration, either alone or combined with other lipid lowering agents, significantly reduces total mortality and coronary events, retards progression, and induces regression of coronary atherosclerosis. Following administration, niacin rapidly inhibits adipocyte lipolysis apparently through the inhibition of hepatic diacylglycerol acyltransferase 2: it leads to the inhibition of triglyceride synthesis and to the decrease of apolipoprotein B-containing lipoproteins; this is accompanied by a similarly rapid drop in plasma levels of free fatty acids[56]. The net effect is a reduced catabolism of HDL and a decreased accumulation of cholesterol esters in LDL particles. It

has also been suggested that niacin may directly inhibit the uptake and catabolism of apolipoprotein AI-containing HDL particles, thus acting to further increase plasma levels of HDL[56].

The main problem with the use of niacin is connected to its side effects: administration of pharmacological doses of niacin is accompanied by unwanted effects, primarily a cutaneous reaction called flushing, which occurs in up to 90% of patients. Niacin-induced flushing is mediated primarily by the production of prostaglandins D2 and E2 by dermal/epidermal immune cells, leading to vasodilation of blood vessels and resulting in the symptoms of redness, warmth, tingling and itching[56]. Several gastrointestinal adverse effects, such as nausea, vomiting, dyspepsia and abdominal pain, can also occur. Moreover, the most severe niacin-induced side effect is hepatotoxicity, which is accompanied by an increase in hepatic transaminase levels[11].

Despite evidence for an atheroprotective effect of niacin from previous small clinical studies, the large outcome trials, AIM-HIGH and HPS2-THRIVE did not reveal additional beneficial effects of niacin on top of statin treatment[57]. Thus the administration of this medication especially in elderly people would deserve more attention.

FIBRATES

Fibrates have been used in clinical practice for about half century due to their ability to substantially decrease triglyceride levels and increase HDL, less effects are exerted as LDL lowering agents. All fibrates are peroxisome proliferators-activated receptors (PPARs)α agonists. Fibrates enhance the oxidation of fatty acids in liver and muscle and reduce the rate of hepatic lipogenesis, thereby decreasing the secretion of VLDL. The increased uptake of triglyceride-derived fatty acids in muscle cells results from an increase in lipoprotein lipase (LPL) activity in adjacent capillaries and a decrease in the amount of apolipoprotein CⅢ (Apo CⅢ), which is transcriptionally mediated by PPARα. The decrease in Apo CⅢ reduces the inhibition of LPL activity. The enhanced catabolism of VLDL generates surface remnants, which are transferred to HDL. HDL concentrations are further augmented by an increase in PPARα - mediated transcription of apolipoprotein A Ⅰ (Apo A Ⅰ) and Apo AⅡ. Ultimately, the rate of HDL-mediated reverse cholesterol transport may increase[58]. Although the beneficial effects of fibrates, their use is associated with a slightly increased risk (< 0.0%) for myopathy, cholelithiasis, and venous thrombosis[59]. A study in which a subgroup analysis by age was performed showed a significant decrease in total cardiovascular disease events in patients aged < 5 years, but not in patients aged ⩾ 5 years. Furthermore, in a study of 1568 men with lower extremity arterial disease, bezafibrate had no significant effects on cardiovascular events in patients aged ⩾ 5 years, compared with placebo[60].

APOB100 ANTISENSE OLIGONUCLEOTIDES

The recent development of antisense oligonucleotides (ASOs) that can target a specific mRNA and suppress the translation of its protein in the liver has opened up a novel therapeutic window for reducing levels of atherogenic lipoproteins. Mipomersen (ISIS 301012) an antisense inhibitor directed to human apoB-100, was recently documented in phase 2 clinical trials to lower plasma apoB-100 and LDL cholesterol levels in humans. The side effects exerted by ASOs are mainly associated to transaminase[61]. No studies have been performed to understand if differences exist in the efficacy or in the side-effects exerted by these molecules in adults and aged people.

BILE ACID SEQUESTRANTS

Bile acids are amphiphilic molecules synthesized from cholesterol in the liver; in their transit through the intestinal lumen they emulsify diet fats, aiding in their absorption. Bile acids are then reabsorbed by active ileal uptake and recycled through the enterohepatic circulation. Bile acid sequestrants, such as colestipol, colestyramine and colesevelam, are anion exchange resins being similar in mechanism of binding bile acids in the intestinal lumen.

They interrupt enterohepatic circulation of cholesterol-rich bile acids and increase their fecal excretion, leading to the depletion of intrahepatic cholesterol, which causes LDLr upregulation[62]. Hepatic LDL uptake is thereby raised, resulting in augmented LDL particle clearance and in the reduction of plasma LDL-C by 15% around. However, the side effects of gastrointestinal intolerance including bloating, abdominal pain and constipation, as well as the inhibition of the absorption of medications like levothyroxine or warfarin, reduce patient compliance. Therefore, bile acid sequestrants are often prescribed with other lipid-regulating agents, most commonly statins[63]. None overall differences in safety or effectiveness of bile acid sequestrant administration between aged and young subjects were observed[52].

INHIBITION OF PCSK9

Proprotein convertase subtilisin kexin 9 (PCSK9), is a 72-kDa protease, highly expressed in the liver[64]. nce secreted, PCSK9 binds LDLr inducing the redistribution of the receptor from the cell surface to lysosomes: indeed PCSK9 appears to change the itinerary of the LDLr, diverting internalized LDLr to degradation in lysosomes and preventing them from recycling to the cell surface[64].

PCSK9 inhibition is considered an attractive target for therapy against hypercholesterolemia. The current drug approaches tested to pharmacologically inhibit PCSK9 in humans are mainly focused on gene silencing that targets both PCSK9 intra- and extra-cellular functions, and on mimetic peptides and monoclonal antibod-

ies that exclusively target circulating PCSK9 and therefore its extracellular function. Other approaches such as orally active cell permeable small molecules that target PCSK9 processing have not reached preclinical development[65]. The subcutaneous administration of the PCSK9 ASO produced by different pharmaceutical companies led to an increase of LDLr hepatic expression and to a concomitant reduction in circulating total cholesterol levels in mice[66] and in non-human primates[67]. Peptides mimicking the epidermal growth factor A (EGFA) domain of the LDLr interacting with PCSK9 at the plasma membrane have also been developed to inhibit PCSK9 function. A synthetic EGFA peptide reduced in a dose-dependent manner the cellular degradation of the LDLr induced by exogenously added recombinant PCSK9[68]. Duff *et al*[69] were able to reverse the PCSK9 mediated effect on cell surface LDLr by using antibodies that recognize epitopes on PCSK9 in the vicinity of the region within the catalytic domain interacting with the LDLr. Evaluation of treatments over the long term will determine whether the beneficial effects of PCSK9 inhibition on LDL-C levels will directly translate into coronary artery disease risk reduction[70-72].

Chen *et al*[72] identified PCSK9 as a specific SEC24A-dependent COPII cargo: indeed as trans-membrane and soluble proteins co-translationally inserted into the endoplasmic reticulum (ER), it is packaged into transport vesicles coated with coat protein complex Ⅱ (COPⅡ) for the export from the ER and the delivery to the Golgi for further processing[73].

Chen *et al*[72] reported that complete deficiency of SEC24A is compatible with normal development and survival in mice. However, these animals exhibit markedly reduced plasma cholesterol and increased hepatic LDLr levels due to selective blockade in the secretion of PCSK9. Consistent with these genetic data, hepatic LDLr levels are up-regulated in SEC24A deficient mice as a consequence of a specific dependence of PCSK9 on SEC24A for efficient exit from the ER.

The loss of SEC24A disrupts PCSK9 secretion without affecting other COPⅡ-dependent processes such as SREBP activation. PCSK9 represents the first example of a soluble vertebrate cargo that is differentially regulated by specific interaction with selective components of the COPⅡ machinery. Although complete deficiency of SEC24A in mice is compatible with embryonic development or survival to adulthood, no human patients have yet been identified with genetic deficiencies in any of the four SEC24 paralogous genes. The SEC24A-deficient phenotype suggests a potential role for genetic variation at the SEC24A locus in the control of plasma cholesterol in humans, a key determinant of risk for myocardial infarction and stroke.

Complete deficiency of SEC24A is compatible with survival and normal development in mice, suggesting that pharmacologic inhibition of hepatic SEC24A expression/function to achieve reduction in plasma cholesterol may be well tolerated as a potential approach to inhibit PCSK9 secretion[72].

