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EDITORIAL

Clinical and biological significance of precursor lesions of intrahepatic cholangiocarcinoma

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Abstract

Cholangiocarcinoma (CC) is primarily a malignant tumor of older adults most prevalent in Southeast Asia, where liver fluke infestation is high. However the etiology in western countries is unknown. Although the incidence of extrahepatic cholangiocarcinoma has remained constant, incidence of intrahepatic CC (ICC) which differs in morphology, pathogenesis, risk factors, treatment and prognosis is increasing. While this increase is associated with hepatitis C virus infection, chronic nonalcoholic liver disease, obesity, and smoking, the pathogenesis of ICC and molecular alterations underlying the carcinogenesis are not completely elucidated. Benign biliary lesions such as biliary intraepithelial neoplasia, intraductal papillary neoplasm of the bile duct, von Meyenburg complex or bile duct hamartoma, and bile duct adenoma have been associated with ICC. For each of these entities, evidence suggests or supports a role as premalignant lesions. This article summarized the important biological significance of the precursor lesions of ICC and the molecular mechanisms that may be involved in intrahepatic cholangiocarcinogenesis.

Key words: Intraepithelial neoplasia; Von Meyenburg complex or bile duct hamartoma; Bile duct adenoma; Intrahepatic cholangiocarcinoma; Intraductal papillary neoplasm of the bile duct

Core tip: This manuscript highlights the important development in the research field of intrahepatic cholangiocarcinogenesis, and summarizes some key points related to progression from the precursor lesions to the intrahepatic cholangiocarcinoma, including their molecular genetics. Each individual precursor or potential precursor is linked to the cancer by the clinical, histological and molecular association.

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INTRODUCTION

Intrahepatic cholangiocarcinoma (ICC) is responsible for 10%-20% of primary liver cancers worldwide[1]. ICCs are classified as perihilar or peripheral type. The perihilar type, involving the large bile ducts, is composed of a large tubular component or papillary proliferation of tall columnar epithelium with mucin production. The peripheral type, involving the smaller ducts and segmental branches, is composed of a proliferation of relatively small, tubular, closely packed cord-like structures or ductular pattern lined by small cuboidal epithelium[2]. Grossly, both perihilar and peripheral ICC are firm and white to tan[1]. Another classification scheme divides ICCs into conventional ductal carcinoma, bile ductular type, intraductal neoplasm and rare variants[3], highlighting similarities between intraductal neoplasms and pancreatic intraductal papillary-mucinous neoplasms, and differences between bile ductular type and the conventional type on the origin of tumor cells. These classification schemes reflect the postulated cells of tumor origin with similarities between the perihilar type, conventional ductal carcinoma, bile duct type and mucin-producing type, and between the peripheral, bile ductular, cholangiolar and cholangiolocellular types[2].

ICCs may evolve from two types of premalignant biliary lesions: Biliary intraepithelial neoplasia (BilIN) and intraductal papillary neoplasm of the bile duct (IPNB)[3] as in the case of perihilar large duct type ICCs[4]. ICC may also evolve without definite premalignant lesions. No intraepithelial or intraductal preneoplastic, dysplastic or neoplastic lesions have been demonstrated in the small bile ducts or ductules[5]. Von Meyenburg complex (VMC) is suggested to be a possible premalignant lesion of ICCs due to the occasional association of VMC with ICCs and reports of VMC-like cystic ICCs[6-8]. Progression of bile duct adenoma (BDA) to ICC has also been reported[9]. The major clinical and histological characteristic features of above lesions are summarized in Figure 1 and Table 1. In addition to these precursor lesions, incidence is also increased in patients with risk factors such as chronic viral hepatitis, infection with parasites such as clonorchis and opistorchis, and hepatolithiasis[1].

BILIN

BilIN is an epithelial lesion and a precursor to both ICCs (the intrahepatic bile ducts and peribiliary gland) and to extrahepatic cholangiocarcinoma (the extrahepatic bile ducts and gallbladder)[10,11]. BilIN is characterized by epithelial cells with nuclear pseudostratification and atypia, often with micropapillary projections into the bile duct lumen[10]. It is not grossly identifiable[10]. BilIN has often been described as the biliary counterpart to pancreatic intraepithelial neoplasia (PanIN), partially due to the similar embryonal development and morphology of the pancreatic and biliary duct systems[12]. Grading of BilIN follows a similar 3-tiered pattern to that of PanIN, which has proven similar in several morphologic and immunohistochemical respects[13]. BilIN is subdivided into BilIN-1, BilIN-2 and BilIN-3 according to consensus criteria proposed in 2005 and accepted by an international group in 2007[14,15]. BilIN-1 shows relatively uniform, mildly irregular nuclei confined to the lower two thirds of the epithelium. In BilIN-2, nuclei are full-thickness and can be found apically, and loss of cellular and nuclear polarity is present but not diffuse. Nuclear irregularity is increased compared to BilIN-1, but mitoses remain rare. BilIN-3 is notable for marked cellular and nuclear atypia and loss of polarity, with cytological resemblance to invasive cholangiocarcinoma albeit without basement membrane invasion. Cribriforming and budding of cell clusters as well as mitoses are often seen[11,15]. Macroscopic and radiologic identification of these lesions is not possible[11].

BilIN has been associated with several conditions which can predispose to cholangiocarcinoma. There is no solid datum about the prevalence of BilIN. While estimates of overall prevalence of BilIN are difficult to determine, one study for margin status in biliary carcinomas showed that 4 of 19 ICCs had BilIN at the margin[16].

BilIN is frequently diagnosed in cases of hepatolithiasis[17], and primary sclerosing cholangitis and choledochal cyst have also been associated with BilIN[15,18]. BilIN has also been shown to arise in autoimmune pancreatitis, chronic hepatitis B and C, and alcoholic cirrhosis[19-21].

BilIN shares several molecular alterations with cholangiocarcinoma, and study of BilIN alongside cholangiocarcinoma has helped to elucidate several of the key molecular mechanisms in cholangiocarcinogenesis. Many of these molecular changes accumulate in conjunction with increasing grade of BilIN[11]. KRAS has been shown to occur early in cholangiocarcinogenesis and is present in approximately 33% of BilIN lesions including in 25% of cases of BilIN-1[22]. Also, p21, p53, cyclin D1 and EZH2 expression has been shown to increase and expression of Dcp4 and $p16^{INK4A}$ decreases in tandem with increasing grade of BilIN[3]. Importantly, p53 overexpression has been shown in BilIN-3 but reports show no increased expression in BilIN-1/2[17,22]. Overexpression of EZH2 has been related to hypermethylation of the p16 promoter, which could explain the correlating increase in EZH2 expression and decrease of p16 expression with increasing grade of BilIN[23]. Overexpression in EZH2 and decreased p16 expression are associated with deregulation of cellular senescence, and deregulation of autophagy has been shown through in vitro studies to precede cellular senescence[24,25]. Related to these findings, it is notable that a recent study of expression of autophagy-related proteins showed that light chain 3β (LC3), beclin-1 and p62 showed increased expression in BilIN-1, BilIN-2 and BilIN-3, suggesting that deregulated autophagy plays a role in occurrence and development of BilIN[17]. Decreased membranous expression of β-catenin similarly correlates with increasing grade of BilIN, while E-cadherin is decreased in some cases of BilIN but in a smaller proportion of cases compared to

Table 1 Summary of the clinical and pathological features of intrahepatic cholangiocarcinoma and its precursor lesions

	ICC	BilIN	IPNB	VMC/BDH	BDA
Incidence/ prevalence	10%-20% of primary liver cancers[1]	No published data	9%-38% of bile duct carcinomas[28]	5.6% of adults, 0.9% of children (autopsy series)[40]	0.00008%-0.006% of patients (autopsy series)[52,53]
Risk factors	Chronic viral hepatitis, clonorchis, opistorchis, hepatolithiasis[1]	Hepatolithiasis, primary sclerosing cholangitis, choledochal cyst, autoimmune pancreatitis, chronic viral hepatitis, alcoholic cirrhosis[15,17-21]	Hepatolithiasis, clonorchis[31,32]	Congenital hepatic fibrosis, polycystic liver disease[40]	No known risk factors
Gross appearance	Firm, white to tan[1]	Not grossly identifiable[10]	Dilated bile ducts filled with soft, papillary white to red to tan lesions without invasion[10]	Well-circumscribed unencapsulated nodules, < 5 mm[1]	Subcapsular, well-circumscribed unencapsulated gray to white, yellow or tan firm nodules, ⩽ 2 cm[47,48]
Histologic appearance	Perihilar type: Involves large bile ducts, composed of large tubules or papillae lined by columnar epithelium. Peripheral type: Involves smaller ducts and segmental branches, composed of small, tubular cords or ductular pattern lined by cuboidal epithelium[2]	Epithelium with nuclear pseudostratification and atypia (increasing from BilIN-1 to BilIN-2 to BilIN-3), often with micropapillary projections into the bile duct lumen[10]	Noninvasive papillary or villous biliary neoplasm covering delicate fibrovascular stalks (subtypes pancreatobiliary, intestinal, gastric, oncocytic)[10]	Irregular dilated to branching low cuboidal epithelium-lined ductules within fibrous stroma, often adjacent to portal areas[1]	Small uniform cuboidal epithelium-lined ductules within fibrous stroma[47,48]
Molecular alterations	Activating mutations in KRAS (22%) occurs early in cholangiocarcinogenesis[63]	Activating mutation of KRAS present in approximately 33% of BilIN lesions including in 25% of cases of BilIN-1[22]	Increased expression of Cyclin D1 and p21[26,35]	Loss of heterozygosity at key loci (5q21, 9p21, 10q23, 17p13) harboring APC, p53, p16, and PTEN[44]	BRAF V600E mutation (53%)[46]
	Loss-of-function mutations in TP53 (15%), BRAF and EGFR mutation (7% and 2%)[55,61,65,67]	Increased expression of p21, p53, cyclin D1 and EZH2[3,17,22-25]	Aberrant expression of p16[30]		
	Rare NRAS and PI3K mutations have been[55,61-72]	Decreased expression of Dcp4 and p16INK4A. Loss of SMAD4/DPC4 associated with higher grade[3,30,35]	Inactivation of p53 associated with increasing grade of dysplasia and invasion[30]		
	IDH1 and IDH2 mutations co-occurring with increased TP53 expression and associated with DNA hypermethylation[62,66]	Decreased membranous expression of β-catenin with increasing grade of BilIN[26]	C-myc mutations in over 50% of cases[26]		
	Chromosomal aberrations including gains at 7p and 8q and losses at 1p, 4q and 9p[68,69,73-76]	Decreased expression of E-cadherin in some cases of BilIN[26]	Loss of SMAD4/DPC4 associated with higher grade[30,35]		
	Aberrant methylation of p16INK4a/CDKN2 (47%), RASSFIA (56%) and APC (29%)[70,71,76]	S100P: Increased immunohistochemical expression in BilIN-2 and BilIN-3[27]			

BilIN: Biliary intraepithelial neoplasia; IPNB: Intraductal papillary neoplasm of the bile duct; VMC: Von Meyenburg complex; BDH: Bile duct hamartoma; BDA: Bile duct adenoma; ICC: Intrahepatic cholangiocarcinoma.

invasive cholangiocarcinoma[26]. S100P also showed increased immunohistochemical expression in BilIN-2 and BilIN-3 as compared to reactive benign epithelium and BilIN-1[27]. The S100P immunohistochemical marker is of particular interest as it has been suggested for use in clinical diagnosis and grading of BilIN[11].

IPNB

IPNB, defined as a precursor to cholangiocarcinoma according to the World Health Organization 2010 classification, has been described as the biliary counterpart to the intraductal papillary mucinous neoplasm (IPMN) of the pancreas[10,28]. Similarly to IPMN, the neoplasm is characterized by thin fibrovascular cores lined with noninvasive papillary or villous epithelium and filling a dilated bile duct lumen[10]. Grossly, IPNB shows dilated, fusiform to cystic bile ducts with soft papillary lesions ranging from white to tan to red. Histologically, the aforementioned fibrovascular cores are lined by any of four cell types: Pancreatobiliary, intestinal, gastric and oncocytic types, parallel to those defined in IPMN[10]. However, there are important differences between IPNB and IPMN. Only approximately 1/3 of IPNB show

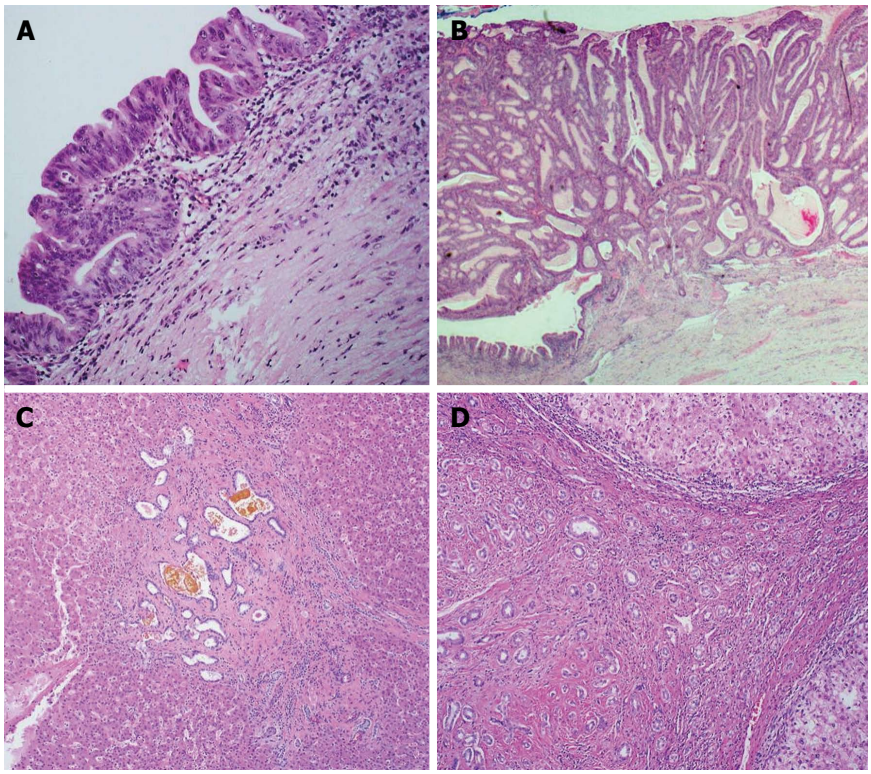

Figure 1 Premalignant biliary lesions. A: Biliary intraepithelial neoplasia. Biliary epithelium with nuclear pseudostratification and atypia; B: Intraductal papillary neoplasm of the bile duct. Papillary proliferation of neoplastic cells lining thin fibrovascular cores; C: Von Meyenburg complex. Small well-circumscribed lesion consisting of dilated irregular bile ducts embedded within dense collagenous stroma; D: Bile duct adenoma. Well-circumscribed proliferation of small uniform tubules surrounded by fibrous stroma.

macroscopic mucin production, while only rare IPMNs are not associated with mucin hypersecretion[28,29]. Also, pancreatobiliary type epithelium is most common and gastric type epithelium is rare in IPNB, whereas IPMN least commonly shows pancreatobiliary differentiation[10] and gastric is among the more common patterns[30]. Just as grading of BilIN parallels that of PanIN, grading in IPNB follows that of IPMN. Low, intermediate and high grade are assigned based on cytologic and architectural characteristics, where low- and intermediate-grade tumors comprise one diagnostic entity, high-grade tumors another, and IPNB with associated invasive carcinoma a final separate entity[10]. Studies of histomorphologic, molecular and other features of these tumors have shown disparate results to some extent due to the different features of IPNB with different etiology.

IPNB comprises 9%-38% of bile duct carcinomas and is most common in patients from far eastern countries in their 6th and 7th decades[28]. In these patients who often have risk factors such as hepatolithiasis and clonorchiasis, intestinal subtype is most common (47% and 38% in two large series)[31,32]. However, Western cohorts more commonly show pancreatobiliary type epithelium (36%, 69% and 45% of patients in three recent series from the United States and western Europe)[30,33,34]. Series in all populations have shown uncommon oncocytic and gastric subtypes.