CONCLUSION

Atherosclerosis is the major cause of heart disease, stroke, and death during aging in both developed and developing countries, for which the rise of lipid levels into the bloodstream represents a primary risk factor. Epidemiological data have indicated that dyslipidemia and coagulation disturbances are among the most remarkable functional alterations leading to the development of atherosclerotic condition.

Oxidative stress has recently been involved in the pathogenesis of several diseases such as atherosclerosis. The production of free radicals has been found to be a major causative factor for the peroxidative damage to plasma lipoprotein which are responsible for the initiation and progression of atherosclerosis in hyperlipidemic subjects[13]. Both the conditions predisposing to artery diseases occur in the elderly which is defined as the series of the deteriorative changes occurring during the adult period of life that underlie increased vulnerability to challenges and decreased survival[12].

The strategies used to reduce plasma cholesterol levels in elderly people are mainly addressed to the inhibition of the rate limiting enzyme of cholesterol biosynthetic pathway, HMGR, in order to increase LDLr membrane exposure and LDL clearance from the circulation. Indeed current therapies are mostly based on the prescription of statins (HMGR inhibitors) that are pretty effective but that exert several side effects. More attention should be given to potential drug interactions, potential age-related changes in drug pharmacokinetics, adverse effects such as myopathy and competing risks when statins are prescribed to old patients. In combination or in alternative to statin therapy, other agents might be required to reduce LDL-C levels. Among the available drugs, the most commonly prescribed are those addressed to reduce cholesterol absorption, to modulate lipoprotein lipase activity and bile acid sequestrants: even these pharmacological interventions are not exempt from side effects. As an example, the efficacy, safety, and tolerability of a nutraceutical-based protocol (containing berberine 500 mg, policosanol 10 mg, red yeast rice 200 mg, folic acid 0.2 mg, coenzyme Q10 2.0 mg, and astaxanthin 0.5 mg) in elderly hypercholesterolemic patients previously intolerant to statins have been recently demonstrated[74].

The use of antioxidants or organoselenium compounds $(PhSe)_2$ and the discovery of new proteins able to modulate exclusively LDLr recycling such as PCSK9 and SEC24 offer new pharmacological approaches to selectively reduce the main causes of dyslipidemia. In order to be effective, pharmacological interventions should be aimed at deleting the causes of hypercholesterolemia considering their age and gender dependence. Indeed, very few research studies are dedicated to elderly (Table 1).

Thus, the selectivity of the different pharmacological targets according to the causes at the root of the pathology could increase the risk/benefit ratio of the prescribed medications. If on one hand elderly people entered epidemiological studies assessing the tolerability

Table 1 Percentage of studies performed on hypocholesterolemic drugs and elderly

Drugs	Number of PubMed papers key word		
	Hypercholesterolemia	Hypercholesterolemia and ageing	
Statins	5980	89	1.48%
Omega3 fatty acids	246	14	6%
Resveratrol	35	2	6%
Flavonoids	229	6	3%
Coumarins	40	0	0%
Organioselenium compounds	4	0	0%
Ezetimibe	658	6	1%
Niacin	397	8	2%
Fibrates	860	9	1%
Bile acid sequestrants	117	0	0%
PCSK9	283	2	1%

and the effectiveness of drugs in this physiological condition, on the other hand researches aimed at evaluating the efficacy of medications in women, and in particular in elderly women are still missing. Thus further studies aimed at evaluating the putative efficacy and safety of the therapeutic approaches in alternative to statin treatment to lower hypercholesterolemia and in turn artery disease risks in elderly people are needed.

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ORIGINAL ARTICLE

Clinical course and prognostic factors of hepatorenal syndrome: A retrospective single-center cohort study

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Abstract

AIM: To investigate clinical and biochemical features of hepatorenal syndrome (HRS), to assess short and long-term survival evaluating potential predictors of early mortality.

METHODS: Sixty-two patients with liver cirrhosis and renal failure, defined as a serum creatinine value > 1.5 mg/dL on at least two measurements within 48 h, admitted to our tertiary referral Unit from 2001 to 201, were retrospectively reviewed. Among them, 33 patients (53.2%) fulfilled the revised criteria of the International Ascites Club for the diagnosis of HRS. Twenty-eight patients were treated with combinations of terlipressin and albumin, two with dopamine and albumin, and three with albumin alone. No patients were suitable for liver transplantation. Complete response was defined as normalization of creatinine levels to less than 1.5 mg/dL, partial response as a decrease of at least 50% but not to less than 1.5 mg/dL, no response as no reduction in creatinine or a decrease of less 50% compared to pre-treatment values. All of the patients were followed up for at least 1 year until January 2013.

RESULTS: HRS type 1 was diagnosed in 15 patients (45.5%). Hepatitis C virus infection was the primary etiology (69.6%), followed by alcohol (15.2%), and cryptogenesis (15.2%). Complete response to therapy was obtained in only 3 cases (9.1%) and partial response in 7 patients (21.2%). Median survival was 30 d (range: 10-274) without significant differences between type 1 and type 2 HRS. By univariate analysis, Child-Pugh class C (P = 0.009), presence of hepatocellular carcinoma (P = 0.04), low serum sodium (P = 0.02), high bilirubin values (P = 0.009) and high Model for End-stage Liver Disease (MELD) score (P = 0.03) were predictive factors of 30-d mortality. By multivariate analysis, only serum sodium < 132 mEq/L (OR = 31.39; P = 0.02) and MELD score > 27 (OR = 18.72; P = 0.01) were independently associated with a survival of less than one month.

CONCLUSION: HRS still has a poor prognosis, even when vasoactive drug therapies are extensively used.

Key words: Hepatorenal syndrome; Liver cirrhosis; Hepatitis C virus; Vasoactive drugs; Mortality

Core tip: Hepatorenal syndrome (HRS) is a life-threatening complication of advanced liver disease. The aims of this study were to investigate the clinical and biochemical features of HRS, to assess short- and long-term survival, and to evaluate the presence of potential predictors of early mortality. Thirty-three patients with

liver cirrhosis and HRS were retrospectively reviewed. Median survival was 30 d. By univariate analysis Child-Pugh class C, hepatocellular carcinoma, low serum sodium, high bilirubin and high Model for End-stage Liver Disease (MELD) score were predictive factors of 30-d mortality. By multivariate analysis, only serum sodium < 132 mEq/L and MELD score > 27 were independently associated with survival of less than one month.

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INTRODUCTION

Hepatorenal syndrome (HRS) is a life-threatening complication of advanced liver cirrhosis. It is characterized by functional renal failure, which develops as a result of portal hypertension, splanchnic vasodilatation and consequential deterioration of all systemic circulatory function[1]. Its incidence is approximately 8% per year in cirrhotic patients with ascites, but its incidence is greater in patients with advanced Child-Pugh scores[2]. The development of portal hypertension is associated with vascular arterial vasodilation in the splanchnic district, as a result of increased local production of nitric oxide and other vasodilators[3]. In the early stages of liver cirrhosis, the reduction of vascular resistance is compensated for by the development of hyperdynamic circulation, characterized by increased cardiac output and heart rate[4]. HRS usually develops during the terminal stages of liver disease, as soon as the hyperdynamic circulation is no longer able to compensate for the relative hypovolemia caused by splanchnic storage of blood[5].

Some recent studies have shown a marked reduction of cardiac output in patients with cirrhosis and HRS, in comparison to patients with cirrhosis without HRS, thus reappraising reduction in cardiac function as an important cofactor in the pathogenesis of HRS[6,7]. Several lines of evidence have finally proved that the results of systemic vasoconstriction can also cause significant hypoperfusion in other organs, such as the skin, muscles, brain and the liver itself. HRS can therefore be better considered a complex syndrome with systemic involvement[8].

There are two well-recognized types of HRS[9]. Type-1 HRS is characterized by rapid progression of renal failure, with doubling of serum creatinine values to greater than 2.5 mg/dL in less than 2 wk. It is often triggered by a precipitating event, mainly bacterial infections, and it is associated with rapid deterioration of circulatory status, with arterial hypotension and multiorgan failure. Type-1 HRS has a poor prognosis, with median survival of only 2 wk, and its main consequence is represented by hepato-renal failure and death. In contrast, type-2 HRS is characterized by gradual renal failure, with a moderate increase in serum creatinine to between 1.5 and 2.5 mg/dL. It represents the natural functional renal failure that develops in patients with End-stage Liver Disease, as a result of the natural history of portal hypertension, and it often does not recognize a specific trigger. Type-2 HRS has a better prognosis compared to type-1, with a median survival of 6 mo, and its principal consequence is represented by refractory ascites[10].