Molecular changes in IPNB remain poorly characterized, although several recent studies have begun to explore various molecular mechanisms in these precursor neoplasms. Comparisons between IPNB and BilIN have shown conflicting results. Cyclin D1 and p21 expression increase occurs in IPNB just as it does in in BilIN; a study by Itatsu *et al*[26] showed higher expression in IPNB than in BilIN while Nakanishi *et al*[35] did not find this differential expression. Another cell cycle protein, p16, has also shown aberrant expression in IPNB and this aberrant expression was shown to be more frequent in cholangiocarcinoma than in IPNB[30]. Inactivation of p53 has been shown to increase with increasing grade of dysplasia and with invasion in one study[30], while other studies have shown different results[35,36]. C-myc mutations have been shown to be common as well with expression in over half of cases in one study[26]. Similar to pancreatic neoplasms, loss of SMAD4/DPC4 has been shown in IPNB and BilIN with increased loss associated with higher grade[30,35]. GNAS mutation has been seen mostly in intestinal subtype and mucin hypersecretion-associated cases, and has been recognized more frequently in studies involving Asian cohorts where intestinal subtype is more common[30,37]. KRAS mutations on the other hand appear more common in early and low-grade lesions[28,30].

VMC (BILE DUCT HAMARTOMA)

VMCs, also known variously as bile duct or biliary hamartomas or as biliary microhamartomas, are small

(generally < 5 mm), well-circumscribed unencapsulated nodules which consist of irregularly shaped, often dilated bile ducts lined by a low cuboidal epithelium, embedded within a dense collagenous stroma. VMCs are thought to result from persistence of the embryonic ductal plate and are generally adjacent to portal areas[1]. Multiple case reports showed exist of VMCs with histologic evidence of transformation to cholangiocarcinoma, showing benign, hyperplastic and dysplastic epithelium with adjacent progression to invasive carcinoma[7,8,38,39].

These lesions are fairly common with prevalence of 5.6% in adults and 0.9% of children in one autopsy series, and are especially prevalent in patients with adult polycystic kidney disease or congenital hepatic fibrosis[40]. VMCs are generally considered benign, but several case reports suggest that these lesions are capable of transformation to cholangiocarcinoma, showing benign, hyperplastic and dysplastic epithelium with adjacent progression to invasive carcinoma, particularly in patients with multiple VMCs[8,38,39,41-43]. This phenomenon has been corroborated with molecular evidence of mutations shared between cholangiocarcinoma and VMCs[44]. Further suggesting this link is a radiologic study of 6 patients with multiple VMCs in which one of the six (17%) patients with initial imaging evidence of VMCs developed cholangiocarcinoma within 2 years[45].

Specific molecular genetic changes for transformation of VMCs to cholangiocarcinoma have not been definitively established[38]. In contrast to biliary duct adenomas, BRAF V600E mutations seen in a subset of ICC are not present in VMCs[46]. However, recent studies have identified multiple findings suggestive of the sequential genetic changes required for transformation of benign VMCs to invasive cholangiocarcinoma[38,44]. In one study of two patients with multiple VMCs and cholangiocarcinoma, loss of heterozygosity (LOH) was examined at 20 key genetic loci in the cholangiocarcinoma tumor, in VMCs distant from the tumor, and in intermediate lesions where VMCs showed transformation to cholangiocarcinoma. LOH was seen in VMCs at some of the same key loci as seen in cholangiocarcinoma, affecting oncogenes p16, p53, APC and PTEN which have been shown to play a role in the development of cholangiocarcinoma[44]. In contrast, immunohistochemical expression of p16INK4A was shown to be lost in cholangiocarcinoma in one report while adjacent uninvolved VMC ducts retained expression of p16INK4A[38].

BDA OR PERIBILIARY GLAND HAMARTOMA

BDA are small (< 2 cm) benign gray to white, yellow or tan lesions usually located directly beneath the liver capsule. They are firm, well-circumscribed and not encapsulated. Histologically, they are comprised of uniform tubular or curvilinear ductules within a fibrous stroma[47,48]. The ductules are lined by cuboidal cells with bland, round to oval nuclei and without mitotic activity, and sometimes show mucinous metaplasia, 1-antitrypsin droplets and neuroendocrine differentiation. Previously synonymous with bile duct hamartoma (BDH)[49] - BDA and BDH have overlapping features - histological findings and immunohistochemical properties aid in distinguishing between the two. It has been reported that BDAs originate from peri-biliary glands and not bile ductules or ducts[50]. This finding is supported by the monoclonal antibody identification of antigens D10 and IF6 and the presence of mucin cells; a shared profile amongst peribiliary glands and BDAs but not VMCs. Malignant transformation of BDA has not been unequivocally demonstrated. ICC in a background of BDA mixed with minor component of BDH is reported in the literature[51] (also present in authors' unpublished case report). There has also been a report of progression of BDA to ICC[9]. BDA is a rare lesion and has been found in 0.0008%-0.006% of patients in two large autopsy series[52,53].

Molecular characterization of BDAs is incomplete. The BRAF V600E mutation was identified by PCR and immunohistochemistry in 53% (8/15) cases of BDA in one series[46]. Interestingly, both wild-type BRAF and BRAF V600E lesions coexisted in patients with multiple BDAs. Identification of oncogenic mutations in BDA supports a benign neoplasm rather than reactive process and suggests that BDA may be an early lesion in the pathogenesis of ICC, which has been shown to harbor BRAF V600E mutations[54,55]. Furthermore, the finding of coexistence of benign lesions, dysplastic lesions and carcinoma (authors' unpublished data) and progression of BDA to ICC[9] strongly suggests an adenoma-dysplasia-carcinoma pathway, as seen in colorectal carcinogenesis. BRAF V600E mutation was not identified in von Meyenberg complexes, supporting distinct etiologies with different molecular and biological processes[46].

BILIARY ADENOFIBROMA

Few cases of biliary adenofibroma[56-59], a benign tumor with complex tubulocystic nonmucin secreting biliary epithelial and an abundant fibroblastic stromal components, have been reported to date. The immunohistochemical profile (cytokeratins 7, 8, 18, 19 and D10 positive, and 1F6 negative) suggests a large bile duct or interlobular duct origin[59,60]. Malignant transformation of the epithelial component has been reported[56].

MOLECULAR GENETICS OF ICCS AND PRECURSORS

Molecular alterations underlying ICC and the pre-malignant biliary lesions are not completely elucidated. Activating mutations in KRAS (22%), loss-of-function mutations in TP53 (15%), BRAF and EGFR mutation (7% and 2%), and rare NRAS and PI3K mutations have been reported[55,61-72]. IDH1 and IDH2 mutations co-occurring

with increased TP53 expression and associated with DNA hypermethylation has also been reported, although the functional relevance is unknown. Chromosomal aberrations including gains at 7p and 8q and losses at 1p, 4q and 9p were identified by comparative genomic hybridization studies[69,73-76]. Aberrant methylation of p16INK4a/CDKN2 (47%), RASSFIA (56%) and APC (29%) genes have been demonstrated in ICC although the exact role in the pathogenesis is also not delineated. Investigation of genetic and epigenetic alterations in benign intrahepatic biliary lesions will further highlight mechanisms of carcinogenesis.

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EDITORIAL

Defining acute-on-chronic liver failure: East, West or Middle ground?

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Abstract

Acute-on-chronic liver failure (ACLF), a newly recognized clinical entity seen in hospitalized patients with chronic liver disease including cirrhosis, is associated with high short- and medium term morbidity and mortality. None of the definitions of ACLF proposed so far have been universally accepted, the two most commonly used being those proposed by the Asia-Pacific Association for the Study of Liver (APASL) and the European Association for the Study of Liver - Chronic Liver Failure (EASL-CLIF) consortium. On paper both definitions and diagnostic criteria appear to be different from each other, reflecting the differences in cut-off values for individual parameters used in diagnosis, the acute insult or precipitating event and the underlying chronic liver disease. Data directly comparing these two criteria are limited, and available studies reveal different outcomes when the two are applied to the same set of patients. However a review of the literature suggests that both definitions do not seem to identify the same set of patients. The definition given by the APASL consortium is easier to apply in day-to-day practice but the EASL-CLIF criteria appear to better predict mortality in ACLF. The World Gastroenterology Organization working party have proposed a working definition of ACLF which will identify patients from whom relevant data can be collected so that the similarities and the differences between the two regions, if any, can be clearly defined.

Key words: Acute-on-chronic liver failure; Chronic liver disease; Cirrhosis; Ascites; Hepatic encephalopathy

Core tip: Acute-on-chronic liver failure, a relatively new clinical entity seen in patients with chronic liver disease including cirrhosis, is associated with high morbidity and mortality. The two most commonly used definitions given by the Asia-Pacific Association for the Study of Liver and the European Association for the Study of Liver - Chronic Liver Failure consortium, are different and appear to identify different set of patients. Because of limited data on these definitions, the World Gastroenterology Organization working party has proposed a new definition to identify patients from

whom data can be collected to ultimately arrive at a uniform definition.

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INTRODUCTION

Patients with cirrhosis who develop superadded insults like infections or organ failure from any other cause, have a poorer prognosis as compared to those who do not[1,2]. Though transient and potentially reversible, such events, either directly affecting the liver or involving another part of the body, can dramatically worsen the liver reserve in patients with pre-exiting stable liver disease. This worsening differs from the progression of the primary chronic liver disease (CLD) which leads to chronic decompensation, being largely irreversible in the majority of cases because of the accompanying parenchymal extinction. Patients in the former group tend to be younger, more commonly alcoholic, show higher levels of white blood cell counts and C-reactive protein and have a higher prevalence of infections as compared to decompensated cirrhotics[3].

Increasing realization of the differences between the two scenarios lead to the recognition of acute-on-chronic liver failure (ACLF), a term used for the first time in 1995 to describe a condition in which two liver insults are present concurrently, *i.e.,* one ongoing and chronic, and the other, recent and acute[4]. A typical feature of ACLF is its rapid progression and the association with high short and medium term mortality (30%-60%)[3,5]. ACLF is frequently encountered in day-to-day practice and has been reported to occur in up to 30% of cirrhotics[3]. Intensive medical care is necessary for most patients with ACLF thus increasing the per-patient costs significantly.

DEFINITIONS FOR ACLF

Since the term ACLF was used for the first time, up to thirteen different definitions have been suggested, contributing to a great deal of confusion regarding what constitutes the condition[6]. The two most commonly used definitions have been provided by the Asia-Pacific Association for the study of Liver (APASL) and the European Association for the study of Liver - Chronic Liver Failure (EASL-CLIF) consortium (Table 1).

The definition provided by the APASL in 2009 characterizes ACLF as an "acute hepatic insult manifesting as jaundice [serum bilirubin $\geqslant$ 5 mg/dL (85 micromoles/L) and coagulopathy international normalized ratio (INR) $\geqslant$ 1.5, or prothrombin activity < 40%] complicated within 4 wk by clinical ascites and/or encephalopathy in a patient with previously diagnosed or undiagnosed CLD/cirrhosis"[7]. This was based on data collected from 200 patients. A subsequent consensus meeting evaluated the as yet unpublished data on approximately 1300 patients from 14 countries from the APASL ACLF research consortium (AARC) database along with newer evidence and altered the definition only to the extent of additionally mentioning the associated high 28-d mortality[8].

Experts in Europe and United States, on the other hand, have characterized ACLF as "a syndrome that defines a subgroup of cirrhotic patients who develop organ failure following hospital admission with or without an identifiable precipitating event and have increased mortality rates"[9]. In view of paucity of any established evidence-based definition of ACLF, investigators of the EASL-CLIF consortium performed the multicenter, prospective observational CANONIC (CLIF Acute-on-Chronic Liver Failure in Cirrhosis) study which defined acute decompensation as an acute development of hepatic encephalopathy, large ascites, bacterial infections or gastrointestinal hemorrhage, or any combination of these. It also defined cut-off levels for diagnosing extra-hepatic organ failure and stratified patients with ACLF into 4 subgroups characterized by increasing mortality (Table 1)[3]. There are many differences between the two definitions including the underlying CLD, the type of acute worsening and its time frame, and prior decompensation raising the question as to which of these might be appropriate for clinical use.

The APASL definition is liver centered. Simply put, the condition basically reflects acute liver failure with two additional requirements - the serum bilirubin should be 5 mg/dL or above and ascites should be present in case encephalopathy is not. On the other hand, the EASL-CLIF consortium defines ACLF based on the failure of one or more organs of which liver is only one.

DIFFERENCES IN THE APASL AND EASL-CLIF DIAGNOSTIC CRITERIA

The individual parameters that make up the diagnostic criteria differ considerably between the two definitions (Table 2). For example, the cut off for serum bilirubin level to define liver failure was found to be 12 mg/dL in the CANONIC study as levels above this were associated with a 28-d mortality above 15%. However, the bilirubin level by itself was not important, since the mortality was only 4% even among patients with elevated serum bilirubin levels if they did not have extra-hepatic organ failure[10]. The cut-off level for serum bilirubin was kept at 5 mg/dL in the APASL criteria ACLF because patients with bilirubin between 5 and 10 mg/dL included in the AARC data, had a mortality of about 38%[8].

Similarly, coagulation failure was defined as INR $\geqslant$ 2.5 as per the CANONIC study, and $\geqslant$ 1.5 as per the APASL criteria[3,8]. INR reportedly reflects the acute liver failure as per APASL; however, in the CANONIC study, all patients had acute decompensation of cirrhosis and

Table 1 Diagnostic criteria of acute-on-chronic liver failure as per the chronic liver failure acute-on-chronic liver failure in cirrhosis study

No ACLF - This group consists of 3 subgroups
Patients with no organ failure
Patients with a single "non-kidney" organ failure (*i.e.*, single failure of the liver, coagulation, circulation, or respiration) who had a serum creatinine level < 1.5 mg/dL and no hepatic encephalopathy
Patients with single cerebral failure who had a serum creatinine level < 1.5 mg/dL
ACLF grade 1 - This group consists of 3 subgroups
Patients with single kidney failure
Patients with single failure of the liver, coagulation, circulation, or respiration who had a serum creatinine level ranging from 1.5 to 1.9 mg/dL and/or mild to moderate hepatic encephalopathy
Patients with single cerebral failure who had a serum creatinine level ranging from 1.5 and 1.9 mg/dL
ACLF grade 2 - This group consists of patients with 2 organ failures
ACLF grade 3 - This group consists of patients with 3 organ failures or more
Definitions of organ failures - CANONIC study
Liver failure - serum bilirubin level of 12.0 mg/dL or more
Kidney failure - serum creatinine level of 2.0 mg/dL or more, or the use of renal replacement therapy
Cerebral failure - grade Ⅲ or Ⅳ hepatic encephalopathy
Coagulation failure - an international normalized ratio of more than 2.5 and/or a platelet count of 20×10^9/L or less
Circulatory failure - use of dopamine, dobutamine, or terlipressin
Respiratory failure - ratio of partial pressure of arterial oxygen to the fraction of inspired oxygen (FiO_2) of 200 or less or a pulse oximetric saturation to FiO_2 ratio of 200 or less

Adapted from Moreau *et al*[3]. ACLF: Acute-on-chronic liver failure; CANONIC: Chronic liver failure acute-on-chronic liver failure in cirrhosis.

Table 2 Principle differences in the definition and diagnostic criteria of acute-on-chronic liver failure between Asia-Pacific and West

	APASL definition	EASL-CLIF definition
Total bilirubin	5 mg/dL or more	12 mg/dL or more
INR	1.5 or more	2.5 or more
Hepatic encephalopathy	Any grade	Only grade Ⅲ and Ⅳ
Ascites	May be present	Not included
Duration between insult and ACLF	4 wk	Not defined
Acute event - sepsis	No	Yes
Acute event - variceal bleeding	No unless producing jaundice and coagulopathy defining ACLF	Yes
Extra-hepatic organ involvement	No	Yes
What is chronic disease	Chronic liver disease with/without only compensated cirrhosis	Only cirrhosis, including those with prior decompensation

APASL: Asia-Pacific Association for the Study of Liver; EASL-CLIF: European Association for the Study of Liver-chronic Liver Failure; INR: International normalized ratio; ACLF: Acute-on-chronic liver failure.

causes other than liver failure including sepsis may have contributed equally to coagulopathy. Additionally, platelet count ($\leqslant 20 \times 10^9$/L) was also used to define coagulation failure in the CANONIC study and not as per the APASL consensus.

Hepatic encephalopathy, irrespective of its grade, is an important criterion for diagnosis as per the APASL criteria, whereas, as per the CANONIC study, mild to moderate encephalopathy (Grade 1 or 2) would be important only if associated with another organ failure (liver, coagulation, circulation or respiration)[3,8].

Clinically detectable ascites was present in 91% of patients with ACLF in the AARC database and it was included as a diagnostic criterion as per the APASL consensus[8]. In the CANONIC study, ascites was significantly more common in those with, compared to those without ACLF (78.7% and 63.4%; $P \leqslant 0.001$); however, it did not differ among the three grades of the former suggesting thereby that its presence per se may not have influenced the outcome[3].

Renal failure was observed in 55.8% of the patients included in CANONIC study[3]. On the contrary, studies based on the APASL definition reported renal failure in only 30%-35% of patients with ACLF[5,11]. Associated renal dysfunction has been well recognized to worsen the outcome in decompensated cirrhosis. The high mortality in ACLF was irrespective of the creatinine level as per the AARC data as mentioned in the APASL consensus statement. Hence the APASL criteria do not include serum creatinine level to define ACLF.

ACUTE INSULT

The term precipitating event is generally used in the West to refer to the acute insult, and the major events recorded are primarily non-hepatic, most often being

Table 3 Acute insult/precipitating event in patients with acute-on-chronic liver failure

As per APASL criteria	As per EASL-CLIF criteria
Hepatotropic viral infections	Bacterial infection
Reactivation of HBV	Gastrointestinal hemorrhage
HEV super-infection	Active alcoholism within the past 3 mo
Active alcohol consumption (within last 28 d)	Other precipitating events
Drug induced liver injury	Transjugular intrahepatic portosystemic shunting
Complimentary and alternative medicines	Major surgery
Severe autoimmune hepatitis	Therapeutic paracentesis without use of intravenous albumin
Flare of Wilson's disease	Hepatitis
Non-hepatotropic insults (if producing direct hepatic insult)	Alcoholic hepatitis (liver biopsy required for diagnosis)
Surgery	No precipitating event identified
Trauma	
Viral infections	
No acute insult identifiable	

Adapted from Sarin *et al*[8] and Moreau *et al*[3]. ACLF: Acute-on-chronic liver failure; APASL: Asia-Pacific Association for the Study of Liver; EASL-CLIF: European Association for the Study of Liver-chronic Liver Failure; HEV: Hepatitis E virus; HBV: Hepatitis B virus.

bacterial infections and sepsis. Spontaneous bacterial peritonitis (SBP) and bacterial pneumonia were the most common precipitating events, seen in 32.6% of patients in the CANONIC study[3]. Superadded Hepatitis A or E or reactivation of hepatitis B infections is seldom, if ever encountered in the West. On the other hand, APASL consensus contends that the acute insults should be hepatic, since liver failure is the core part of ACLF. Among these, super-added hepatitis E virus infection and reactivation of hepatitis B virus (HBV) are the leading causes of acute insult in ACLF (Table 3)[12-15]. Among the non-infectious etiologies, alcohol-related liver injury is the major cause of acute worsening of CLD and this is equally reported in studies from the two regions[3,5]. Events outside the liver, whether related to the underlying liver disease *per se* (*e.g.,* SBP and variceal bleeding), or not (*e.g.,* pneumonia or urinary infections), do not qualify as acute insults leading to ACLF as per the APASL definition unless the liver is secondarily affected so as to cause jaundice, coagulopathy and ascites or hepatic encephalopathy.

Both documents recognize that an acute insult may not be identifiable in a significant proportion of patients with ACLF as was seen in 43.6% in the CANONIC study[3]. Interestingly, the identification or otherwise of a precipitating event was unrelated to the severity of ACLF as well as short term mortality.

UNDERLYING CLD

The diagnosis of CLD in the context of ACLF is primarily made by history, physical examination and laboratory, radiologic or endoscopic investigations[16]. However, most of the ACLF patients in the Asia-Pacific region present with liver failure without having been previously evaluated for liver disease. Unlike the EASL-CLIF definition, the APASL includes non-cirrhotic, CLD s such as chronic hepatitis and non-alcoholic fatty liver disease/non-alcoholic steatohepatitis (NAFLD/NASH) also as underlying CLD. This is because even such patients carry a poor prognosis with mortality rates being as high as 33% at 4 wk once they meet the other threshold criteria for ACLF[8]. On the other hand, both the CANONIC and North American Consortium for End-Stage Liver Disease studies included only patients with cirrhosis[3,17].

DOES PRIOR DECOMPENSATION MATTER?

In the CANONIC study, up to 50% of the patients with ACLF had prior history of decompensation or developed ACLF in 3 mo or less after the first episode of decompensation. Previous studies from the West have concluded that hepatic decompensation in the past was an independent predictor of mortality in patients with ACLF[18]. Contrarily, in the CANONIC study, patients with ACLF and no prior acute decompensation had a higher prevalence of organ failure and a more severe grade of ACLF as compared to those with acute decompensation in the past. As expected, the former group also showed a significantly higher mortality at 28 d (42.2% *vs* 29.6%; $P = 0.03$). Despite this difference however, patients with previous decompensation are not excluded since they too have a mortality above the 15% cut off considered relevant in the study. Also, for any given value of leucocyte count, the probability of mortality was significantly higher in those without prior decompensation compared to in those with[3]. This could imply that those without previous decompensation have an inappropriately exaggerated inflammatory response and immune dysfunction leading to worse outcome than those with previous decompensation. However, as per the APASL criteria, patients with known previous decompensation with jaundice, ascites and hepatic encephalopathy are excluded from being defined as ACLF based on the AARC data. In a retrospective study, patients who met the APASL criteria for ACLF but also

had prior decompensation in addition were older, more often had non-hepatic insults as a cause for acute worsening and generally had less severe hepatic damage compared to ACLF patients without any decompensation in the past[19]. The 28-d survival was however similarly high (58.9% *vs* 61.4%) in the two groups. A study from India with a smaller number of patients showed similar results[11]. Further research is needed to explore this issue.

Liver biopsy continues to be an important tool to differentiate between the underlying cirrhotic and non-cirrhotic liver disease and to establish the etiology of CLD in certain situations. Excluding patients with prior decompensation and including those with chronic hepatitis and NAFLD as it does, the APASL definition would necessitate a more frequent need for doing a liver biopsy for diagnosing the underlying CLD. Coagulopathy being a necessary part of the definition, the trans-jugular approach, needing expertise and adding to the cost of care, would be necessary for obtaining the liver biopsy in almost all who need the same. In the absence of liver biopsy, there is a possibility that conditions such as acute Budd-Chiari syndrome or abdominal tuberculosis with hepatic and peritoneal involvement might be mistaken for ACLF, though these conditions are rather uncommon.

DO THE TWO DEFINITIONS IDENTIFY THE SAME PATIENTS?

The differences in the two definitions would not matter if they identified mostly the same patients. Data from the literature however suggests they do not. Two studies from Asia in patients with acute worsening of CLD looked at how the two definitions fared in these patients. Zhang *et al*[20] found that 118 (29.9%) of their 394 patients met both the criteria for ACLF by EASL and APASL, while 276 (70.1%) met only the APASL criteria. On the other hand Dhiman *et al*[11] found that 38 (76%) of their 50 patients met the EASL criteria, whereas only 19 (38%) met the APASL criteria. The relative proportion of patients with ACLF by one or the other definition may vary from study to study depending on the background liver disease population from which they are drawn. But the proportions differing in the same study when the two definitions are applied clearly shows that they identify different patients with some overlap.

Because underlying chronic hepatitis and NASH are considered for inclusion but previous decompensation is not as per the APASL definition, patients meeting these criteria clearly are in an earlier stage of CLD compared to those meeting the EASL-CLIF criteria. This would mean that the former would have a higher inflammatory response from the acute event compared to the latter, other factors being equal. This could also be the reason why the higher thresholds for serum bilirubin and INR come into play in the EASL-CLIF definition. Shi *et al*[21] have shown recently that ACLF precipitated by acute hepatic injury and by extra-hepatic insults are distinct but overlapping conditions which have similarly high transplant-free, 28-d mortality (48.3% *vs* 50.7%; P = 0.22). The former group had compensated cirrhosis, liver and coagulation failure being frequent in them, while the latter had advanced underlying cirrhosis and a high frequency of extra-hepatic organ failure. Thus, conceptually, the acute precipitating events as per EASL-CLIF not directly involving the liver by and large, would need to raise the indicators of liver damage such as bilirubin and INR to a higher level to cause a mortality equivalent to Asian patients with ACLF. As would be expected from these, infections are less frequent in Asian patients with ACLF compared to their Western counterparts[8]. It thus becomes clear that the patients defined by the two definitions actually differ considerably. Further studies using the World Gastroenterology Organization (WGO) consensus definition (see below) would help confirm this.

PREDICTING OUTCOMES IN ACLF

The APASL consensus document stated that in the absence of studies on prognostic parameters to further stratify the outcome in patients with ACLF, the SOFA score may be used[8]. In a study from China, out of the 276 patients who met the APASL criteria, 83 (30.1%) progressed to ACLF as per the EASL-CLIF criteria post-enrollment and the mortality in them was over 50% compared to less than 5% in the rest[20]. When the patients who met the APASL criteria were compared with those who met EASL-CLIF criteria from among cirrhotics with acute worsening, the 90-d mortality between the two groups differed significantly (59.3% *vs* 13.1% respectively)[20]. Dhiman *et al*[11] from India found that the short-term mortality was significantly higher in those with ACLF than those without, if EASL-CLIF criteria were used (47.4% *vs* 8.3%, respectively) but not if the APASL definition was used (36.8% *vs* 38.7%, respectively). They concluded that the former criteria were better than the latter for defining ACLF and that the CLIF-SOFA score, and not the Acute Physiology and Chronic Health Evaluation Ⅱ (APACHE Ⅱ), Child-Pugh (CP) scores and Model for end-stage liver disease (MELD) scores was a significant independent predictor of mortality. The first study was retrospective, and the second one included a small number of patients. Agrawal *et al*[22] showed that simple organ failure count is better than the CANONIC system of severity grading for predicting the in-hospital mortality in Asian patients with ACLF. Jalan *et al*[23] added two other individual predictors of mortality, *i.e.,* age and white blood cell count to the simplified organ function scoring system (CLIF Consortium Organ Failure score, CLIF-C OFs) to develop the CLIF Consortium ACLF score (CLIF-C ACLFs) which was compared and was found to have higher predictive accuracy than MELD, MELD-Sodium (MELD-Na), and CP score. It was subsequently validated in an external cohort and found to perform better than the other prognostic scores for sequential use in stratifying the mortality risk in patients

Table 4 Subtypes of acute-on-chronic liver failure as per World Gastroenterology Organization working party

Type A ACLF - non-cirrhotic chronic liver disease with an acute flare; often indistinguishable from acute or sub-acute liver failure
Reactivation of hepatitis B
Hepatitis A or E superimposed on chronic hepatitis B
Autoimmune hepatitis
Hepatitis E infection in patients at risk for NASH
Type B ACLF - well compensated cirrhosis with an acute insult
Type C ACLF - cirrhosis with previous hepatic decompensation

Adapted from Jalan *et al*[10]. ACLF: Acute-on-chronic liver failure; NASH: Non-alcoholic steatohepatitis.

with ACLF. Further comparative studies and extensive research would be needed to determine the predictors of mortality that can be applied to patients with ACLF as defined by APASL criteria.

ARE THE DIFFERENCES REGION-SPECIFIC?

The possibility exists that the two definitions may be region-specific because of the differences in the pattern of the underling liver diseases and the prevalence of acute events.

This would mean that one is best served by using the definition applicable to one's own region. However, some concerns surface. One of the problems in comparing different studies from the same region would be that the background CLD and the acute insults may differ between them. For example, Zhang *et al*[20] found that CLD from chronic HBV infection constituted 52.5% of the patients while alcohol abuse was the acute insult in only 23.4%. Even within studies from India, the cause of underlying CLD differed, hepatitis B infection being the most common cause in a study from Mumbai (29.6%) and alcohol, in the study from Chandigarh (66%)[22,24]. Similarly the etiology of acute insult was also different, acute viral hepatitis A or E being seen in 33.3% patients in the former, while acute hepatitis E noted in 7% in the latter study[22,24].

EASE OF USE

With its simple clinical parameters, the definition given by the APASL consortium is easier to apply in day-to-day practice. The consensus document also states that it has a high degree of predictive ability[8]. However Dhiman *et al*[11] concluded that the APASL criteria did not predict mortality as well as the CLIF-SOFA criteria and that the latter is better to stratify patients with ACLF so as to predict the outcomes. Thus, lack of validated criteria to stratify the risk of mortality and the possible increased need for liver biopsy are the limitations of the APASL criteria. The practical application of the CLIF-SOFA criteria could, on the other hand, be difficult in the hands of internists, gastroenterologists, or hepatologists[25].

NEW DEFINITION - THE WORLD GASTROENTEROLOGY ORGANIZATION CONSENSUS

Because of the limited prospective data and of the differing definitions offered by APASL consensus and EASL-CLIF consortium, the WGO working party have proposed a definition of ACLF which is primarily only to identify patients from whom relevant data can be collected so as to arrive at a uniform definition which could bridge the gap between the Asia-Pacific region and the West[10].

The working definition characterizes ACLF as "a syndrome in patients with CLD with or without previously diagnosed cirrhosis which is characterized by acute hepatic decompensation resulting in liver failure jaundice and prolongation of the INR and one or more extra-hepatic organ failures that is associated with increased mortality within a period of 28 d and up to 3 mo from onset". Thus, this definition includes patients with chronic hepatitis, compensated cirrhosis as well as cirrhosis with previous decompensation (Table 4). As per the CANONIC study, the 28-d mortality was significantly lower in patients with type C ACLF. It is hoped that future studies will enroll patients as per this definition so that patients falling into the different sub-categories with possible different outcomes can be compared and more useful data would emerge thereby.

CONCLUSION

Despite the diversity of early data on ACLF, two consensus definitions have emerged in recent years which appear to represent two different but overlapping conditions. Several questions still remain to be answered regarding which definition to use and whether there are differences within a region based on the pattern of patients seen in each. Now that a third important definition has emerged, that proposed by the WGO, it is likely that relevant data collected will help clarify many of these issues so as to further improve the management of patients with ACLF ultimately contributing to improved outcomes in these patients.

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TOPIC HIGHLIGHT

2015 Advances in Hepatocellular Carcinoma

Microwave ablation of hepatocellular carcinoma

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Abstract

Although surgical resection is still the optimal treatment option for early-stage hepatocellular carcinoma (HCC) in patients with well compensated cirrhosis, thermal ablation techniques provide a valid non-surgical treatment alternative, thanks to their minimal invasiveness, excellent tolerability and safety profile, proven efficacy in local disease control, virtually unlimited repeatability and cost-effectiveness. Different energy sources are currently employed in clinics as physical agents for percutaneous or intra-surgical thermal ablation of HCC nodules. Among them, radio-frequency (RF) currents are the most used, while microwave ablations (MWA) are becoming increasingly popular. Starting from the 90s', RF ablation (RFA) rapidly became the standard of care in ablation, especially in the treatment of small HCC nodules; however, RFA exhibits substantial performance limitations in the treatment of large lesions and/or tumors located near major heat sinks. MWA, first introduced in the Far Eastern clinical practice in the 80s', showing promising results but also severe limitations in the controllability of the emitted field and in the high amount of power employed for the ablation of large tumors, resulting in a poor coagulative performance and a relatively high complication rate, nowadays shows better results both in terms of treatment controllability and of overall coagulative performance, thanks to the improvement of technology. In this review we provide an extensive and detailed overview of the key physical and technical aspects of MWA and of the currently available systems, and we want to discuss the most relevant published data on MWA treatments of HCC nodules in regard to clinical results and to the type and rate of complications, both in absolute terms and in comparison with RFA.