According to the European Association for the Study of the Liver (EASL) Clinical Practice Guidelines, pharmacological therapy with terlipressin plus albumin should be considered as the first-line therapy[11]. However, the prognosis remains poor even when pharmacological therapy is extensively used, and orthotopic liver transplant (OLT) remains the best treatment for HRS.

The aims of our study were to investigate the clinical and biochemical features of HRS, to assess short-term and long-term survival, and to evaluate potential predictors of early mortality.

MATERIALS AND METHODS

All patients with liver cirrhosis and renal failure, defined as a serum creatinine value > 1.5 mg/dL on at least two measurements within 48 h, admitted to our tertiary referral unit from 2001 to 2012, were retrospectively reviewed. Among them, we selected those who fulfilled the revised criteria of the International Ascites Club for the diagnosis of HRS[12]. The criteria were: (1) cirrhosis with ascites; (2) serum creatinine > 1.5 mg/dL; (3) no improvement in serum creatinine (decrease to a level < 1.5 mg/dL) after two days of diuretics withdrawal and volume expansion with albumin (1 g/kg of body weight up to a maximum of 100 g/d); (4) absence of shock; (5) no current or recent treatment with nephrotoxic drugs; and (6) absence of parenchymal kidney disease, as indicated by a urinary protein concentration < 500 mg/d, a urinary red blood cell count < 50 cells per high power field, and normal renal ultrasonography. Type-1 HRS was defined by a large and rapid increase in serum creatinine to final values greater than 2.5 within 2 wk, while type-2 HRS was defined by a slower and more moderate increase in serum creatinine. The following were considered precipitating factors for type-1 HRS: spontaneous bacterial peritonitis and other infections diagnosed by standard methods; gastrointestinal bleeding; acute hepatitis superimposed on cirrhosis; and major surgical procedures.

Terlipressin was administered at a starting dose of between 0.5 and 1 mg every 4 h, increased to a maximum dose of 2 mg every 4 h if there was no reduction in serum creatinine compared to the baseline value by day 3 of therapy. Albumin was administered at an average dose of 40 g/d.

All of the patients were followed up for at least 1 year until January 2013. No patients were suitable for liver transplantation. Complete response was defined as normalization of creatinine levels to less than 1.5 mg/dL, partial response as a decrease of at least 50% but not to less

Table 1 Demographic and clinical features of 33 patients with hepatorenal syndrome *n* (%)

Age (yr), (mean ± SD)	65.9 ± 9.6
Sex	
Male	17 (51.5)
Female	16 (48.5)
Etiology	
HCV	23 (69.6)
Alcohol	5 (15.2)
Cryptogenic	5 (15.2)
Child-Pugh	
B	18 (54.5)
C	15 (45.5)
Esophageal varices	
Absent	4 (14.3)
F1	17 (60.7)
F2	7 (25.0)
Diabetes mellitus	13 (39.4)
Hepatocellular carcinoma	12 (36.4)
Portal Vein Thrombosis	7 (21.2)
HRS	
Type-1	15 (45.5)
Type-2	18 (54.5)

HRS: Hepatorenal syndrome; HCV: Hepatitis C virus.

Table 2 Laboratory features of patients with hepatorenal syndrome at diagnosis

Hemoglobin (g/dL)	10.3 ± 2.2
White blood cells (× 10^3/mmc)	8.5 ± 5.7
Platelets (× 10^3/mmc)	104.8 ± 85.2
BUN (mg/dL)	145.17 ± 61.82
Creatinine (mg/dL)	3.29 ± 1.09
Creatinine clearance	19 (8-25.25)
Sodium (mEq/L)	130.7 ± 5.5
Potassium (mEq/L)	4.94 ± 0.85
Urinary sodium (mEq/L)	6.5 (2-14)
Urinary potassium (mEq/L)	17.5 (11-29)
Total bilirubin (mg/dL)	3.63 (1.63-13.2)
INR	1.47 (1.2-1.62)
MELD score	26 (22-32)

Data are expressed as absolute mean ± SD or median (IQR). IQR: Interquartile range; INR: International normalized ratio; BUN: Blood urea nitrogen; INR: International normalized ratio; MELD: Model for End stage liver disease.

than 1.5 mg/dL, and no response as no reduction in creatinine or a decrease of less 50% compared to pre-treatment values.

Statistical analysis

The results are reported as frequencies, medians or means and standard deviations. Student's *t*-test, the Mann-Whitney and the (χ^2 test were used to compare continuous or categorical variables. Multivariate analysis, including all of the significant baseline variables ($P < 0.05$), was also performed, using binary logistic regression to identify independent predictors of outcomes. For this analysis, continuous variables were categorized using receiver operating characteristic curves. Survival analysis was conducted using the Kaplan-Meier method. The log-rank test was used to compare survival between type-1 and type-2 HRS. All of the statistical analysis was performed using SPSS software, version 20.0 for Macintosh (SPSS Inc., Chicago, IL, United States).

RESULTS

Among 62 patients with liver cirrhosis and acute renal failure, 33 patients (53.2%) fulfilled the revised criteria of the International Ascites Club for the diagnosis of HRS[12]. The main clinical and laboratory characteristics are summarized in Tables 1 and 2. The mean age of the patients was 65.9 years old (range: 39-83), with a comparable sex distribution. Chronic hepatitis C virus infection was the main etiology, and approximately one third of patients were affected by hepatocellular carcinoma (HCC). Type-1 HRS was diagnosed in 15 patients (45.5%).

Twenty-eight patients (84.8%) were treated with terlipressin plus albumin, 2 patients (6.1%) with dopamine and albumin, and 3 patients (9.1%) with albumin alone. The median duration of therapy was 7 d (range: 3-14). All of the patients with partial or no response discontinued treatment within 14 d. No significant adverse events occurred during vasoactive therapy. At the end of treatment, a complete response was obtained in 3 cases (9.1%), one with type-1 and two with type-2 HRS, and partial response was obtained in 7 patients (21.2%), four with type-1 and three with type-2 HRS.

The overall median survival was 30 d (range: 10-274) (Figure 1) without significant differences between type-1 and type-2 HRS ($P = 0.2$ by log-rank test) (Figure 2). Between the two groups of type-1 and type-2 HRS patients, there were no statistically significant differences except for serum creatinine, creatinine clearance, international normalized ratio (INR) values and End-stage Liver Disease (MELD) score (Table 3) (Figure 3).

Comparing the two groups of patients according to 30-d mortality, the variables associated with poorer prognosis were Child-Pugh class C ($P = 0.009$), presence of HCC ($P = 0.04$), low serum sodium ($P = 0.02$), high bilirubin values ($P = 0.009$) and high MELD score ($P = 0.03$) (Table 4). By multivariate analysis, only serum sodium < 132 mEq/L (OR = 31.39; $P = 0.02$) and MELD score > 27 (OR = 18.72; $P = 0.01$) were independently associated with survival of less than one month (Table 5).

DISCUSSION

HRS is a life-threatening complication in patients with advanced liver cirrhosis, and it should always be distinguished from other causes of renal failure. It is not always easy to recognize because there are no specific clinical or laboratory parameters that clearly allow for its diagnosis, so diagnosis is mainly based on the exclusion of other causes of renal failure. Currently, the new criteria of the International Ascites Club are regarded as the gold standard for HRS diagnosis[12]. Nonetheless, a recent study by Salerno *et al*[13] showed that these criteria allow for a correct diagnosis in only two thirds of cases.

According to the EASL Clinical Practice Guidelines,

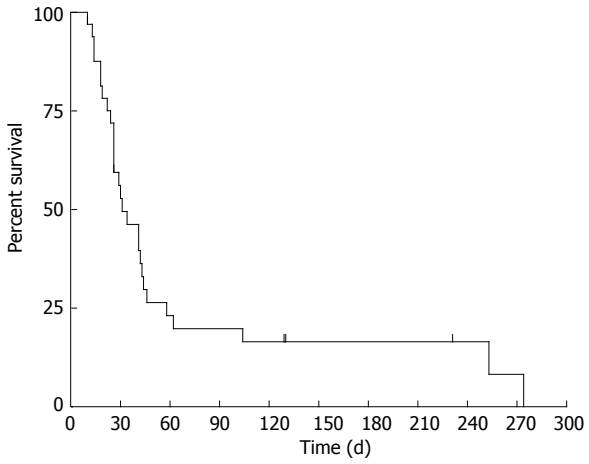

Figure 1 Overall survival of 33 patients with hepatorenal syndrome.

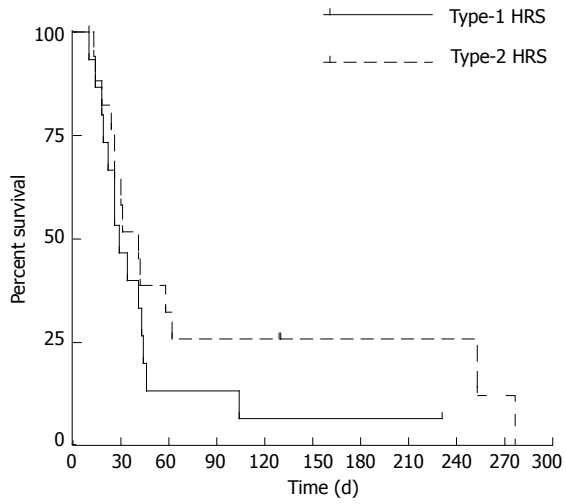

Figure 2 Overall survival of patients according to type-1 and type-2 hepatorenal syndrome (*P* = 0.2 by Log-rank test). HRS: Hepatorenal syndrome.

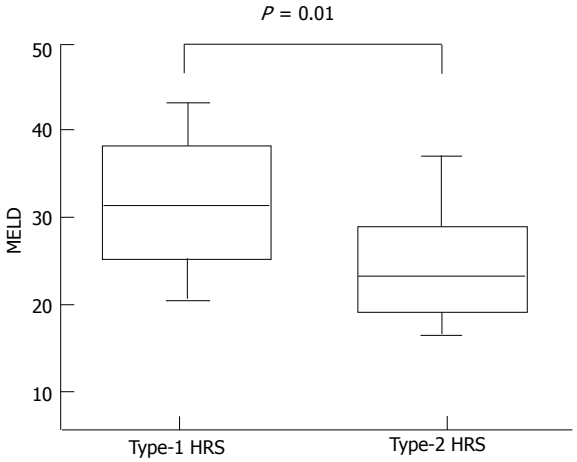

Figure 3 Box plot of distribution of model for end-stage liver disease scores in patients with type-1 and type-2 hepatorenal syndrome at diagnosis. HRS: Hepatorenal syndrome; MELD: Model for end stage liver disease.

Table 3 Comparison between type-1 and type-2 hepatorenal syndrome

	Type-1 HRS (*n* = 15)	Type-2 HRS (*n* = 18)	*P* value
Age (yr)	63.3 ± 8.5	68.2 ± 10.2	0.150
Sex			
Male	8 (53.3)	9 (50.0)	0.800
Female	7 (46.7)	9 (50.0)	
Etiology			
HCV	9 (60.0)	14 (77.8)	0.500
Alcohol	3 (20.0)	2 (11.1)	
Cryptogenic	3 (20.0)	2 (11.1)	
Child-Pugh			
B	8 (53.3)	10 (55.6)	0.900
C	7 (46.7)	8 (44.4)	
Diabetes mellitus	5 (33.3)	8 (44.4)	0.500
Hepatocellular carcinoma	6 (40.0)	6 (33.3)	0.700
Portal vein thrombosis	4 (26.7)	3 (16.7)	0.500
Platelets ($\times 10^3$/mmc)	108.7 ± 96.6	101.6 ± 7711	0.800
BUN (mg/dL)	152 ± 73.4	139.5 ± 51.7	0.500
Creatinine (mg/dL)	3.95 ± 1.13	2.75 ± 0.71	0.001
Creatinine clearance	8 (5.2-17)	24.5 (15-26)	0.030
Sodium (mEq/L)	130.8 ± 4.24	130.7 ± 6.47	0.900
Potassium (mEq/L)	4.9 ± 0.96	4.97 ± 0.78	0.800
Urinary sodium (mEq/L)	11 (2.0-17.7)	6 (2-11)	0.700
Urinary potassium (mEq/L)	19 (7.2-38.2)	16 (11-27.75)	0.900
Total bilirubin (mg/dL)	6.37 (1.6-27.1)	2.62 (1.3-13.2)	0.200
INR	1.51 (1.3-2.2)	1.29 (1.18-1.6)	0.040
MELD score	31 (26-33)	23 (20-26)	0.010

Data are expressed as absolute mean ± SD or median (IQR). IQR: Interquartile range; BUN: Blood urea nitrogen; INR: International normalized ratio; MELD: Model for End stage liver disease.

pharmacological therapy with terlipressin plus albumin should be considered the first-line therapy[11], even if terlipressin is not available in some countries, such as in the United States, and where it is available, there is not a standardized dose, due to the absence of dose-finding studies. However, prognosis remains poor even when pharmacological therapy is extensively used, and OLT remains the best treatment for both type-1 and type-2 HRS.

In our cohort, the prevalence of HRS among patients with liver cirrhosis and renal failure was 53.2%, similar to the incidences found by Salerno *et al*[13] (45.8%) and Martín-Llahí *et al*[14] (43%). However, in contrast to above-cited studies, we found an equal prevalence of type-1 and type-2 HRS (45.5% *vs* 54.5%, respectively). This difference might have been due to the small sample size and to the elevated number of patients with end-stage cirrhosis who are followed in our Unit, who tend to develop mainly type-2 HRS.

Comparing type-1 and type-2 HRS patients, there were no statistically significant differences between the two groups, except for serum creatinine, creatinine clearance, INR values and MELD score. Similarly, we found no differences in survival at 30 d between the groups. A difference in favor of patients with type-2 HRS was observed over 30 d, but the number of patients with survival longer than 30 d was very low, and the difference was not significant. Interestingly, we observed a poor rate of response to vasoactive therapy, although this drug was administered at effective doses and for an

Table 4 Comparison of patients according to 30-d mortality

	< 30-d mortality (*n* = 16)	⩾ 30-d mortality (*n* = 17)	*P* value
Age (yr)	62.9 ± 11.4	68.7 ± 6.6	0.080
Sex			
Male	8 (50.0)	9 (52.9)	0.800
Female	8 (50.0)	8 (47.1)	
Etiology			
HCV	10 (62.5)	13 (76.5)	0.600
Alcohol	3 (18.8)	2 (11.8)	
Cryptogenic	3 (18.8)	2 (11.8)	
Child-Pugh			
B	5 (31.3)	13 (76.5)	0.009
C	11 (68.8)	4 (23.5)	
Esophageal varices			
Absent	2 (14.3)	2 (14.3)	0.900
F1	8 (57.1)	9 (64.3)	
F2	4 (28.6)	3 (21.4)	
Diabetes mellitus	6 (37.5)	7 (41.2)	0.800
Hepatocellular carcinoma	3 (18.8)	9 (52.9)	0.040
Portal vein thrombosis	2 (12.5)	5 (29.4)	0.200
Platelets ($\times 10^3$/mmc)	103.4 ± 87.9	106.1 ± 85.2	0.900
BUN (mg/dL)	134.8 ± 40.5	154.8 ± 76.8	0.300
Creatinine (mg/dL)	3.14 ± 0.8	3.44 ± 1.32	0.400
Creatinine clearance	26 (20-37)	13 (8-24)	0.050
Sodium (mEq/L)	128.5 ± 4.6	132.8 ± 5.5	0.020
Potassium (mEq/L)	4.77 ± 0.87	5.1 ± 0.82	0.200
Urinary sodium (mEq/L)	11 (7-18)	4 (2-13)	0.200
Urinary potassium (mEq/L)	25 (19-29)	15 (10-27)	0.200
Total bilirubin (mg/dL)	10.8 (3.2-21.2)	1.8 (1.5 - 3.6)	0.009
INR	1.6 (1.3-1.7)	1.2 (1.2-1.5)	0.560
MELD score	31 (26-33)	23 (20-26)	0.030
HRS			
Type-1	8 (50)	7 (41.2)	0.600
Type-2	8 (50)	10 (58.8)	

Data are expressed as absolute *n* (%), mean ± SD or median (IQR). IQR: Interquartile range; BUN: Blood urea nitrogen; INR: International normalized ratio; MELD: Model for End stage liver disease; HRS: Hepatorenal syndrome.