Key words: Thermal ablation; Hepatocellular carcinoma; Microwave ablation; Percutaneous microwave ablation; Laparoscopic microwave ablation; Complications

Core tip: In clinical practice there is an increasing interest in the use of microwave radiations as ablative technique for the treatment of small and intermediate hepatocellular carcinoma nodules. No literature data are

already available about a direct comparison between radiofrequency ablation and microwave ablation; in this review we provide an extensive and detailed overview on the technical and engineering aspects of microwave devices, and we critically expose the most relevant clinical data about the experience in microwave ablation, also by making a comparison with radiofrequency ablation.

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INTRODUCTION

The purpose of thermal ablative treatments is to destroy solid tumors by raising their temperature above a lethal threshold (60 ℃ for instantaneous coagulative necrosis, 50 ℃ for prolonged exposure to heat)[1] through direct energy deposition, which eventually turns into heat within a limited and controlled range of action. Interstitial thermal ablation is currently used for the treatment of a large variety of tumors, including liver[2], lung[3], kidney[4], bone[5], thyroid[6] and breast malignancies[7]. Despite the constantly increasing use of thermal ablation in extra-hepatic applications[8], the treatment of hepatocellular carcinoma (HCC) nodules remains its most common clinical target. Resection is still the favored treatment option for early-stage HCC in patients with well compensated cirrhosis, but thermal ablation techniques provide a valid non-surgical treatment alternative, thanks to their minimal invasiveness, excellent tolerability and safety profile, proven efficacy in local disease control, virtually unlimited repeatability and cost-effectiveness[9,10].

Different energy sources are currently employed in clinics as physical agents for percutaneous or intra-surgical thermal ablation of HCC nodules. Among them, radiofrequency (RF) currents (*i.e.,* alternating electric currents in the 400-500 kHz frequency range) are the most used[11], while microwave (MW) radiations (*i.e.,* non ionizing electromagnetic fields in the 1 GHz frequency range) are becoming increasingly popular[12]. Other thermal ablation agents - such as laser radiations and high intensity focused ultrasound beams[13] - are also employed, but apparently provide less flexibility of use and a globally inferior performance in terms of maximum ablation volume attainable per probe and/or per treatment time unit compared to RF ablation (RFA) and to MW ablation (MWA)[14].

MWA was initially introduced in the Far Eastern clinical practice in the 80s' and 90s'[15], showing promising potential but also severe limitations in the controllability of the emitted field and in the high amount of power employed for the ablation of large tumors, resulting in a poor coagulative performance and a relatively high complication rate[16]. Starting from the 90s', several RFA systems were developed in the United States and in Europe, showing safe, effective and repeatable coagulative performance[17-19]. RFA rapidly became the gold standard in ablation, especially in the treatment of small HCC nodules, at first flanking and eventually replacing percutaneous ethanol injection (PEI) treatments[10]. However, RFA exhibits substantial performance limitations in the treatment of large lesions and/or tumors located near major heat sinks[20,21]: Over the last 5 years, these limitations have been effectively tackled by second and third generation MWA systems, with considerably enhanced characteristics both in terms of treatment controllability and of overall coagulative performance[22-25].

The purpose of this review is: (1) to provide a brief overview of the key physical and technical aspects of MWA and of the currently available systems; and (2) to gather and discuss the most relevant published data on MWA treatments of HCC nodules in regard to clinical results and to the type and rate of complications, both in absolute terms and in comparison with RFA.

TECHNIQUE

Physical differences between RFA and MWA

RF heating relies on the ohmic dissipation effects related with the circulation of alternating electric currents within target tissues. RF effectiveness depends on the electrical conductivity of the treated tissues, which, in turn, is strongly correlated with their water content[26]. Dehydration and subsequent carbonisation of tissues occurring at temperatures above 100 ℃ is, therefore, an intrinsic barrier to further RF heating[27]. This upper temperature threshold in the active heating zone (*i.e.,* the inner treatment region, where heating is mainly due to absorption and dissipation of the energy delivered by the ablation probe) limits and slows down also the indirect peripheral heating (*i.e.,* the passive heat transfer by mere thermal conduction from the active zone outwards), accounting for the limited coagulative performance of a single probe, the poor response of tissues with low electric conductivity and the high sensitivity to heat sinking effects typical of RFA[28]. These limitations are overcome altogether when moving from an ohmic (*i.e.,* based on electric power dissipation within a conductive medium) to a dielectric (*i.e.,* not requiring electric currents circulation) heating modality, as in MWA[29]. Electromagnetic radiation at industrial, scientific and medical (ISM) frequencies (*i.e.,* portions of the electromagnetic spectrum left open for applications in the Industrial, Scientific and Medical fields, in the neighbourhood of 900 MHz, 2.4 GHz and 5.8 GHz, respectively) propagating through a biologic tissue induces a fast switching rotation of the electric dipoles at the atomic or molecular level. Such microscopic charge displacement - not generating any macroscopic electric current - is countered by inter-

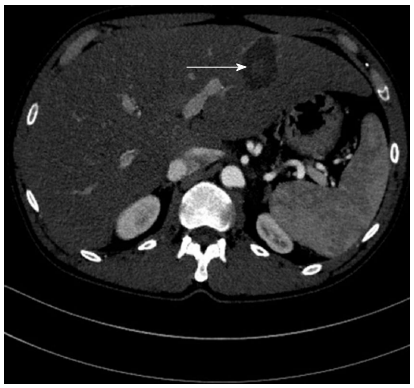

Figure 1 Contrast-enhanced computed tomography scan shows, in the region surrounding what was the probe active tip position during the ablation (white arrow), an inner hyper-dense core contrasting with an outer thicker and hypo-dense annulus.

dipolar interactions, producing frictional heat[30]. The dielectric heating mechanism is particularly effective in polar (*i.e.,* featuring an intrinsic dipolar momentum) molecules, such as water. Therefore, tissues rich in water content are effectively heated by MWs, while tissues poor in water content (which would hinder RF currents circulation) absorb a smaller fraction of the applied MW field energy, allowing further propagation to the next tissue layer[31]. Tissue carbonisation is not, therefore, an insurmountable barrier to the MW heating process, and temperatures far higher than 100 ℃ may be reached within the target tumor, allowing enhanced active and passive tissue heating, larger coagulation zones and more effective rejection of heat sinking effects[32,33]. When high power MWA treatments are performed, several qualitative and quantitative differences are observed in terms of RFA: (1) the hyper-echogenic spot around the probe-active tip detectable on ultrasound-scanning during a thermal ablation procedure forms and expands at a much higher rate, providing a visual feedback of the ongoing vaporization process; (2) post-MWA follow-up scans [either computed tomography (CT) or magnetic resonance imaging (MRI)] usually show, in the region surrounding what was the probe active tip position during the ablation, an inner hyper-dense core contrasting with an outer thicker and hypo-dense annulus, the former being charred tissue (not present on RFA) and the latter being the coagulated but not carbonized zone typical of any thermal ablation modality (Figures 1 and 2); and (3) due to massive water evaporation, MWA treatments induce substantial contraction in target tissues (30%-70% in volume, according to several *ex-vivo* and *in vivo* experimental observations[34-36]), far more than their RFA counterparts[37]. If the appropriate shrinkage correction factor is used for accurately calculating the actual ablation volume, the coagulative performance gap between MWA and RFA widens further. Since the amount of tissue contraction relates with the initial water content of the target tissues, one may expect liver tumors of equal size and location, but featuring a different water content (*e.g.,* due to the absence or presence and degree of cirrhosis), to give different responses to the same thermal treatment, as shrinkage phenomena would not affect the final ablation volume and aspect ratio in the same fashion. This adds to the well-known oven effect, *i.e.,* the higher energy deposition and enhanced heating observed within (pseudo-) capsuled nodules[38,39], in accounting for different technical and clinical outcome generally observed for the thermal treatments of HCC nodules and of hepatic metastases (typically non capsuled and not on a cirrhotic background), beyond the obvious differences in histology, morphology and vascularization.

All antennas are bipolar by definition: Therefore, MWA differs from monopolar RFA treatments also for the absence of neutral electrodes applied to the patient, intrinsically ruling out the risk of skin burns at the grounding pads site[40]. Moreover, a MW field at ISM frequencies in the 30-100 W power range propagating across a biological tissue is almost completely absorbed within just a few centimetres. Therefore, long-range non thermal effects induced by MWA probes are excluded and, unlike with RFA, patients with pacemakers or metallic prosthesis are not at risk[41].

Key components of a MWA system and current implementations

A MWA system typically comprises: (1) a programmable energy source, designed to generate the power required and monitor energy delivery to the patient[42,43]; (2) an interstitial antenna, usually a semi-rigid coaxial cable emitting MW radiations from its exposed - *i.e.,* uncovered by the outer conductor - distal end, embedded into a needle-like device; and (3) a power transmission line linking the energy source output ports to the antennas: indeed, MWA allows simultaneous multi-probe operation, either in phased-array mode (*i.e.,* exploiting synchronized field emissions in order to obtain the desired interference patterns in the individual radiation diagrams) or asynchronously - with still substantial thermal synergy - obtaining in both cases remarkably increased and more spherical ablation volumes compared to sequential, contiguous, single-probe ablations[44,45]. On the contrary, RFA allows only switched (*i.e.,* sequential, unparalleled) multi-probe operation due to potential cross-electrode interference, with sensibly/considerably reduced synergistic performance[46]. The MW generator consists of an oscillator working at the selected frequency of operation - either 915 MHz or 2.45 GHz in commercially available systems - and an amplifier - ranging between 40 W and 190 W output power in current systems - either magnetron-based (*i.e.,* resonating cavities built out of high-powered vacuum tubes, very much like commercial MW ovens) or in solid state (*i.e.,* transistor-based) technology. Magnetrons are less expensive but are considerably heavier (up to 3-5 times) and bulkier (up to 4-5 times in volume) than their solid state counterparts. The frequency of operation affects the antenna design and the type of interaction between the electromagnetic field with biological tissues: The higher the frequency, the shorter the corresponding radiation wavelength and

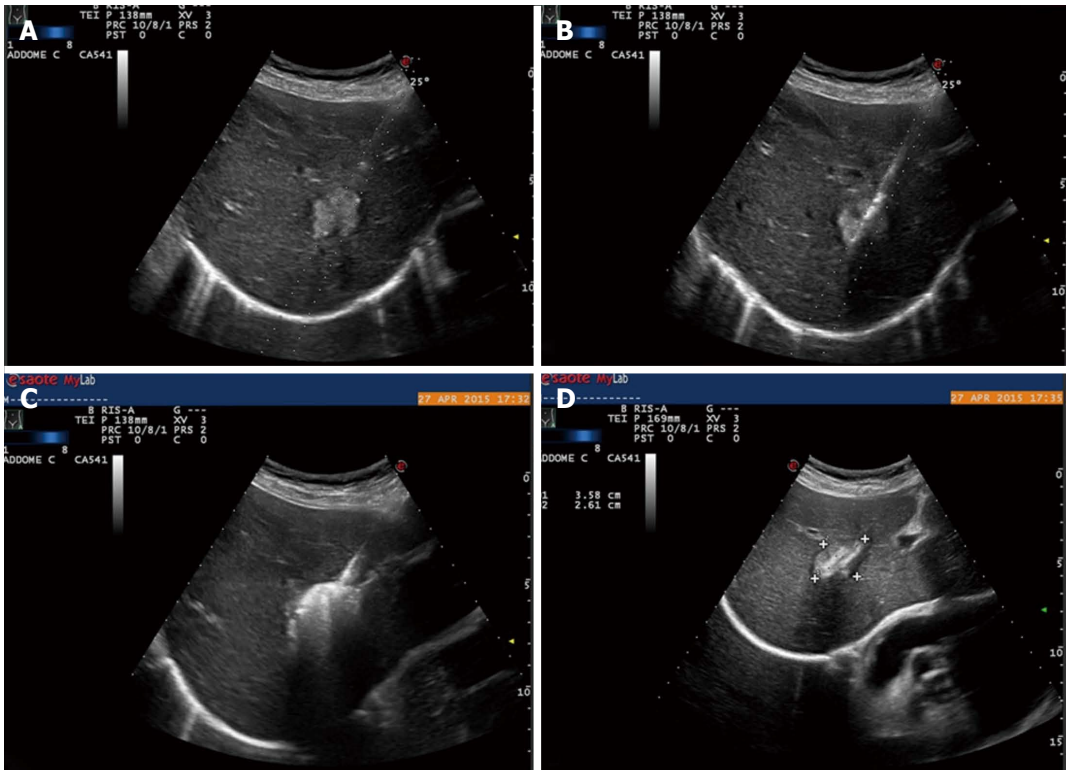

Figure 2 Time-lapse of ultrasound-guided percutaneous microwave ablation of medium-sized hepatocellular carcinoma of the right lobe. A: Ultrasound evaluation before ablation; B: Needle insertion; C: Hyperechoic boiling effect in the ablation area during the procedure; D: One month later ultrasound evaluation: The inner hyperechoic track corresponds to the position of the active probe.

the key lengths in the antenna geometry, the smaller the field penetration into the target tissues, and the higher the MW energy absorption rate by water molecules[47]. The selection of the operating frequency is, therefore, a trade-off between conflicting requirements, which accounts for the almost identical number of commercially available MWA systems operating in the 915 MHz and in the 2.45 GHz frequency bands. Hoffmann *et al*[48] shows a thorough *ex-vivo* comparison of 4 different MWA systems, 2 operating at 915 MHz and 2 at 2.45 GHz, suggesting that the latest generation of internally cooled high power 2.45 GHz systems provides an overall higher performance compared to earlier low power 915 MHz systems (either in single or multi-probe configuration, cooled or not cooled) as for ablation volume, transversal diameter (*i.e.,* the coagulation size perpendicular to the antenna) and sphericity (*i.e.,* the linear or quadratic ratio between the radial and longitudinal axis of the ablation zone). However, the preliminary experience of Liu *et al*[49] in the treatment of large HCC nodules (> 4 cm) with both high power, internally cooled 915 MHz antennas (21 patients) and with equivalent 2.45 GHz antennas (19 patients) showed that the former were able to achieve a lower local tumor progression rate (14.3% *vs* 26.3%) with fewer probe insertions (3.69 ± 0.6 *vs* 4.71 ± 1.61).

Simo *et al*[50] came to opposite conclusions upon a series of 48 patients with 124 hepatic tumors, out of which 72 were treated with a 915 MHz system (average nodule size: 1.7 ± 0.1 cm) and 52 with a 2.45 GHz system (average nodule size: 2.5 ± 0.2 cm): The 2.45 GHz system achieved equivalent, but more predictable and faster ablations using a single antenna.

Internally cooled MWA probes seem to provide large, more spherical and more consistent ablations over their not cooled counterparts[51]. MW power dissipation along the coaxial cable feeding the distal antenna active tip is very high (up to 15%-30% per meter length at room temperature for operating frequencies around 1 GHz, in cables of approximately 1-mm outer diameter) and puts the probe at severe risk of shaft overheating. Either lowering the radiated power or pulsing MW energy delivery to prevent this risk has proven to excessively reduce the probe performance in terms of the maximum ablation zone achievable and/or the overall treatment duration. Newer generations of MWA probes feature either water[51] or gas[52] cooling within the applicator shaft, allowing very high power treatments (even up to 100 W radiated power) and large ablations (up to 5 cm perpendicular to the probe in 10 min, with a single antenna, in *ex-vivo* bovine liver), while not enlarging the probe size (still in the 13 G-17 G range).

The high power attenuation rate also affects the coaxial cables used for transferring MW energy from the generator output port to the interstitial antenna. Low attenuation cables are generally thicker and heavier[42]: For a reasonable trade-off/compromise between ergonomics and power handling, most MWA manufacturers opt for relatively short (1.5 to 2.5 m) and fairly flexible coaxial cables, exhibiting an overall insertion loss in the 1.5-3.0 dB range (= 30%-50% loss). When setting the

working parameters for a MWA procedure, the majority of currently available systems refer to the nominal MW power at the generator output port. However, the only clinically relevant quantity is the power irradiated into the target tissues, which is significantly lower than the nominal power, and generally differs from system to system even for equal nominal power levels.