Table 5 Risk factors associated with 30-d mortality in patients with hepatorenal syndrome

Variable	< 30-d mortality *n* (%)	⩾ 30-d mortality *n* (%)	Crude OR (95%CI)	Adjusted OR (95%CI)	*P* value
Age, (yr)					
< 65	7 (43.8)	4 (23.5)	1	-	
⩾ 65	9 (56.2)	13 (76.5)	2.53 (0.57-11.26)		
Child-Pugh class					
B	5 (31.2)	13 (76.5)	1	11.98 (0.97-148.23)	0.053
C	11 (68.8)	4 (23.5)	7.15 (1.53-33.37)		
Sodium					
⩾ 132 mEq/L	3 (18.8)	10 (58.8)	1		
< 132 mEq/L	13 (81.2)	7 (41.2)	6.19 (1.27-30.17)	31.39 (1.54-641.83)	0.020
Total bilirubin					
⩽ 3.6 mg/dL	4 (25.0)	12 (70.6)	1		
> 3.6 mg/dL	12 (75.0)	5 (29.4)	7.20 (1.55-33.56)	0.69 (0.17-28.06)	0.800
Hepatocellular carcinoma					
Absent					
Present	13 (81.2)	8 (47.1)	1		
	3 (18.8)	9 (52.9)	4.88 (1.01-23.57)	1.37 (0.13-14.91)	0.700
MELD score					
⩽ 27	4 (25.0)	14 (82.4)	1		
> 27	12 (75.0)	3 (17.6)	14.0 (2.60-75.41)	18.72 (1.63-214.56)	0.010

Crude and adjusted odds ratio (OR) deriving from multiple logistic regression analysis. CI: Confidence interval; MELD: Model for End stage liver disease.

adequate period of time. In particular, a response was observed in only 30.3% of cases (complete response: 9.1%; partial response: 21.2%). These results were lower compared to those observed in the literature[15-21]. In a recent study, Salerno *et al*[13] achieved a response in 50% of cases, with a higher percentage of complete than partial response (30% *vs* 20%, respectively). This finding might be secondary to the differences between the patients in our cohort and those recruited for prospective studies, particularly for randomized controlled trials. In addition, the numbers of patients with hepatocellular carcinoma and with portal vein thrombosis were not specified in Salerno and colleagues' paper; however, patients with advanced HCC and advanced cirrhosis did not receive any treatment. In our cohort, 36.4% of patients had HCC, most of whom were in advanced stages, and 21.2% had a portal vein thrombosis, none of whom were suitable for liver transplantation. Consequently, we likely obtained a worse response to therapy because of the worse pre-treatment prognosis of our patients.

Comparing the two groups of patients with survival shorter and longer than 30 d, Child-Pugh class C, presence of HCC, low serum sodium, high bilirubin values and high MELD scores were associated with lower survival (Table 4). By multivariate analysis, only serum sodium less than 132 mEq/L, and MELD score greater than 27 were independently associated with 30-d mortality.

Overall, these results confirm that prognosis was negatively influenced by the severe impairment of liver function, highlighting the role of MELD score as a prognostic factor in patients with cirrhosis and renal failure, as already described in other studies[22-24].

This study had several limitations. First, the sample size was small. Second, there were a relatively large number of patients with co-morbidities that adversely affected the prognosis, such as hepatocellular carcinoma, as confirmed by the ineligibility of any patients for OLT. Third, none of the patients included were treated with a combination of midodrine and octreotide. This combination would have been useful to compare the outcomes of patients treated with terlipressin to those treated with midodrine and octreotide. Finally, none of the patients underwent liver transplantation, so we were not able to evaluate the impact of vasoactive therapy as a bridge to OLT in our cohort.

HRS still has a poor prognosis, even when drug therapy is extensively used. The impact of vasoactive drugs is poor, and the true effectiveness of these drugs is in prolonging short-term survival, as a bridge to transplan-

tation, in patients suitable for OLT. In this setting, the use of TIPS has been limited by the absence of large, prospective studies and by restricted use in patients with end-stage cirrhosis[25,26]. Prognostic factors for short-term mortality (low serum sodium and high MELD score) could be used to choose candidate patients for OLT as soon as possible after the onset of HRS.

COMMENTS

Background

Hepatorenal syndrome (HRS) is a life-threatening complication of advanced liver cirrhosis. It is characterized by functional renal failure, which develops as a result of portal hypertension, splanchnic vasodilatation and consequential deterioration of all systemic circulatory function.

Research frontiers

HRS still has a poor prognosis, even when vasoactive drug therapies are extensively used. The aims of the study were to investigate the clinical and biochemical features of HRS, to assess short-term and long-term survival, and to evaluate potential predictors of early mortality.

Innovations and breakthroughs

Comparing the two groups of patients with survival shorter and longer than 30 d, Child-Pugh class C, presence of hepatocellular carcinoma, low serum sodium, high bilirubin values and high Model for End-stage Liver Disease (MELD) scores were associated with lower survival. By multivariate analysis, only serum sodium less than 132 mEq/L, and MELD score greater than 27 were independently associated with 30-d mortality.

Applications

The impact of vasoactive drugs is poor, and the true effectiveness of these drugs is in prolonging short-term survival, as a bridge to transplantation, in patients suitable for orthotopic liver transplant (OLT). Prognostic factors for short-term mortality (low serum sodium and high MELD score) could be used to choose candidate patients for OLT as soon as possible after the onset of HRS.

Terminology

HRS is a life-threatening complication in patients with advanced liver cirrhosis, and it should always be distinguished from other causes of renal failure. It is not always easy to recognize because there are no specific clinical or laboratory parameters that clearly allow for its diagnosis, so diagnosis is mainly based on the exclusion of other causes of renal failure. Currently, the new criteria of the International Ascites Club are regarded as the gold standard for HRS diagnosis.

Peer review

In this manuscript, the authors investigated the clinical and biochemical features of HRS, the short and long-term survival of HRS patients, and the potential predictors for early mortality. This manuscript may provide useful information for the clinicians. The data analysis and presentation were appropriate, and the manuscript was well prepared.

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CLINICAL PRACTICE

Acute cytomegalovirus infection in liver transplant recipients: An independent risk for venous thromboembolism

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Abstract

Acute cytomegalovirus (CMV) infection is a commonly encountered complication in the post liver transplant setting. We present a case of a 71-year-old male with acute CMV infection, initially presenting with a gastrointestinal bleed due to acute CMV gastritis and later on complicated by acute venous thromboembolism occurring as an unprovoked event in the post liver transplant period. Traditional risk factors for venous thromboembolism have been well described in the medical literature. Sporadic cases of thromboembolism due to CMV infection in the immune compromised patients have been described, especially in the post kidney transplant patients. Liver transplant recipients are equally prone to CMV infection particularly in the first year after successful transplantation. Venous thromboembolism in this special population is particularly challenging due to the fact that these patients may have persistent thrombocytopenia and anticoagulation may be a challenge for the treating physician. Since liver transplantation is severely and universally limited by the availability of donor organs, we feel that this case report will provide valuable knowledge in the day to day management of these patients, whose clinical needs are complex and require a multidisciplinary approach in their care and management. Evidence and pathophysiology linking both the conditions is presented along with a brief discussion on the management, common scenarios encountered and potential impact in this special group of patients.

Key words: Cytomegalovirus; Venous thromboembolism; Orthotopic liver transplantation; Thrombocytopenia; Hypersplenism

Core tip: Liver transplant recipients are a special group of individuals whose clinical needs are complex due to the use of immunosuppressive agents. They are prone to several opportunistic infections which are not commonly encountered in regular clinical practice. Cytomegalovirus (CMV) infection is a well-recognized complication in the post-transplant setting which can affect the graft function and increase morbidity and mortality. Venous thromboembolism occurring in the setting of acute CMV infection in this group of patients is an important complication and we attempt to delineate the pathophysiology, discuss evidence linking both the conditions and provide practical points in the management of these complex individuals.

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INTRODUCTION

Liver transplantation is a complex surgical procedure requiring extensive evaluation of these patients in the pre-transplant period and specialized care in the post-transplant period. Immunosuppression in these patients is extremely important to prevent graft loss as a result of rejection. Various complications can occur in these patients, especially in the immediate post-operative period and can include technical complications from surgery, rejection of the graft, metabolic derangements, bacterial infections and viral infections from profound immunosuppression. Cytomegalovirus (CMV) infection in the post-transplant setting is a well-recognized complication that can affect graft function and can also predispose these patients to venous thromboembolism. Here we present a case of acute CMV infection, complicated by unprovoked deep vein thrombosis and discuss the evidence, pathophysiology and practical aspects of management.