Early MWA technologies suffered from the poor predictability of the radiated field pattern and from uncontrolled back-heating effects (often referred to in literature as "comet effect"[53,54]) due to the reflected waves (*i.e.,* MW radiations not absorbed by the target tissues and back-propagating along the probe shaft outer walls) generated by the inevitable impedance mismatches between the antenna and the tissues. On the one hand, this caused unwanted, deep cauterisation of tissues along the probe shaft, increasing the risk of complications; on the other hand, it reduced the antenna efficiency, dispersing the MW field longitudinally rather than focussing it on the probe distal end. Newer MWA probes have solved this major performance issue, finally enabling a safe delivery of large, spherical and controllable ablations, through a number of design variants, such as monopole or dipole antennas featuring a miniaturized choke (*i.e.,* an impedance transformer superimposed to the coaxial antenna, which traps reflected waves through a destructive interference pattern)[55,56], or triaxial antennas (*i.e.,* with the main coaxial line encompassed by an outer coaxial line, serving for reflected waves absorption)[57].

Whichever the antenna design, MWA probes are intrinsically less mechanically robust than RFA electrodes. The latter are monolithic metal tubes, either loaded with multi-tines or with a sharp metal penetration tip integral with the electrode shaft; the former necessarily exhibits a transition from a metallic to a non-metallic (typically, plastic or ceramic) material around the antenna emitting tip. Mechanic breakings of this junction have been reported, especially when targeting hard cirrhotic livers or when hitting the ribs, although recent advancements in the material selection and assembly have substantially mitigated this risk.

Unlike pronged RFA electrodes, straight RFA and MWA applicators are potentially prone to migration from their target. MWA probes exhibit an even higher risk of displacement, due to the heavier and more rigid extension cables. This problem is partly alleviated - only during the probe insertion manoeuvre, but not during the ablation treatment itself - when cryogenic cooling is used, which causes and fixes an ice-ball at the probe tip[58].

Ultimately, it is worth noting that currently available MWA systems exhibit a great variability in key technical features (antenna design, frequency of operation, use of single or multiple probes, energy delivery algorithms, maximum deliverable power, *etc.*), offering a wider and more heterogeneous technological landscape than RFA, which further contributes to the complexity of an exhaustive and conclusive evaluation of MWA in the interventional oncology scenario, beyond the still limited clinical experience.

CLINICAL APPLICATION IN HCC

Local ablation is considered the first-line treatment option for patients with early-stage HCC, not suitable for surgical therapy[59]. For many years, PEI has been the main technique for percutaneous treatment of HCC. However, PEI is occasionally ineffective when there is intra- or extra-capsular invasion, as fibrotic tissues hinder ethanol diffusion[60]; moreover, the effectiveness of PEI is rapidly impaired with increasing nodule size. Thermal ablative techniques - including RFA, MWA and laser ablation - have shown higher efficacy compared to PEI in the loco-regional treatment of HCC, leading to a better disease control and a survival benefit for lesions larger than 20 mm[61-65]. RFA is currently the most popular and widely used thermal ablation modality: It provides a reasonable compromise between a number of highly heterogeneous and often conflicting requirements, such as safety, tolerability, efficacy, ease of use and cost-effectiveness. RFA has proven to be particularly effective for HCC lesions smaller than 3 cm, with the best reported rate of complete necrosis approaching 99% of treated lesions, offering a 5-year overall survival (OS) of around 40%[66]. However, despite the high percentage of necrosis reported by various authors, the recurrence rate is highly variable, from 2% to 39%, depending on the technique used[67,68]. The main limitations of RFA are related to poor energy propagation into tissues with high electric and thermal impedance, to the intrinsic 100 ℃ upper temperature threshold that prevents tissue charring, and to the relatively slow tissue heating mechanism that leads to tissue sensitivity to convective heat sinking effects induced by blood or bile circulation in proximity of the ablation target. MWA overcomes all these limitations, due to its dielectric (*i.e.,* not related to electric currents circulation) heating mechanism rather than the ohmic (*i.e.,* based on electric power dissipation within a conductive medium) modality typical of RFA. However, higher heating velocity and efficacy are achieved through a somewhat increased technological complexity and costs compared to RFA, both for generating the required amount of energy and monitoring energy delivery to tissues, and for designing and manufacturing safe, effective and minimally invasive disposable probes suitable for percutaneous use.

Early MWA systems suffered from several technical problems, ranging from inadequate power handling to exceeding probe gauge, poor predictability of the radiated field pattern and uncontrolled back-heating effects. MWA was first used clinically in the Far East. Lu *et al*[69] in 2001 reported their results in 107 HCCs ranging in size from 0.8 to 6.4 cm (mean: 2.7 ± 1.5 cm), treated with MWA using a single antenna insertion in 46 nodules ⩽ 2 cm, or multiple antennae insertions in 61 nodules > 2 cm. Technical success was achieved in 98% of tumors ⩽ 2 cm and in 92% of nodules > 2 cm, while local recurrence was found in 2% of nodules

⩽ 2 cm and in 8% of nodules > 2 cm after a follow-up of only 9 mo. Dong *et al*[70] reported the long-term results of 339 HCC nodules of a mean tumor size of 4.1 ± 1.9 cm treated with MWA. After a mean follow-up period of 27.9 mo, the 1-, 3- and 5-year cumulative survival rates were 92.70%, 72.85%, and 56.70%, respectively. Even if obtained using first generation, non-optimized MWA systems, these results showed similar effectiveness and survival in the treatment of small HCC nodules compared to RFA. Shibata *et al*[71] in 2002 published a study comparing percutaneous RFA with percutaneous MWA. Using a first generation microwave device capable of obtaining a necrotic area of 24 mm × 16 mm for single needle insertion, the authors showed no statistically significant differences in the rate of complete ablations between patients treated with MWA and patients treated with a latest generation radiofrequency device, while the number of treatment sessions was significantly lower in the RF ablation group. Moreover the study showed no significant difference in the local recurrence rate between the 2 groups even if RF ablation group recurrence rate at 1 and 2 years was 4% and 10%, respectively, while 12% and 24% in the MWA group; the absence of statistical significance might have been due merely to the small number of patients treated.

In 2005, Lu *et al*[72] reported the results of a retrospective study comparing percutaneous MWA with RFA. The mean diameter of HCC nodules was 2.5 ± 1.2 cm in MWA group and 2.6 ± 1.2 cm in RFA group. They used a 2.45 GHz microwave generator connected to a 14-gauge electrode with a power output of 10-80 W. A single insertion was applied for tumors of < 2.0 cm diameter, while for > 2.0 cm tumors multiple insertions were employed. RFA was performed by using a 290 KHz-RF generator with a maximum power output of 200 Watts. Complete ablation rates were 98.6% in < 3.0 cm tumors and 83.3% in > 3.0 cm tumors in the MWA group and 98% and 81% in the RFA group: the differences between the 2 groups were not statistically significant. Moreover, they found a non-significant difference in local recurrence of 11.8% for MWA compared to 20.9% for RFA. Complications and long-term survivals were also equivalent in the 2 groups. In opposition to these data, Ohmoto *et al*[16] published a retrospective study comparing RFA with MWA: RFA resulted more useful for the treatment of small HCCs, obtaining a lower local recurrence rate and a higher survival rate compared with MWA.

More recent studies with newer microwave system have confirmed the efficacy of MWA in the treatment of HCC. Iannitti *et al*[73] published the data from the first clinical trial in the United States using MWA and a 915 MHz generator. The mean single antenna ablation volumes obtained were 10.0 mL (range 7.8-14.0 mL), and clustered antennae ablation volumes were 50.5 mL (range 21.1-146.5 mL). They treated 87 patients (45% ablations were performed open, 7% laparoscopically, and 48% percutaneously) with both HCC and metastatic disease: they reported a local recurrence at the ablation site in 2.7% of tumors, and an OS rate for all tumor types of 47%, and for HCC of 74% at 19 mo. More recently Qian *et al*[74] compared the performance of MWA using a cooled-shaft antenna to the performance of RFA with a cooled electrode both *in vivo* porcine liver tissues and in patients with small HCCs (diameter range: 1.2-3.0 cm). They used a 2450 MHz MW generator (MTC-3) connected to a 14-gauge cooled-shaft antenna with a power output of 100 W (Qinghai Microwave Electronic Institute, Nanjing, China) and a Cool-tip™ RF ablation system (Valleylab, Boulder, CO, United States) connected to a 17-gauge internally cooled needle electrode with a maximum power output of 200 W. In an *in vivo* animal study a single MW ablation induced a significantly increased ablation volume compared to single RF ablation (33.3 ± 15.6 cm^3 *vs* 18.9 ± 9.1 cm^3, $P < 0.001$). Similarly, in clinical study the ablation volume of MW ablation, shown on contrast enhanced CT or MRI, was significantly larger than that of RF ablation (109.3 ± 58.3 cm^3 *vs* 48.7 ± 30.5 cm^3, $P < 0.001$). The most interesting finding of the study is that all 3 axes of the ablation volume obtained by MWA were greater than those of RFA, confirming that the technological evolution of MW devices obtains more spherical ablation areas. Poggi *et al*[23] reported their preliminary results on the feasibility and efficacy of thermal ablation of HCC using a new 2.45 MHz microwave generator delivering energy of 40-100 W through a 14- or 16-G internally cooled, coaxial antenna featuring a miniaturized quarter-wave impedance transformer (mini-choke) for reflected wave confinement (AMICA-GEM, HS Hospital Service SpA, Aprilia, Italy). Complete ablation was achieved in 183 lesions (94.3%), after a mean of 1.03 percutaneous MWA sessions. To estimate the amplitude of the ablation zone obtained with MWA, the authors calculated the difference between the volume of the ablation zone and the baseline volume of each treated lesion: This difference was called Δ volume. To assess how the ablated area was similar to a spherical shape, they calculated the greater and the smaller diameter ratio. For small HCCs they obtained a median Δ volume of 11.2 cm^3, representing an increase of almost 100% of the volume of a 3 cm diameter lesion and they achieved nearly spherical ablations areas with a mean diameter ratio of 1.1. Using the same MWA device Di Vece *et al*[75] compared the ablation area produced by a single application of MWA with that produced by an internally cooled RFA system in 40 patients with both primary and secondary inoperable liver tumors. They found that long- and short-axis diameters of the ablation areas produced by MWA were significantly greater than those produced by RFA: 48.5 ± 6.7 mm *vs* 30.9 ± 1.1 mm ($P < 0.0001$) and 38.5 ± 4.6 mm *vs* 26.8 ± 2.9 mm ($P < 0.0001$), respectively. The results of clinical trials with new generation MW ablation devices seem to confirm the expectations of larger and faster ablation volumes with microwave compared to radiofrequency. Yet it is particularly difficult to compare the different technologies

available, due to the availability of many different MW devices, the constant and rapid technological upgrade and the lack of clinical outcome standardization. In this regard, in a recently published review North *et al*[76] stated that currently the most powerful prognostic factor for ablation success that can be converted into improved progression-free survival remains the completeness of the initial ablation. However, standards of optimal MWA have not been defined yet. The authors selected 18 clinical studies, published between 2007 and 2013, on MWA of primary and secondary hepatic tumors with a sample size of at least 20 patients and a follow-up period of at least 6 mo. For each study they evaluated the proposed definitions for the effectiveness of the procedure, local recurrence, distant recurrence, morbidity, mortality and OS. Ablation success turned out to be the highest quality reporting standard while local recurrence remained highly variable, without a clearly defined distance from the initial target ablated lesion.

Given that nine microwave systems are currently available on the market with differences in the frequency used, the power supplied, the diameter of the probe, the availability of a probe-cooling system or a miniaturized device to decrease MW reflection[77], standardization of clinical criteria for reporting MWA outcomes is pivotal to compare the different methods.

The upgrade of MW devices enabled the new frontier of percutaneous thermal ablation to treat medium and large HCCs. Preclinical data support this hypothesis. Brace *et al*[57] and Strickland *et al*[78] obtained, in an *in vivo* porcine liver model, ablation zones with mean diameters up to 6.5 cm and ranging from 3 to 6 cm respectively. Early clinical trials also reported promising results of MWA in treating hepatic tumors > 3 cm. Yin *et al*[79] treated with percutaneous RFA or MWA 109 patients with HCCs measuring between 3.0 cm and 7.0 cm. They reported a complete ablation of 92.6%, a local recurrence in 22% of patients and a 3-year survival rate of 30.9% and they found no significant difference in the complete ablation rate between RFA and MWA. Kuang *et al*[80] reported a complete ablation rate of 91% of tumour measuring 3-5 cm. Likewise Poggi *et al*[23] obtained 90% of complete ablation in 49 HCC measuring 3.1-5.0 cm. More recently Sun *et al*[81] reported retrospective data of patients with a single medium-sized HCC who underwent percutaneous MWA. The OS rates were 89%, 74%, 60% while cumulative recurrence-free survival was 51%, 36% and 27% at 1, 2, and 3 years respectively. Patient age and tumor diameter were independent factors associated with local tumor recurrence while serum albumin level and the appearance of a new lesion were independently associated with OS. Therefore, despite the high percentage of complete ablation reported, the recurrence rate for HCCs larger than 3 cm is still quite high and is often directly related with the size of the lesion. To date few studies have evaluated the role of TACE combined with MWA in the treatment of medium and large HCC. TACE can reduce blood flow, creates ischemia, increases the chemotherapeutic agent local effect on tumor cells and increases the sensitivity of neoplastic cells to hyperthermia, resulting in synergy with the thermal ablation effect. Liu *et al*[82] compared TACE followed by MWA and TACE alone in 34 consecutive patients with large unresectable HCC (> 5 cm). They found that the mean survival rates were significantly higher in the former than in the latter group of patients (11.6 mo *vs* 6.1 mo). Poggi *et al*[83] reported their preliminary results on feasibility and effectiveness of the combination of MWA and TACE in 36 unresectable HCCs > 3 cm (size 3-11 cm, mean 4.78 cm), achieving a technique effectiveness in 83.3% of the lesions. Complete ablation was obtained in 100% of intermediate-sized HCCs. Local tumor progression was found in 3 lesions (8%) 9 mo after the procedures.

MWA is also performed through a laparoscopic approach. Hepatic lesions close to the gastrointestinal tract, gallbladder and bile ducts can be safely treated in this way. Laparoscopic MWA can also be a viable therapeutic option for patients unsuitable for hepatic resection due to impaired liver function or concurrent comorbidities. In a prospective cohort study, Cillo *et al*[84] treated 50 HCC in 42 patient with laparoscopic MWA. They obtained a complete ablation rate of 100% in < 3.0 cm tumors and of 80% in > 3.0 cm tumors. The two-year survival rate was 81% and the two-year recurrence rate was 55% with no peri-operative mortality and a median post-operative hospital stay of three days. Cillo *et al*[85] have recently described an innovative use of laparoscopic MWA in 2 patients affected by multiple liver metastases and a large HCC, respectively. The Authors developed a novel variation to the staged hepatectomy in which laparoscopic portal vein ligation was associated to laparoscopic MWA on the future hepatic transection plane. This modified procedure allows a complete hypertrophy of the non-occluded future liver remnant preventing the development of interlobar portoportal shunts that impair the remnant liver hypertrophy[85,86]. Image-guided tumor ablation can also have a role in HCC "bridge" to orthotopic liver transplantation (OLT), reducing the risk of list drop-out and in HCC "down-staging" to fit patients into OLT criteria. Particularly, Zanus *et al*[87] reported that out of 6 cases of HCC patients which underwent laparoscopic MWA before OLT, 4 had received it as a bridge to OLT to prevent neoplastic disease diffusion, and 2 as HCC down-staging to fit into OLT criteria. In all 6 cases no peritoneal or nodal HCC macroscopic and microscopic diffusion was observed intraoperatively at the time of laparotomy for OLT. Gringeri *et al*[88] reported 1 case of laparoscopic MWA of a single small HCC on liver graft. A complete ablation of the tumor was achieved and after 24 mo the patient was still free from local or distant recurrence, showing that MWA can be safely and effectively applied to treat HCC in liver transplant recipients.