PRESENTATION OF CASE

A 71-year-old Caucasian male presented to our post transplant clinic at 6 mo post orthotopic liver transplantation (OLT) for follow up. He complained of swelling of the right lower extremity for the last three days. There was no history of trauma. Physical exam revealed swelling of the right leg below knee, ankle edema and warmth with some discomfort to palpation. Acute deep vein thrombosis was suspected and venous doppler confirmed extensive deep vein thrombosis involving the profunda femoris, femoral, popliteal, anterior and posterior tibial and peroneal veins. Patient was admitted to the hospital for further management.

Three weeks prior to this event he was admitted to the hospital with fever, malaise, diarrhea and melena requiring blood transfusion. Upper gastrointestinal endoscopy at that time revealed a clean based gastric ulcer with antral gastritis. Biopsies revealed acute gastritis with CMV inclusion bodies consistent with acute CMV infection (Figure 1). Serologies confirmed CMV IgM positive titres. He was recently taken off valacyclovir prophylaxis for CMV in the post transplant setting per protocol. Thrombophilia work up revealed increased factor Ⅷ levels and decreased protein S activity at 28%. He did not have any risk factors for venous thromboembolism except for recent hospitalization due to gastrointestinal bleed. During that time he did not receive standard venous thromboembolism prophylaxis other than subcutaneous compression devices due to obvious bleeding. We concluded that he most likely had an unprovoked deep vein thrombosis event precipitated by acute CMV infection and performed a literature search to support our hypothesis.

EVIDENCE

A recent large prospective study reported that CMV IgM seropositivity was independently associated with increased short term risk venous thromboembolism (VTE), following adjustment for age and other confounding factors ($P = 0.003$). There was no clinically significant association with arterial thrombosis in the same study[1].

A case control study of immunocompetent patients with VTE revealed CMV IgG antibodies were found more frequently in patients with VTE as compared to controls ($P = 0.016$). The overall rate of CMV IgM antibodies detection was low but they were more often detected in cases as compared to controls. The difference was noted to be statistically significant in patients with an unprovoked VTE event ($P = 0.017$). With the exception of age no association was found between CMV seropositivity and established VTE risk[2].

VTE in renal transplant patients has been reported in a case series of seven patients with simultaneous acute CMV infection with no other obvious risk factors for the same[3]. Sporadic cases of venous and arterial thromboembolism in the setting of acute CMV infection have been reported in both immunocompromised and immunocompetent patients[4].

PATHOPHYSIOLOGY

CMV infection a frequent complication in the post solid organ transplantation setting is known to modify endothelial phenotype from anticoagulant to procoagulant state[3]. Various potential causative mechanisms that may act directly on the vessel wall or indirectly by triggering thrombosis, have been proposed as the reason for CMV associated thrombosis. *IE84* a gene product of CMV binds to and inhibits P53 mediated apoptosis, increasing smooth muscle proliferation and increased risk for thromboembolism[5]. Other mechanisms involve CMV enhanced platelets and leukocyte adhesion to infected endothelial cells, effects mediated by activating factor X, factor Ⅷ and triggering thrombin generation[6]. The most accepted theory supported by several *in-vitrio* studies conclude that CMV transiently induces production of anti-phospholipid antibodies which is a well known risk factor for venous and arterial thromboembolism[7]. The possible role of other factors associating infection with VTE as an independent risk, decreased physical activity and prolonged fever following acute CMV infection leading to dehydration can explain this observation[1]. CMV IgG positive serostatus was linked to an unexplained factor Ⅷ elevation and other procoagulant factors like fibrinogen, which again can trigger thromboembolism[8,9].

CASE CONTINUED

Due to a recent history of upper gastrointestinal bleed from a pyloric channel ulcer and risk for rebleeding with anticoagulation our patient underwent inferior vena cava filter placement. Repeat endoscopy as a follow up for recently diagnosed gastric ulcer due to the need for anticoagulation revealed normal mucosa with complete heal-

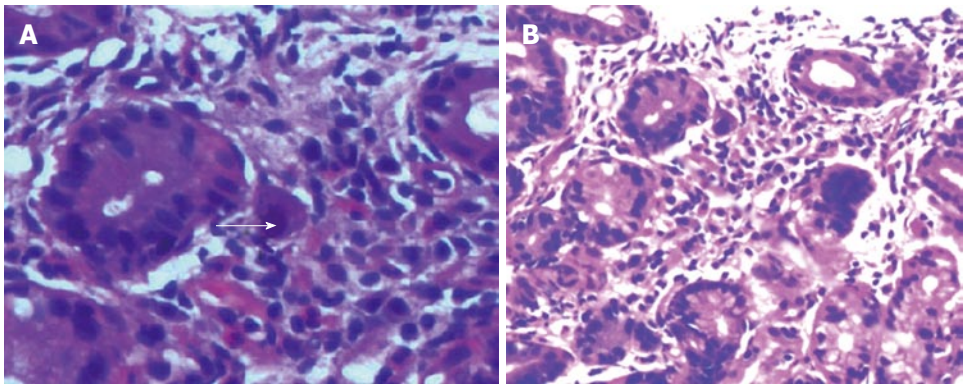

Figure 1 Gastric antral biopsy showing cytomegalovirus. A: High power view, inclusion body (arrow); B: Low power view of cytomegalovirus body.

ing of the pyloric channel ulcer. Due to the extent and severity of VTE, after weighing the risks and benefits of anticoagulation, he was initiated on anticoagulation with warfarin and low molecular weight heparin overlap per standard protocol. For his acute CMV infection he was initiated on oral valacyclovir after initial diagnosis and is symptom free at follow up.

DISCUSSION

Acute CMV infection in the post OLT setting is a well-known complication. It can be asymptomatic or manifest at 2-12 mo post OLT with features that may include fever, malaise, diarrhea, increase in aminotransferases, cytopenias, retinitis and pneumonitis[10]. High risk patients include those who are CMV recipient negative and receive a CMV positive donor liver. It can also result from reactivation of prior infection in a recipient[10].

Patients with end stage liver disease who undergo OLT have severe coagulopathy and thrombocytopenia prior to and in the immediate post-operative period. This usually recovers within the next few days to weeks post transplantation. Thrombocytopenia may persist for several months or may never recover due to severe pre-transplant hypersplenism. Immunosuppressive agents and prophylactic medications for opportunistic infections may contribute to cytopenias, which include persistent thrombocytopenia in the post-transplant period. VTE prophylaxis in these patients with low molecular weight heparin is usually contraindicated due to the high risk for bleeding complications that may affect graft survival and increase morbidity and mortality. These patients are at high risk for hospitalization in the first few months post-transplant due to complications that may include rejection of transplanted organ, surgical, infectious and metabolic complications.

Acute CMV infection in the post OLT setting is routinely encountered and its clear association with VTE should always be considered when the clinical presentation favors or is suspicious for the same. Based on the data provided above and pathophysiological mechanisms that may be involved, there seems to be a clear association between acute CMV infection and the risk for VTE. Several other factors including hospitalization and immobilization may increase the risk of thromboembolic events in this group of patients. Appropriate prophylaxis for VTE with low molecular weight heparin combined with subcutaneous compression devices should be utilized in these patients. Especially during the time of hospitalization in the post OLT period, providing there are no contraindications. Confirmed diagnosis of VTE should be treated aggressively with appropriate anticoagulation and if contraindicated use of vena caval filters is recommended, to prevent the potentially fatal and dreaded complication of pulmonary embolism. Cytomegalovirus infection if confirmed should be treated appropriately using standard guidelines.

CONCLUSION

Liver transplantation is severely limited by the availability of donor livers and the financial burden associated with the whole process is enormous. Transplant hepatologists and surgeons should be vigilant in providing care for this special group of individuals, whose clinical needs are complex and require a multi-disciplinary approach in their management. A high degree of suspicion for common and not so common complications like VTE occurring simultaneously with acute CMV infection should be maintained. Appropriate diagnostic and therapeutic measures should be undertaken in a timely manner so as to prevent the loss of a vital and scarce treatment modality. Future studies looking into the incidence and prevalence of VTE in the post OLT patients, their associations with CMV status before transplant and at the time of occurrence of VTE are warranted to better define the risk and their association.

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CASE REPORT

Hepatocellular carcinoma and synchronous liver metastases from colorectal cancer in cirrhosis: A case report

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Abstract

A 68-year-old Caucasian man with hepatitis C virus-related cirrhosis was admitted to our Unit in February 2010 for a diagnostic evaluation of three centimetric hypoechoic focal liver lesions detected by regular surveillance ultrasound. The subsequent computer tomography (CT) led to a diagnosis of unifocal hepatocellular carcinoma (HCC) in Ⅵ hepatic segment, defined the other two nodules in the Ⅵ and Ⅶ segment as suspected metastases, and showed a luminal narrowing with marked segmental circumferential thickening of the hepatic flexure of the colon. Colonoscopy detected an ulcerated, bleeding and stricturing lesion at the hepatic flexure, which was subsequently defined as adenocarcinoma with a moderate degree of differentiation at histological examination. Finally, ultrasound-guided liver biopsy of the three focal liver lesions confirmed the diagnosis of HCC for the nodule in the Ⅵ segment, and characterized the other two lesions as metastases from colorectal cancer. The patient underwent laparotomic right hemicolectomy with removal of thirty-nine regional lymph nodes (three of them tested positive for metastasis at histological examination), and simultaneous laparotomic radio-frequency ablation of both nodule of HCC and metastases. The option of adjuvant chemotherapy was excluded because of the post-surgical onset of ascites. Abdomen CT and positron emission tomography/CT scans performed after 1, 6 and 12 mo highlighted a complete response to treatments without any radiotracer accumulation. After 18 mo, the patient died due to progressive liver failure. Our experience emphasizes the potential coexistence of two different neoplasms in a cirrhotic liver and the complexity in the proper diagnosis and management of the two tumours.

Key words: Hepatocellular carcinoma; Colorectal cancer; Liver metastases; Cirrhosis

Core tip: A 68-year-old man with hepatitis C virus-related cirrhosis was admitted to our Unit for a diagnostic evaluation of three focal liver lesions detected by regular surveillance ultrasound. Computer tomography scans of abdomen allowed a diagnosis of single nodule hepatocellular carcinoma (HCC) and showed two centimetric liver nodules suspected for metastases and a luminal narrowing with thickening of the colon. The subsequent colonoscopy and ultrasound-guided biopsy of the three focal liver lesions confirmed a diagnosis of colorectal cancer with liver metastases together with a single nodule HCC. Our experience highlights the potential coexistence of two different neoplasms in a cirrhotic liver and the complexity in the proper diagnosis and management of the two tumours.

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INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is currently increasing worldwide, being the leading cause of death in patients with cirrhosis[1,2]. Despite intensive surveillance programs, considerable recent therapeutic advances and the use of potentially radical treatments, prognosis and life expectancy remain poor in this setting[3]. Curative treatments are applicable for early stage tumors only, and include resection, liver transplantation and percutaneous ablation, whereas transarterial chemoembolization and sorafenib are regarded as non-curative treatments able to improve survival in intermediate and advanced stages, respectively[4].

The burden of colorectal cancer (CRC) is huge, since it is the third most common diagnosed cancer in men and the second in women and it is the third leading cause of cancer death in the United States[5]. The liver is the most frequent metastatic site for patients with CRC: up to 50% of patients develop hepatic metastases during the course of the disease[6]. However, few data can be retrieved from the literature about the incidence and management of liver metastases from CRC in patients with cirrhosis.

We describe the coexistence of HCC and liver metastases from CRC in a patient with hepatitis C virus (HCV)-related cirrhosis.

CASE REPORT

A 68-year-old Caucasian man with HCV-related cirrhosis was admitted to our Unit in February 2010 for a diagnostic evaluation of three centimetric hypoechoic focal liver lesions in Ⅵ and Ⅶ segment, detected by surveillance ultrasound. His medical history included type 2 diabetes mellitus requiring insulin treatment and chronic obstructive pulmonary disease diagnosed few years before.

Upon admission the patient was asymptomatic and there was no evidence of hepatic encephalopathy, ascites or peripheral edema. Physical examination was unremarkable, except for hepatosplenomegaly and palmar erythema. Laboratory tests showed microcytic anemia (haemoglobin 9.1 g/dL, haematocrit 30%, mean corpuscular volume 70 fL) and mild hypoalbuminemia (3.2 g/dL), while all other liver function tests were normal. The Child-Pugh score was A6 showing a good residual liver function.

Esophagogastroduodenoscopy detected the presence of medium-size (F2) oesophageal varices and portal hypertensive gastropathy without signs of active bleeding. The subsequent dynamic computer tomography (CT) led to a diagnosis of unifocal HCC in Ⅵ hepatic segment (Figure 1), defined the other two nodules in Ⅵ and Ⅶ segment as suspected metastases (Figure 2), and showed a luminal narrowing with marked segmental circumferential thickening of the hepatic flexure of the colon (Figure 3). Colonoscopy revealed an ulcerated, bleeding and stricturing lesion at the hepatic flexure, which was defined as adenocarcinoma with a moderate degree of differentiation at histological examination. The levels of carcinoembryonic antigen (CEA) and α-fetoprotein were 21.5 ng/mL (NV < 5) and 3.2 ng/mL (NV < 5), respectively.

Ultrasound-guided liver biopsy of the three focal liver lesions confirmed the diagnosis of HCC (grading G2 according to Edmondson-Steiner System) for the nodule in the Ⅵ segment and characterized the other two lesions as metastases from CRC. Finally, CT scans of the chest did not show metastasis. On one hand, considering the good performance status (PS 0), the preserved liver function (Child-Pugh score A6), and the tumour stage (single nodule < 3 cm, absence of vascular invasion), our patient could be allocated in stage A according to Barcelona Clinical Liver Cancer staging system; on the other hand, tumour stage of colon cancer was Ⅳa (T3, Nx, M1) according to Tumor-Node-Metastasis Classification of Malignant Tumours.

The patient underwent right hemicolectomy with removal of thirty-nine regional lymph nodes (three of them tested positive for metastasis at histological examination), and simultaneous laparotomic radio-frequency ablation (RFA) of either nodule of HCC and metastasis. No operative complications occurred. As a probable consequence of surgery, the patient developed hepatic decompensation after about 2 wk, with onset of moderate degree ascites. Consequently, the option of adjuvant chemotherapy was excluded and the patient began oral diuretic treatment with furosemide and canrenone with complete clinical response. Abdomen CT and positron emission tomography/computerized tomography (PET/CT) scans performed after 1, 6 and 12 mo showed a complete response to treatments according to mRECIST[7,8] for HCC, and RECIST 1.1[9] criteria for measurable target lesion of colon cancer, without any radiotracer accumulation. Finally, determinations of CEA were repeatedly negative.

After 18 mo, the patient died due to hepatic decompensation and progressive liver failure.

DISCUSSION

Multiple primary cancers are quite rare. Their incidence ranges from 0.73% to 11.7%[10], even if it is steadily rising as a result of the continuous improvement of treatments and the consequent increased cancer survival. Coexistence of multiple primary tumors is defined by Warren and Gates Criteria, *i.e.,* demonstrating that (1) each tumor is distinct, (2) each tumor is clearly malignant on histological examination, and (3) one of the two tumors is not a metastasis of the other one. In addition, multiple primary tumors are classified into synchronous and metachronous according to Moertel classification[11] if they occur within or after 6 mo since the diagnosis of the first tumor, re-

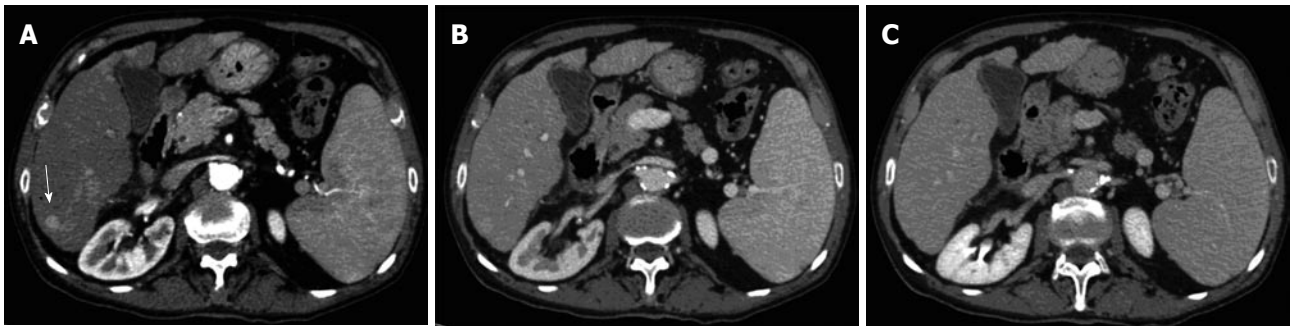

Figure 1 Computer tomography scans of the abdomen performed on admission. A: Arterial phase computer tomography (CT) scan shows small hepatocellular carcinoma nodule with mild enhancement (arrow) in right liver lobe; B: Hepatic venous phase; and C: Delayed phase CT scan at same level shows isoattenuation of lesion to liver parenchyma.