COMPLICATIONS

According to the standardization of terminology and reporting criteria for image-guided tumor ablation by Goldberg *et al*[77], a major complication is an event that

Table 1 Major complications of microwave ablation in literature *n* (%)

	Liang *et al*[91]	Bertot *et al*[90]	Livraghi *et al*[92]	Ding *et al*[89]	Ding *et al*[89]	Lahat *et al*[93]
Major complications	2.6%	4.6%	2.9%	3.1%	2.7%	4.6%
Intra-peritoneal bleeding	1 (0.03)	NA	2 (0.3)	2 (0.31)	NA	NA
Portal vein thrombosis	NA	NA	NA	0.15 (1/654)	NA	NA
Bile leakage	NA	NA	NA	2 (0.31)	NA	NA
Biloma	1 (3)		NA	1 (0.15)		
Bile duct injury	1 (3)		1 (0.1)	1 (0.15)		
Obstructive jaundice	NA		1 (0.1)	NA		
Liver disfunction	NA	NA	3 (0.4)	4 (0.61)	2 (2)	NA
Liver abscess	4 (13)	NA	1 (0.1)	1 (0.15)	NA	NA
Gastrointestinal perforation	2 (7)	NA	2 (0.3)	NA	NA	NA
Haemothorax	NA	NA	1 (0.1)	1 (0.15)	NA	NA
Intractable pleural effusion	NA	NA	3 (0.4)	5 (0.76)	1 (0.8)	NA
Right diaphragmatic hernia	NA	NA	NA	2 (0.31)	1 (0.8)	NA
Pneumothorax	NA	NA	1 (0.1)	NA	NA	NA
Tumor seeding	5 (16)	NA	1 (0.1)	NA	NA	NA

NA: Data not available.

leads to substantial morbidity and disability, increasing the level of care, or results in hospital admission or substantially lengthened hospital stay. All other events are considered minor complications.

As stated by literature data, no statistically significant difference in mortality rates, neither major nor minor complications between the RFA and MWA is detected[89]; in particularly, microwave ablation-associated mortality ranges from 0% to 0.36%, showing that it can be considered a safe technique for the treatment of liver tumors[90-93]. With respect to major complications, data from meta-analysis of comparative studies between RFA and MWA shows that there are not significant differences between the 2 ablative techniques. However, it should be pointed out that there are still few studies focused on a large number of patients, and data are collected both from randomized and observational studies: These bias are still too strong to making a solid conclusion[77].

Major complications can be divided in vascular, biliary, mechanical, infectious and functional.

Vascular complication includes bleeding and thrombosis; bleeding complications (intra-peritoneal bleeding, intra-hepatic haematomas) are mainly due to injury of blood vessels during ablation caused by mechanical trauma with the needle or by of an indirect thermal damage by tissue coagulation and necrosis. To avoid these complications, patients with severe coagulation dysfunctions should not be treated. Moreover, the complete cauterization of the needle track can reduce the risk of major bleeding.

Portal thrombosis that can lead to portal hypertension and liver failure, can occur when the ablated area is close to the portal vein, where blood flow is often already slow due to cirrhotic disease: this condition reduces the "heat-sink" effect that normally protects the vessel wall, through the cooling property of the blood flow.

Bile duct injuries, as bile leakage, biloma formation and obstructive jaundice, mostly occur while treating lesions adjacent to the bile ducts. While bile leakage is often transient, biloma has a high risk of secondary infection, and it should be promptly treated with catheter drainage and antibiotics. Obstructive jaundice can be caused by biliary injury at the porta hepatis and should be treated with stent placement.

Perforation of the gastrointestinal wall related to thermal injury can occur while treating lesions adjacent to a gastrointestinal lumen (*i.e.,* subcapsular lesions or nodules of the left lobe), more frequently with a percutaneous approach in patients with a history of abdominal surgery, intestinal adhesions and anatomical variations.

MWA of lesions adjacent to diaphragm can cause thermal damage, resulting in pleural effusion or, rarely, in diaphragmatic hernia. Moreover, through a percutaneous intercostal approach, the damage to the intercostal or diaphragmatic vessels during needle insertion could cause haemothorax.

Liver abscess is uncommon, but it can occur in high-risk patients, such as patients with diabetes, post-biliary-enteric anastomosis, duodenal sphincterotomy and biliary stent placement. In these categories of patients, a prophylactic antibiotic therapy should be considered to prevent infections.

Tumor seeding can occur when the lesion is near the liver surface, more often when a diagnostic biopsy is performed before the ablation. A complete needle track ablation with cauterization when the antenna is withdrawn may prevent tumor implantation.

Liver failure is more common after the ablation treatment of patients with Child-Pugh Score of B or above, and with multiple lesions.

Table 1 summarizes data of these complications found in literature.

Minor complications include asymptomatic pleural effusion not requiring drainage, liver decompensation requiring only home therapy, subcapsular hematoma, skin burns, slight thickening of the gallbladder wall, asymptomatic portal thrombosis, hemobilia, arterial-portal shunt. Periprocedural pain and fever are con-

sidered symptoms of post-ablation syndrome, and are related to the side of lesion (subcapsular or peri-hilar) and the amount of tissue necrosis.

To reduce the percentage of major complications, the selection of patients and the choice of either percutaneous or surgical approach are fundamental; high-risk patients for infections, coagulation disorders, previous abdominal surgery should be evaluated to establish the right cost-benefit rate of the procedure. Gastrointestinal perforation or thermal biliary injury should be avoided with the use of thermocouples to check the temperature, as to timely interrupt the procedure. Finally, the learning curve of the physicians and the improvement of MW antenna technology have considerably reduced complications due to thermal damage.

CONCLUSION

In conclusion, the recent improvement in ablation microwave technology has significantly improved clinical efficacy of this treatment. The devices of the latest generation allow to obtain faster and larger ablation areas than RFA. However, large-scale randomized prospective clinical trials comparing MWA and RFA are needed to determine the future clinical role of MWA.

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TOPIC HIGHLIGHT

2015 Advances in Hepatitis C virus

Ribavirin contributes to eradicate hepatitis C virus through polarization of T helper 1/2 cell balance into T helper 1 dominance

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Abstract

The mechanism of action of ribavirin (RBV) as an immunomodulatory and antiviral agent and its clinical significance in the future treatment of patients with hepatitis C virus (HCV) infection are reviewed. RBV up-regulates type 1 and/or 2 cytokines to modulate the T helper (Th) 1/2 cell balance to Th1 dominance. Examination of co-stimulatory signaling indicated that RBV down-modulates inducible co-stimulator on Th cells, which contributes to differentiating naïve Th cells into Th2 cells while reducing their interleukin-10 production. The effects on T-regulatory (Treg) cells were also investigated, and RBV inhibited the differentiation of naïve Th cells into adaptive Treg cells by down-modulating forkhead box-P3. These findings indicate that RBV mainly down-regulates the activity of Th2 cells, resulting in the maintenance of Th1 activity that contributes to abrogating HCV-infected hepatocytes. Although an interferon-free treatment regimen exhibits almost the same efficacy without serious complications, regimens with RBV will be still be used because of their ability to facilitate the cellular immune response, which may contribute to reducing the development of hepatocellular carcinogenesis in patients infected with HCV.

Key words: Ribavirin; Forkhead box-P3; T helper 1/2 cell balance; T-regulatory lymphocytes; Inducible co-stimulator; Interleukin-10; Hepatitis C virus infection

Core tip: Ribavirin has the potential to regulate the T-helper (Th) 1/2 cell balance into Th1 dominance by modulating the co-stimulatory signaling between antigen-presenting cells and naïve Th cells as well as the inhibitory activity of T-regulatory cells. These are considered useful in treating hepatitis C virus infection, especially to inhibit hepatocellular carcinoma development.

Nakatsuka K, Atsukawa M, Shimizu M, Takahashi H, Kawamoto C. Ribavirin contributes to eradicate hepatitis C virus through polarization of T helper 1/2 cell balance into T helper 1 dominance. *World J Hepatol* 2015; 7(25): 2590-2596 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i25/2590.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i25.2590

INTRODUCTION

About 185 million people worldwide are estimated to be infected with hepatitis C virus (HCV)[1]. Eighty percent of HCV-infected patients will progress to persistent infection[2,3], and 15%-30% of these will develop cirrhosis over a 25- to 30-year period[4]. In addition, hepatocellular carcinoma (HCC) occurs in 8% of cirrhotic patients annually[5-7]. Because persistent HCV infection is closely related to the development of HCC[8,9], the elimination of HCV contributes markedly to preventing the development of this form of cancer[10,11].

The treatment strategy for HCV infection has improved during the 25 years since the introduction of interferon (IFN) therapy. In particular, the rate of persistent elimination of the HCV genotype 1, which was considered to be IFN resistant, improved markedly from 8% to 45% with the introduction of pegylated (PEG)-IFN treatment in combination with ribavirin (RBV)[12]. In addition, the administration of direct antiviral agents (DAAs) with PEG-IFN plus RBV therapy greatly improved treatment efficacy[13-15]. Currently, about 90% of persistent HCV infection can be eliminated by administering the IFN/RBV/DAA regimen.

In 2014, an IFN-free regimen featuring a combination of the HCV-NS5A inhibitor daclatasvir (DCV) and NS3/4A protease inhibitor asnaprevir (ASV) was approved. This IFN-free regimen appears to have efficacy similar to that of the previous standard regimen without serious side effects[16,17]. Thus, IFN-free regimens will play a leading role in future HCV treatment. However, the potential of RBV to modulate the immune response is considered useful in treating HCV infection, especially to inhibit HCC development[18-22]. This paper reviews the immunological activity of RBV and considers the clinical significance of this antiviral agent in future HCV treatment.

IMPORTANCE OF THE HOST CELLULAR IMMUNE RESPONSE IN ELIMINATING HCV

Abrogation of infected cells is necessary to eliminate persistent viral infection, and up-regulation of the host cellular immune responses triggered by the activation of T helper (Th) 1 cells is thought to be essential for eliminating persistent HCV infection[23-26]. Among the various mechanisms by which IFN eradicates viruses, modulation of the host immune system may be critical, along with its antiviral activity[27]. IFN could enhance host immune responses *via* the activation of natural killer cells, $CD4^+$ Th cells, and $CD8^+$ cytotoxic T cells and the up-regulation of major histocompatibility complex molecule expression to stabilize the presentation of antigeneic epitopes from the infected cells[28]. Unfortunately, although it has these abilities for modulating immune systems, IFN monotherapy shows only limited efficacy against HCV infection. Numerous investigations have attempted to elucidate why IFN alone fails to eliminate HCV, and it appears that HCV can escape[29] or inhibit[30] the host immune response to establish persistent infection. Hence, additional techniques were needed to enhance the host cellular immune response.

EFFECTS OF RBV ON THE TH1/2 CELL BALANCE

The synthesized purine nucleotide analogue RBV, developed as antiviral reagent[31,32], is well known to contribute to HCV elimination in combination with IFN[33]. The mechanism of action of RBV is not fully understood, and it has been reported to: (1) induce viral RNA-error catastrophe[34]; (2) inhibit RNA polymerase[35]; (3) reduce RNA pooling *via* nicotinamide adenine dinucleotide phosphate inhibition[36,37]; and (4) alter the Th1/2 balance to Th1 dominance[38,39]. Among the putative mechanisms of the enhancement of viral elimination by RBV, it is notable that RBV polarizes the Th cell balance into Th1 cell dominance because this supports the importance of the activation of the host cellular immune response in eliminating HCV. However, it remains unclear how RBV modulates the Th1/2 balance. Many groups examined the effects of RBV on type 1 and 2 cytokine production from T lymphocytes. Some reported that RBV directly up-regulates Th1 cells through the activation of type 1 cytokines, such as interleukin (IL)-12[40-42]. In contrast, others indicated that RBV may maintain Th1 cell activity through interference with immunosuppressive cytokines such as IL-4 or IL-10[43-46]. From the viewpoint of type 1 and 2 cytokine profiles, it remains controversial whether RBV up-regulates Th1 cells directly or indirectly through the inhibition of Th2 cell activity.

POTENTIAL OF RBV TO MODULATE CO-STIMULATORY SIGNALING

The importance of co-stimulatory signaling to determine the differentiation of naïve Th cells into Th1 or 2 cells is well established[47]. The signaling from CD80 on professional antigen presenting cells (APCs) to its counterreceptor CD28 on $CD4^+$ T cells promotes differentiation of naïve Th into Th1 cells[48]. On the other hand, the signaling from *B7-H2* on APCs to its counterreceptor inducible co-stimulator (ICOS) on $CD4^+$ T cells promotes differentiation into Th2[49]. It would be interesting to determine whether RBV exerts specific effects on co-stimulatory signaling, although

only a few reports have addressed this aspect. Cheng *et al*[50] reported that CD28 was up-regulated by IFN plus RBV therapy in both treatment responders and nonresponders. Atsukawa *et al*[51] demonstrated that RBV down-modulates ICOS on human $CD4^+$ T cells, which is associated with decreased IL-10 production. They also examined the modulation of type 1/2 cytokine fluctuations in the small cohort of patients who received IFN plus RBV treatment and their results indicated that IL-10 production from $CD4^+$ T-cells was decreased in patients whose ICOS were down-modulated by the therapy, which was closely associated with persistent HCV elimination without changing CD28 expression and IFN-γ secretion levels. These results indicated that RBV mainly contributes to inhibiting the differentiation of naïve Th cells into Th2 cells to maintain the activity of Th1 cells by inhibiting stimulation-inducible molecules on the surface of $CD4^+$ T cells. However, these results do not fully explain the role of RBV because other important factors play a role in Th1/2 cell modulation.

POTENTIAL OF RBV FOR MODULATING T-REGULATORY CELL ACTIVITY

It is well known that the activation of host T-regulatory (Treg) cells is critical to allow persistent HCV infection[52]. Treg cells, found at first as antigen-specific inhibitors of autoreactive T lymphocytes[53,54], can identify as $CD4^+CD25^+$, and intracellular forkhead box-P3 (FOXP3)-expressing T cells. Subsequent detailed examinations revealed that the Treg family includes various subpopulations such as naturally occurring Treg (Tr^{nat}), adaptive Treg (Tr^{adapt}), Treg, and Th3 cells[55-58]. Tr^{nat} cells differentiate in the thymus and exhibit inhibitory activity against autoreactive Th cells in a cell contact-dependent fashion, which plays an important role in regulating the autoimmune response[59]. Tr^{adapt} cells differentiate from naïve Th cells under the influence of Tr^{nat} cells in the periphery and exhibit inhibitory activity in a humoral element-dependent fashion[60]. The orchestration of these Treg cells could modulate antigen-specific Th1 activity in the later phase of exogenous pathogen infections to switch the dominant immune response from cellular to humoral[61]. These phenomena play a role in the termination of excessive activation of the Th1 response against exogenous antigens[62]. In addition, Tr1 and Th3 cells exhibit inhibitory activity against Th1 cells in a humoral element-dependent fashion[56-58].

According to previous reports, the effect of antiviral therapy on the activity of Treg cells remains uncertain[63,64]. Recently, Kobayashi *et al*[65] examined the effects of RBV on the inhibitory activity of Treg cells *in vitro* and found that it down-modulates the inhibitory activity of peripheral $CD4^+CD25^+CD127^-$ T cells (= $FOXP3^+$ Treg cells). Intracellular FOXP3 expression of $CD4^+CD25^-$ T cells decreased when they were incubated with RBV-preincubated Treg cells. In addition, RBV reduced the inhibitory effect of Treg cells in an IL-10-dependent, but not tumor growth factor (TGF)-β-dependent, manner[65]. These data indicate that RBV-treated Treg cells would lose their ability to differentiate naive Th cells into Tr^{adapt} cells. Moreover, the activity of IL-10-dependent Treg cells such as Tr^{adapt} and Tr1 was mostly inhibited in the presence of RBV. Although that in vitro study clearly indicated the effects of RBV against Treg cells, it remained controversial whether RBV could regulate Treg cells in clinical studies. Langhans *et al*[66] showed that the activity of Treg cells was down-modulated in the clinical course of HCV treatment with PEG-IFN and RBV. In contrast, Lee *et al*[67] found that RBV did not impair the inhibitory activity of Treg cells. More detailed studies are needed to confirm the effects of RBV on Treg-cell activity *in vivo*.

Based on these findings, RBV may indirectly maintain and/or up-regulate Th1 activity by inhibiting the differentiation of naive Th cells into Th2 cells. The potential to down-modulate the inhibitory activity of Treg cells would be closely associated with this regulatory cascade. The potential mechanism by which RBV modulates the Th1/2 balance-regulatory cascade is shown in Figure 1. Because both ICOS and FOXP3 are enhanced after cell stimulation, it is also possible that RBV affects the expression of these inducible factors. Further studies are needed to elucidate how RBV is associated with these intracellular signaling pathways.