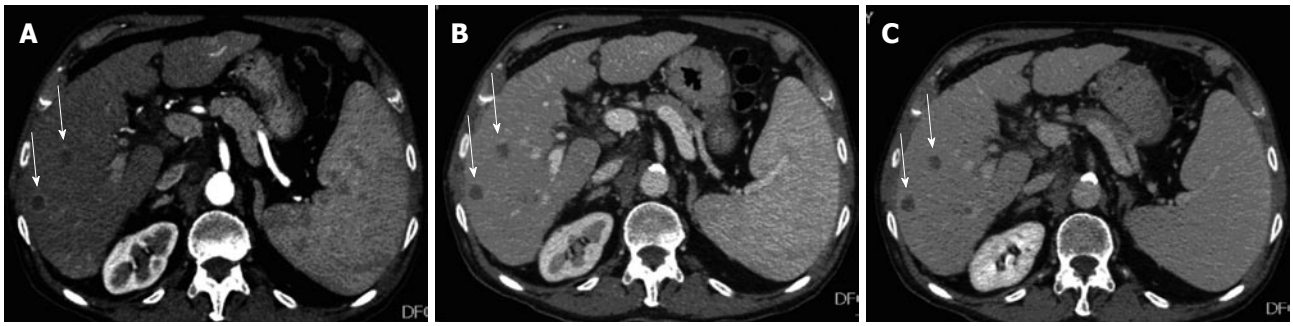

Figure 2 Computer tomography scans of the abdomen performed on admission. A: Arterial phase computer tomography (CT) scan shows intense heterogeneous rim hyperenhancement of two round metastatic lesions in right liver lobe (arrows); B: Hepatic venous phase; and C: Delayed phase and CT scan show persistent rim enhancement around lesions (arrows). Diagnosis was confirmed at biopsy.

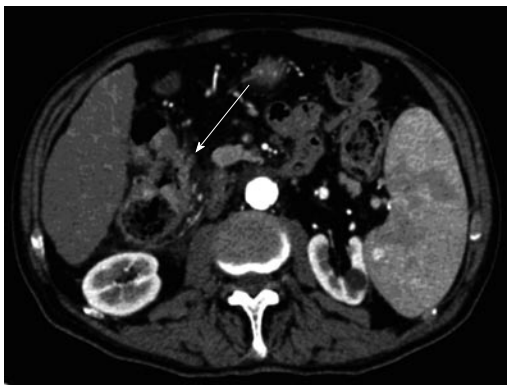

Figure 3 Computer tomography scans of the abdomen performed on admission. Contrast material-enhanced axial computer tomography scan shows luminal narrowing and marked segmental circumferential thickening of the hepatic colon flexure. Adenocarcinoma was confirmed at colonoscopy and biopsy.

spectively.

We described a case of synchronous HCC and CRC with liver metastases. As already mentioned, the presence of multiple primary tumors is rare, and even rarer the presence of synchronous neoplasms. In addition to the rarity of the case, the complexity in distinguishing the simultaneous presence of HCC nodules and metastases in a cirrhotic liver must be emphasized. Indeed, the baseline probability of a new focal liver lesion to be a HCC in cirrhosis increases according to nodule size, ranging from 66% for nodules between 1 and 2 cm, to 80% for nodules between 2 and 3 cm in size and up to 95% for nodules larger than 3 cm[12]. Nevertheless, it is possible to find metastases from tumors aroused from extra-hepatic sites in a cirrhotic liver, although unlikely; consequently, an accurate and complete characterization of the focal liver lesions is mandatory in this setting. In our case, we reached the diagnosis through dynamic CT scans and subsequent histological confirmation.

The presence of liver cirrhosis, if the disease is compensated, does not change *per se* the therapeutic indications for non-hepatic tumors[13]. In view of the good residual function of the liver disease, and after screening for portal hypertension and fluid retention, our patient was surgically treated with laparotomic right colectomy, without operative complications.

The standard of care for patients with colorectal hepatic metastases (CRHM) is the systemic chemotherapy with or without biologic agents. When metastasis are confined to liver and there is no vascular invasion or regional lymph node involvement, surgical resection is the standard of care. In patients with cirrhosis, as in non surgical candidates, loco-regional therapies as RFA may be regarded as one of the best options for both curative and palliative approaches[6]. In our patient, the single nodule of HCC, as well as the two CRHM, were simultaneously treated during the laparotomic session with RFA.

Finally, adjuvant chemotherapy was excluded because of the post-surgical onset of ascites, whereas CT and PET scans performed after six and twelve months showed a complete response to treatments.

Our experience highlights the possible coexistence of two different neoplasms in a cirrhotic liver, although rare, and the complexity in the proper diagnosis and management of the two tumors. Even if dynamic CT or MR play a key role in the diagnosis of HCC, the identification of synchronous metastasis may be difficult, due to the inherent morphological characteristics of the cirrhotic liver and to the high pre-test probability of a new lesion to be a HCC. Therefore, despite the potential risk of tumour seeding in this setting, ranging from 10% to 19% according to several reports[14], liver biopsy remains a critical diagnostic tool recommended in doubtful cases and/or when hepatic metastases are suspected, in order to confirm the histological origin of the primary neoplasm and to provide the best therapeutic algorithm based on the correct diagnosis.

In absence of recommendations regarding the best combined therapeutic approach, it is desirable to carry out the best available therapy for each tumor, when feasible. In this line, RFA can be regarded as one of the best options for both curative and palliative approaches of CRHM in compensated cirrhosis[6].

COMMENTS

Case characteristics

Upon admission the patient was asymptomatic, and physical examination was unremarkable, except for hepatosplenomegaly and palmar erythema.

Clinical diagnosis

The patient presented with microcytic anemia and imaging evidence of three hepatic focal lesions on a background of liver cirrhosis.

Differential diagnosis

Differential diagnosis was performed between hepatocellular carcinoma and hepatic metastases from colorectal cancer, using dynamic computer tomography (CT) and histology of the primary lesion and the three hepatic nodules.

Laboratory diagnosis

Laboratory tests showed microcytic anemia (haemoglobin 9.1 g/dL, haematocrit 30%, mean corpuscular volume 70 fL) and mild hypoalbuminemia (3.2 g/dL); all other liver function tests were normal, whereas levels of carcinoembryonic antigen and α-fetoprotein were 21.5 ng/mL (NV < 5) and 3.2 ng/mL (NV < 5), respectively.

Imaging diagnosis

Dynamic CT showed a unifocal hepatocellular carcinoma in VI hepatic segment and other two nodules suspected for metastases, and it pointed out a luminal narrowing with marked thickening of the hepatic flexure of the colon. This latter was further detected by colonoscopy as an ulcerated, bleeding and stricturing lesion at the hepatic flexure.

Pathological diagnosis

Histological examination defined the stricturing lesion of the colon as adenocarcinoma with a moderate degree of differentiation, whereas biopsy of the three focal liver lesions confirmed a diagnosis of single nodule hepatocellular carcinoma (HCC) together with two liver metastases from colorectal cancer.

Treatment

The patient underwent laparotomic right hemicolectomy with removal of thirty-nine regional lymph nodes, and simultaneous laparotomic radio-frequency ablation of both nodule of HCC and metastases.

Related reports

This case report emphasizes the possible coexistence of two different neoplasms in a cirrhotic liver, although rare, and the complexity in the proper diagnosis and management of the two tumors.

Experiences and lessons

Even if dynamic CT or colorectal cancer play a key role in the diagnosis of HCC, the identification of synchronous metastasis may be difficult, due to the inherent characteristics of the cirrhotic liver and to the high pre-test probability of a new lesion to be a HCC. Therefore, liver biopsy remains a critical diagnostic tool recommended in doubtful cases and it is mandatory when a liver metastasis from another primary tumour is suspected.

Peer review

This manuscript is interesting and presents a careful observation and discussion regarding diagnosis and management of primary HCC and liver metastasis from colorectal cancer.

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15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23243641.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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