RBV MAY MODULATE INTRACELLULAR SIGNALING TO INHIBIT EXPRESSION OF FOXP3

The results of various studies indicated that RBV affects intracellular signaling, contributing to the elimination of HCV. However, it remains unclear how RBV modulates intracellular signaling to inhibit the differentiation of Th cells into Tr^{adapt} cells. Some investigators reported that RBV promotes signal transducer and activator of transcription (STAT) 1 and 3 phosphorylation[68,69]. In addition, overexpression of STAT-3 in suppressor of cytokine signaling (SOCS)-1-knockout murine Treg cells led to the down-modulation of FOXP3 expression[70]. Although no report directly demonstrated the relationship between SOCS-1 and FOXP3, one possible hypothesis is that RBV promotes STAT3 phosphorylation *via* interference with SOCS1, which leads to the suppression of FOXP3, with resultant inhibition of the differentiation of naïve Th cells into Tr^{adapt} cells. RBV can also reduce intracellular RNA pooling by suppressing inosine-5'-monophosphate dehydrogenase activity[71], which appears to support this hypothesis.

CLINICAL USEFULNESS OF RBV IN THE FUTURE TREATMENT OF HCV

As described above, the agonistic effects of RBV on the cellular immune response plays a major role in the elimination of HCV-infected hepatocytes in combination with IFN and protease inhibitors. Because this effect

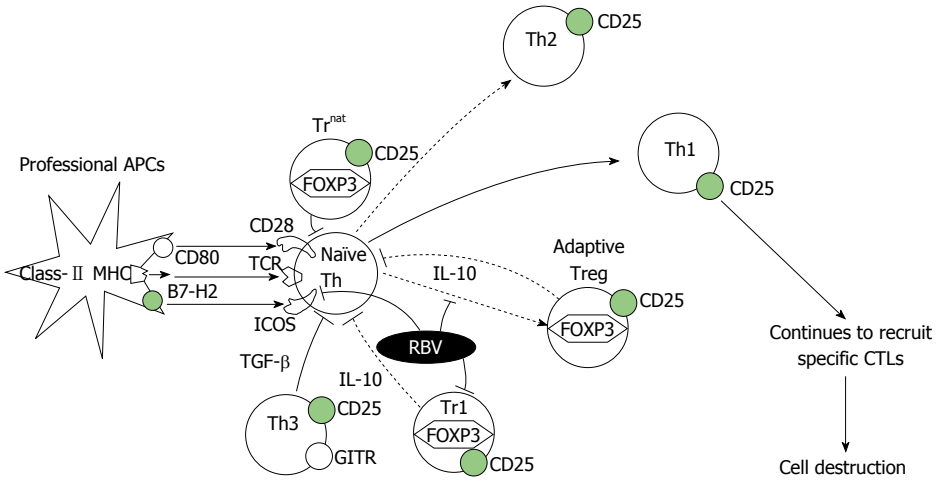

Figure 1 Schema of the potential mechanism of action of ribavirin on T-regulatory cells in the T helper 1/2-regulatory cascade. RBV interferes with FOXP3 expression in naïve Th cells, making them unable to differentiate into adaptive Treg cells. RBV also disrupts the inhibitory activities of adaptive Treg and Treg 1 cells by suppressing their IL-10 production. In addition, RBV down-modulates ICOS, expressed on naïve Th cells after stimulation, to inhibit the differentiation of naïve Th cells in to Th2 cells. The combination of these affects may contribute to maintain Th1 activity against exogenous antigens, which would contribute to the elimination of HCV-infected cells *via* the activation of specific CTLs. RBV: Ribavirin; ICOS: Inducible co-stimulator; IL: Interleukin; HCV: Hepatitis C virus; CTL: Cytotoxic T cell; FOXP3: Forkhead box-P3; Treg: T-regulatory; Th: T helper; TGF: Tumor growth factor; MHC: Major histocompatibility complex; TCR: T cell receptor; GITR: Glucocorticoid-induced tumour-necrosis-factor-receptor-related protein; APC: Antigen presenting cells.

will promote the autoimmune response, therapeutic regimens featuring RBV may be not recommended for patients with certain autoimmune disorders. On the other hand, because the role of the cellular immune response is important in cancer immunological surveillance, this ability of RBV may be indispensable to protect HCC development after eliminating HCV. However, IFN-free regimens will be the main strategy for the treatment of HCV infection in the near future because their efficacy in eliminating the virus is equivalent to that of IFN-based regimens without serious complications. Although it is still controversial whether IFN-free regimens can protect against HCC to the same degree as IFN and RBV-based regimens, serum alpha-fetoprotein levels were reduced in patients administered the DAA regimen featuring DCV and ASV, indicating that IFN-free regimens could also decrease the rate of HCC development. Therefore, the clinical significance of RBV-featuring regimens will be limited. However, it is still interesting whether combining RBV and DAA therapy can decrease the development of HCC more. Large-scale clinical studies will be needed to establish the efficacy of RBV for protecting HCC.

CONCLUSION

This review of the potential of RBV as a modulator of the Th1/2 balance cascade indicates that the agonistic effects of this antiviral agent on Th1 activity contributes greatly to the elimination of HCV in combination with IFN and protease inhibitors. In addition, it is possible that RBV may assist in the prevention of HCC development by accelerating cancer immunological surveillance.

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MINIREVIEWS

Dietary supplements and pediatric non-alcoholic fatty liver disease: Present and the future

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children. High prevalence of pediatric obesity and sedentary lifestyle has augmented the incidence of NAFLD in children. Obesity is associated with an increased risk of NAFLD through various mechanisms such as intensification of insulin resistance and increased levels of inflammatory markers. There is no approved medical intervention for treatment of pediatric NAFLD; the only proven strategy in management of pediatric NAFLD is lifestyle modification. Recently, the effects of nutritional supplements have been examined in the management of pediatric NAFLD. The purpose of this review is to summarize the studies evaluating the effects of nutritional supplements on pediatric NAFLD and explain the future direction in this field.

Key words: Pediatric non-alcoholic fatty liver disease; Diet; Nutrition; Dietary supplement; Fatty liver

Core tip: The purpose of this review is to report the existing evidences and future directions on dietary supplements that can be used for management of pediatric nonalcoholic fatty liver disease. Also we tried to explain the properties of these supplements and needs for future studies.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in children. This disorder includes a wide spectrum from a simple steatosis with the mortality rate lower than one percent to a critical illness such as cirrhosis and hepatic fibrosis that may ultimately lead to hepatocellular carcinoma and death[1,2]. If NAFLD is not treated at early stages, it can progress to nonalcoholic steatohepatitis (NASH), which is characterized by lobular inflammation and hepatocellular injury which may definitely progress to cirrhosis and liver fibrosis[3]. Coincident with the rise in prevalence of childhood obesity over the past few decades, NAFLD has become the leading cause of liver disease in children. Insulin resistance and oxidative stress are involved in the pathogenesis of NAFLD[4].

PREVALENCE

Several conditions such as obesity, sedentary lifestyle and the use of high-calorie foods have caused a sharp increase in the NAFLD prevalence[5]. The prevalence of NAFLD is different from 5% to 20% in general population and above 40% in obese and type 2 diabetes patients[6,7]. Also, its prevalence is different between the men and women and increase in the older people. According to the recent studies, NAFLD prevalence in normal children is 3%, rising up to 50%-70% among obese children[8]. The prevalence in developed countries also increased remarkably from 16.9% to 23.8% for boys and from 16.2% to 22.6% for girls, while in developing countries increased from 8.1% to 12.9% for boys and from 8.4% to 13.4% for girls. In most of the communities, parallel with the rising in prevalence of the obesity, and chronic diseases such as diabetes and dyslipidemia, the prevalence rate of the NAFLD is also increasing[9].

According to the American Association for the Study of Liver Disease guideline, liver biopsy is a gold standard for diagnoses of NAFLD[10]; however, because of the invasive nature of this method, it is usually recommended to use the other methods such as magnetic resonance spectroscopy and Fibroscan for screening and staging of this disease[11].

PATHOPHYSIOLOGY

Pathogenesis of NAFLD is closely associated with obesity and insulin resistance[12,13]. Obesity especially abdominal obesity can lead to insulin resistance. Insulin plays various roles in metabolism such as oxidative distress and carbohydrate metabolism. In obesity and some chronic diseases such as type 2 diabetes and dyslipidemia, insulin cannot function properly, so lipolysis pathway will be activated leading to an increased influx of free fatty acids to the liver[14]. Also, several studies have confirmed that endogenous lipid synthesis is increased in patients with NAFLD[15]. Hepatic fat derivatives such as malondialdehyde (MDA) can contribute in the progression of NAFLD[16,17]. In addition, the expression of certain proinflammatory cytokines such as tumor necrosis factor (TNF)-α increases in obese people, which can cause insulin resistance through deterioration of insulin receptors[15].

Free fatty acids that released from the adipose tissue can inhibit the Cytochrome P450 (an enzymatic system that involved in various pathways specially free fatty acids beta-oxidation), which results in the production of active oxygen radicals and depletion of body antioxidant reserves (such as glutathione and vitamin E)[18].

Moreover, genetic predisposition plays an important role in NAFLD pathogenesis. The genes involved in the mitochondrial and fatty acids metabolism, affect the disease progression[19].

MANAGEMENT OF NAFLD

One of the main strategies in the management of pediatric NAFLD is modification of life style and dietary pattern. Several studies have found that gradual weight loss and regular physical activity can help in the treatment of NAFLD through improving the insulin sensitivity, reduction in the body inflammation and resolution of hepatic steatosis[20-22]. It should be considered that weight loss in obese children (5% to 10% of basal weight) must be quite gradual and the diet must be balanced[23].

The result of pharmacological interventions in treatment of pediatric NAFLD is contradictory. The main family of medications that evaluated for treatment of pediatric NAFLD are insulin sensitizers specially metformin[24]. These medications play a positive role in the treatment of NAFLD by improving insulin sensitivity; however, these beneficial effects have not been shown in long term treatment[25]. Recently, the beneficial effects of some supplements specially vitamin E[9], probiotics[26] and omega-3 fatty acids[27] in NAFLD management have been shown.

The purpose of this review is to report the effectiveness of some dietary supplements in the management of the pediatric NAFLD and future directions in this field.

LITERATURE STUDY

A MEDLINE, PubMed and Cochrane Review database search used a combination of keywords, including NAFLD, pediatric, treatments, lifestyle changes, NASH, supplements, nutrition, vitamins, minerals, vitamin E, vitamin D, polyunsaturated fatty acids, probiotic, symbiotic, polyphenols, curcumin, resveratrol, quercetin, anthocyanin, herbal medicine, green tea polyphenols, cinnamon, ginger and their related MeSH terms. The articles were selected based on their relevance to the review.

POLYNUNSATURATED FATTY ACIDS

Several studies have evaluated the beneficial effects of Omega-3 long-chain polyunsaturated fatty acids on the treatment of NAFLD[27]. Janczyk *et al*[28] have shown that supplementation with long chain omega-3 for 6 mo resulted in a significant decrease in aspartate aminotransferase and gamma-glutamyl transpeptidase levels compared with the control group; however, it did not significantly reduce the levels of alanine amino-transferase (ALT) and liver steatosis. Nobili *et al*[29] have reported that Docosahexaenoic acid (DHA) supplementation for 24 mo resulted in a significant reduction in triglyceride and ALT levels and improved liver steatosis compared with the control group. As noted, one of the major risk factors that involved in NAFLD pathogenesis is insulin resistance[13]. Nobili *et al*[30] have shown that DHA increased insulin sensitivity and decreased triglyceride levels in children with NAFLD. Di Minno *et al*[27] have also proposed that omega-3 supplementation has a therapeutic effect on pediatric NAFLD. Also in another study, St-Jules *et al*[31] have shown that lack of fish and long-chain omega-3 fatty acid intake in children was associated with greater portal and lobular inflammation in children with NAFLD. It seems that omega-3 fatty acids supplementation has beneficial effects on pediatric NAFLD through its anti-inflammatory and insulin sensitizer effects; however, no study has examined its long term effects.

Vitamin E

Several previous studies have evaluated the beneficial effects of vitamin E, as a potent antioxidant, in the management of pediatric NAFLD. Nobili *et al*[32] prescribed vitamin E in children with NAFLD and observed a similar decrease in the levels of ALT and HOMA-IR in the intervention and control groups after 12 mo. A large, well designed, clinical trial[25] has shown that vitamin E is not superior to placebo in attaining the sustained reduction in ALT level in patients with pediatric NAFLD after 96 wk; however vitamin E could significantly reduce the ALT levels at weeks 24, and 48. Moreover, it could significantly improve the histopathological features of the disease at week 96. Finally, Sarkhy *et al*[33] in a systematic review and meta-analysis have shown that vitamin E supplementation decreases level of ALT. It seems that vitamin E can improve the characteristics of pediatric NAFLD and specially NASH patients in short time, but not in the long term supplementation.

Prebiotics and probiotics

Probiotics are among the major supplements which have attracted the attention of many investigators regarding their application for the treatment of NAFLD and NASH[34]. Probiotics can modify some NAFLD risk factors such as insulin resistance, liver fat and oxidative stress[26,35-37]. Prebiotics are chemicals that induce the growth or activity of microorganisms. In some animal studies, researchers have shown that mice fed with prebiotic products exhibited a lower plasma of inflammatory markers such as TNF-α and interleukin-1α in comparison to controls[38]. Some of the prebiotics such as lactulose and galacto-oligosaccharides can promote the growth of certain intestinal bacteria such as Lactobacillus and down-regulate levels of inflammatory factors. In a clinical trial, researchers showed that supplementation with oligofructose decreased levels of liver enzymes specially ALT[39]. Alisi *et al*[40] performed a clinical trial and prescribed VSL#3 [a mixture of eight probiotic strains including: *Streptococcus thermophilus*, *Bifidobacteria* (*B. breve*, *B. infantis*, *B. longum*), *Lactobacillus acidophilus*, *L. plantarum*, *L. paracasei*, and *L. delbrueckii* subsp. *Bulgaricus*] in children with NAFLD for four months and evaluated changes in fatty liver severity after 4 mo as detected by ultrasonography. The results of this study showed that probiotic supplementation reduced the severity of NAFLD but it did not cause significant differences in levels of triglyceride, ALT and HOMA-IR in comparison to the placebo group. The authors discussed that this beneficial effect is due to the increased levels of glucagon-like peptide 1. Vajro *et al*[41] proposed that supplementation with *Lactobacillus* rhamnosus strain GG decreases the level of ALT in children with persistently elevated aminotransferases and echogenic livers. Further clinical trials are needed to find the best combination of probiotics for achieving the best results from supplementation.

Vitamin D

Vitamin D is a crucial nutrient for children's health and development[42]. Several studies have reported the vitamin D deficiency in obese children[43]; on the other hand, NAFLD is more prevalent in obese children than normal weight children[8]. These data urged the investigators to evaluate the association of plasma vitamin D levels with NAFLD pathogenesis. Misra *et al*[43] evaluated the vitamin D level in children with biopsy-proven NAFLD. They reported a high prevalence of vitamin D deficiency in children with NAFLD; however, they did not find any association between vitamin D deficiencies and the severity of NAFLD. Black *et al*[44] analyzed the association of vitamin D and NAFLD severity in adolescents with NAFLD; they have reported a high vitamin D deficiency in these patients. In addition, they showed an inverse association between serum vitamin D levels and severity of NAFLD. In a similar study, Nobili *et al*[45] have demonstrated a reverse association between vitamin D level in serum and severity of NAFLD and fibrosis in 73 children with elevated serum aminotransferase levels and hyperechogenic liver on ultrasonography.

Little is known about the effect of vitamin D supplementation on NAFLD in children; however, a clinical trial in adults resulted in a reduction in MDA and hs-CRP levels without any significant change in ALT levels[46]. Conducting clinical trials in children are needed.

Polyphenols

Polyphenols are plant-derived compounds which have been used for treatment of NAFLD due to their anti-

inflammatory and antioxidant properties[47]. One of the main members of polyphenols family is resveratrol; supplementation with resveratrol has been shown to be useful in reduction of the severity of the disease. It has been shown that resveratrol reduces the level of liver enzymes and inflammatory cytokines[48,49] and attenuates hepatic steatosis[50]. Other members of the polyphenols family such as curcumin[47], quercetin[51], anthocyanin[47] and green tea polyphenols[52] have been also indicated to have promising outcomes in the treatment of adults NAFLD. The beneficial roles of polyphenols in adults NAFLD propose its effectiveness in pediatric NAFLD. Thus, clinical trials in children are needed to confirm it.

INSULIN SENSITIZERS AND LIPID LOWERING AGENTS

Herbal medicine has been used in traditional medicine of many countries for treatment of different disorders[53]. Since these medications can improve the insulin sensitivity and lipid profile, they are proposed for treatment of NAFLD[54]. Several studies have shown satisfactory results from these insulin sensitizers and lipid reducing agents such as cinnamon, curcumin[55] and ginger[56] in the management of NAFLD; however, studies have not conducted to examine the effects of these supplements on pediatric NAFLD. More extensive investigations are needed to fully determine the therapeutic potential of these components for treating NAFLD.

CONCLUSION

Due to the sharp rise in obesity prevalence in children and a dramatically increase in pediatric NAFLD prevalence, therapeutic strategies are urgently needed. Dietary supplements play a pivotal role in pediatric management through insulin sensitizer, anti-oxidant and anti-inflammatory properties. In recent years, the beneficial effects of some of these supplements have been shown; however, several of them have not yet been evaluated. Further clinical trials are needed to support the use of supplements, either as preventative or therapeutic agents in pediatric NAFLD.

More studies related to the pediatric NAFLD performed to assess changes in children lifestyle[57]. Recently, the beneficial effects of nutritional supplements have been shown in both pediatric and adult NAFLD[9]; however, several of these supplements' effects are assessed only in adults. Thus, evaluating the effects of these potentially beneficial supplements in pediatric NAFLD is highly recommended.

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MINIREVIEWS

Hepatocellular adenoma: An update

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Abstract

Hepatocellular adenomas (HCA) are rare benign liver tumors. Recent technological advancements have helped in the early identification of such lesions. However, precise diagnosis of hepatocellular incidentalomas remains challenging. Studies at the molecular level have provided new insights into the genetics and pathophysiology of these lesions. These in turn have raised questions over their existing management modalities. However, the rarity of the tumor still restricts the quality of evidence available for current recommendations and guidelines. This article provides a comprehensive review on the etiology, molecular biology, pathophysiology, clinical manifestations, and complications associated with HCA. It also elaborates on the genetic advancements, existing diagnostic tools and current guidelines for management for such lesions.

Key words: Liver adenoma; Focal liver lesion; Benign liver lesion

Core tip: Hepatocellular adenomas despite being benign liver neoplasms often pose diagnostic and therapeutic challenges. Studies at the molecular level have provided new insights into the genetics and pathophysiology of these lesions. These in turn have raised questions over their existing management modalities. This article provides a comprehensive review and update on the topic.

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INTRODUCTION

Hepatocellular adenomas (HCA) are rare monoclonal benign liver tumors of presumable epithelial origin that usually develops in healthy liver. It constitutes 2% of all liver neoplasms; with an incidence of 3/1000000 per year in Europe and North America[1]. In spite of recent technological and radiological advances, these seemingly benign lesions often pose diagnostic challenges. The advancements at molecular levels that predict the clinical courses of these lesions on the basis of their genotypic

and phenotypic characteristics have further complicated the management of such lesions.

ETIOLOGY AND PRESENTATION

The true prevalence of hepatocellular adenoma is not easy to access as uncomplicated adenomas often lack symptoms. Often they are encountered as incidental findings in patients undergoing radiological work up for unrelated or non-specific symptoms. Majority (70% to 80%) of HCA are solitary, and are usually located in the right liver lobe[2]. They vary in size from 1 to 30 cm. Patients with larger tumors are more likely to report with symptoms such as vague abdominal pain. A palpable intra-abdominal mass or enlarged liver may be noted in < 30% of the cases[2]. Jaundice if present is usually due to pressure effect of the tumor on intrahepatic biliary system. Elevation levels of gamma glutamyl transferase and alkaline phosphatase may be noted in some patients. High serum alpha-fetoprotein should raise suspicion for malignant changes. Hemorrhage and malignant transformation remain the most notorious complications associated with these lesions. Bleeding from a ruptured HCA is more anticipated with large sub capsular lesions, particularly during pregnancy.

With the widespread use of oral contraceptives (OCPs) in the 70's the annual incidence of HCAs had reached approximately 3-4 women per 100000 users[3]. Over the years, a reduction in drug potency has reduced their frequency to about 1/100000. In comparison, the annual incidence in women who have never used OCPs is nearly 1/1000000[3]. The role of estrogen in the development of these lesions and its causal relationship has been well established through multiple studies. The natural history of HCAs closely corresponds with the dose and duration of oral contraceptive used. HCAs tend to regress with the withdrawal of estrogen therapy. However those that don't regress can be explained by the variable expression of estrogen receptors (26%-73%) in these lesions. Recent reports have noted epidemiological differences in HCA patients between East and West. The female preponderance and degree of association with oral contraceptive use was reportedly absent in the Eastern population[4].

Similar to OCPs, use of anabolic steroids predisposes the emergence and growth of HCA, as well as, tends to regress with their withdrawal. Anabolic steroids are used primarily for the treatment of Fanconi syndrome, impotence, to gain muscle mass and in transsexuals. In general, adenomas secondary to exogenous hormone therapy are single, large and encapsulated. In addition to exogenous hormone therapy, high levels of endogenous androgens or estrogens puts both sexes at risk of developing HCA.

Type Ⅰ and type Ⅲ Glycogen storage diseases (GSD) result in impaired glycogenesis and excessive hepatic intracellular glycogen deposits. Both are inherited autosomal recessive disorders. HCAs are seen in nearly 22%-75% of patients with type Ⅰ disease compared to 4.4%-25% of the patients with type Ⅲ disease[5]. They usually occur in second decade, with a higher proportion among males (2:1). They are typically small, multiple and un-encapsulated. Type Ⅰ GSD is usually associated with inflammatory subtype of HCA[6]. The possibility of malignant conversion is minimal in type Ⅲ as compared to nearly 10% in type Ⅰ GSD[5]. Disease regression has been noted in patients adhering to specific diet (continuous nocturnal feeding) with correction of insulin, glucose, and glucagon levels[7].

In the Western population, obesity and related metabolic syndrome manifestations such as diabetes mellitus, insulin resistance, dyslipidemia and high blood pressure are becoming increasingly postulated as risk factors for development and progression of HCA[8]. Men with metabolic syndrome are at a much higher risk (10 times more likely than females) for malignant transformation[9].

GENETICS AND PATHOPHYSIOLOGY

Advancements in molecular biology have enabled classification of hepatic adenomas into 4 major subgroups based on genotypic and phenotypic characteristics[10]: (1) Adenomas inactivated for hepatocyte nuclear factor 1α: They account for 35%-50% of HCA's and are considered the least likely to undergo malignant transformation (15%)[10,12]. This subtype involves biallelic inactivation of *TCF1* gene[11]. Mutations are predominantly somatic (85%) with remaining 15% being in part somatic and in part hereditary. It is associated with marked steatosis, metabolic disease and absence of liver fatty acid binding protein expression. These also lack cytologic abnormalities or inflammatory infiltrates[10,12]; (2) β-catenin activated adenomas (β-HCA): These mutations account for 15%-18% of HCA's and is highly correlated with malignant transformation into hepatocellular carcinoma (HCC)[10,12]. *CTNNB1* gene alterations, Exon 3 deletions and changes in amino acid chain mostly characterize these mutations. Their development has been linked to male sex, androgenic hormone administration or glycogenesis. These mutated liver cell adenomas overexpress β-catenin (nuclear and cytoplasmic) and glutamine synthetase. They have cytoplasmic changes and lower rates of steatosis; (3) Inflammatory adenomas: These represent the most common subtype (40%-55%). Hepatocellular proliferations noted in these lesions are secondary to sustained activation of janus kinase (involved in the JAK-STAT pathway). It is characterized by inflammatory changes such as sinusoidal dilatation, dystrophic arteries and dystrophic vessel ductular reaction and immunohistochemically positivity for serum amyloid A and C reactive protein[13]. It is more common in females on OCPs, but has also been associated with obesity and alcohol abuse. Telangiectatic hepatocellular adenoma which was once referred as telangiectatic focal nodular hyperplasia has now been reclassified under inflammatory HCA. Ten percent of these HCAs have been reported to harbour

mutations in the β-catenin gene are hence also at danger of malignant changes; and (4) Unclassified Adenomas: The unclassified type (< 10%) includes patients whose tumors did not exhibit genetic or inflammatory diseases[13]. It usually is a diagnosis of exclusion.

Several other genetic markers are being studied to clarify their roles in the pathogenesis of hepatic adenomas. These include micro RNAs (miR-224, miR-122a, miR-107, miR-375), tumor suppressor proteins (p16INK4a, p14ARF), *APC* gene and interleukin-6-Gp130 signaling pathway[14].

DIAGNOSIS AND ROLE OF IMAGING

Accurate diagnosis of HCA is sometimes not feasible with a single diagnostic study. Often the clinical setting is combined with imaging studies and/or surgical resection to enable a comprehensive diagnosis. When lesions do not exhibit typical radiological features characteristic for an adenoma, a definitive diagnosis is impractical; it can be, at most, strongly suspected. The definitive diagnosis then demands tissue examination either by biopsy or resection. Herman *et al*[15] reported a radiological diagnostic yield of 90% for HCA *vs* 77% for FNH.

Percutaneous biopsy of a suspected liver adenoma is not favored as this can induce bleeding and tumor dissemination. Further, it is often inaccurate and absence of abnormal tissue does not exclude malignancy. Charny *et al*[16] in his study reported about 33% accuracy rate for biopsies taken for suspicious liver lesions. Biopsy should be considered only if imaging is inconclusive and a biopsy would possibly bring about a change in management. With advancement at the molecular level, the role of preoperative biopsy could influence management of adenomas in the future to a greater degree based on their genetic pathophysiological outcome[17]. In the near future, the molecular biology of the tumor might guide management of such lesions.

Open/laparoscopic excision biopsy remains the gold standard method for diagnosis[1]. However, this modality is only used if there are doubts regarding the diagnosis after magnetic resonance imaging (MRI) and/or percutaneous biopsy. A common dilemma is the differentiation of an adenoma from focal nodular hyperplasia (FNH) and well differentiated HCC. Considering varying management strategies for the several liver lesions, the implications of a prompt and accurate diagnosis cannot be emphasized more.

Accurate tissue diagnosis may at times be tricky and challenging. Incorporating more recent tissue studies such as QBend 10- and erbB2-immunostaining, comparative genomic *in situ* hybridization, and fluorescence *in-situ* hybridization may help overcome this issue[18-21]. Microscopically adenomas typically appear as monotonous sheets of hepatocytes, which lack biliary structures, dysplasia or fibrosis.

Ultrasound

The sonographic features of HCAs lack specificity and could mimic other benign or malignant liver lesions[22]. Adenomas may appear well-demarcated and present as iso echoic, hypo echoic (20%-40%), or hyper echoic in up to 30%, often due to high lipid content of hepatocytes[22,23]. Central necrosis, intratumoral bleeding and calcifications may give rise to heterogeneous echogenicity. Necrosis may appear as hyperechoic areas with acoustic shadows. Focal fat sparing usually appear as a hypo echoic halo. Color Doppler may show the presence of intra tumoral vessels and peri lesional sinusoids in the absence of a central arterial signal, which can aid in distinguishing HCA from FNH[23]. Contrast-enhanced ultrasonography, when available can augment ultrasonic diagnosis.

Computed tomography

Multi-phase computed tomography (CT) angiography enables dynamic sequencing by obtaining images during both contrast and non-contrast phases. HCA characteristically shows early phase peripheral contrast enhancement and subsequent centripetal contrast enhancement during the portal venous phase[23,24]. Adenomas are typically well-demarcated and are isodense on non-contrast images. Post contrast, the lesion may become isodense and then hypodense. They are often heterogeneous due to hemorrhage, necrosis and fibrosis. Previous hemorrhagic areas may appear as calcifications in less than 10% of cases[24].

Magnetic resonance imaging

Most adenomas are heterogeneous on both T1 and T2 weighted sequences. Presence of enhanced T2 weight images that further enhance on gadolinium administration is highly characteristic of HCA[25]. Studies of late have tried to characterize these lesions into its four genotypic types based on MRI features. However their specificity is debatable, owing to the highly variable appearance of HCA. In comparison to the existing gadolinium-based contrast agents for MRI, gadobenate dimeglumine or gadoxetate disodium enhanced liver MRI (hepatobiliary specific contrast agents) have both renal and hepatobiliary clearance. Owing to variable contrast uptake observed in hepatobiliary phase, HCA (being hypointense) can be differentiated from FNH (being iso- or hyperintense on delayed imaging)[25].

Isotope scanning (Technetium Tc-99m sulfur colloid)

HCAs usually have very few to no Kupffer cells, which are mostly nonfunctional. Hence most adenomas do not take up technetium Tc-99m sulfur colloid leaving a "cold" spot in the liver[26]. However upto 23% of adenomas take up colloid uptake, making them indistinguishable from FNH (vivid tracer uptake).

Angiography

This modality is sparingly used to diagnose hepatic adenomas. HCAs demonstrate pattern of perfusion starting at the periphery in contrast to the central vessel seen in FNH[23]. However necrosis and hemorrhage

within the HCA may make these features less obvious.

COMPLICATIONS

Rupture/bleeding - How to deal?

Of all the complications associated with HCAs, bleeding is most common. Adenomas morphologically consist of dilated sinusoids, thin-walled blood vessels with minimal connective tissue support[1]. These compounded by the high pressure arterial flow and lack of substantial fibrous capsule make them prone to bleed. More recent studies have associated pregnancy, adenoma size > 3.5 cm, visualization of lesional arteries, left lateral lobe location, and exophytic growth as risk factors for spontaneous bleeding[27].

Bleeding can be subclassified as intra-tumoral, intra-hepatic or extra-hepatic (hemoperitoneum). The risk of spontaneous bleeding in HCA is ill-defined; however the reported rates range between 20% and 40%[1]. Bleeding in most cases are contained and < 10% report with life threatening hemodynamic instability. Intratumoral bleeding accounts for most patients who present with abdominal pain.

Over the past few decades, management of bleeding adenomas has changed drastically. Earlier, the threshold for emergency laparotomy and hepatic resection was very low, however with poor outcomes. Now, the trend is for a multidisciplinary approach to such events. Management of such patients is often dictated by the severity of the bleeding and hemodynamic status of the patient. Options include emergency resection, resection post hemodynamic stabilization or a delayed resection.

Emergency resection of ruptured hepatic adenomas is associated with a mortality of 5% to 10%, which drops to 1% when resection is elective[28]. With the development of minimally invasive locoregional treatments, selective arterial embolisation has increasingly been applied as initial treatment[29]. Apart from reducing mortality, this therapy facilitates hemodynamic stabilization and reduces extent of definitive elective resection.

Patients who present with hemodynamic instability secondary to ruptured HCA carry mortality risk of up to 20%. Severe hemodynamic instability or failure of interventional radiology may warrant emergency laparotomy for damage control and control of hemorrhage. Emergency hepatic resection in this setting is often debated with opponents of this advocating time for hematoma to resorb before undertaking formal resection[30].

Malignant transformation

The precise risk of HCA transforming into carcinoma is little known. Stoot *et al*[31] in a systematic review noted an overall risk of transformation to HCC to be 4.2%. Prolonged use of exogenous steroid therapy, male gender, increasing size, type I GSD, and adenomas harboring β-catenin mutations are high risk factors for malignant transformation[12,32]. Elevated α-fetoprotein levels may raise suspicion for malignant conversion; however serve as poor indicators of tumor progression.

There is considerable debate on the pathophysiology and genetics involved in HCC arising from hepatic adenomas. Histopathologic evidence of HCCs occurring within regions of otherwise typical adenomas and molecular level evidence of the same nucleotide mutation of β-catenin in the adenoma and HCC part the adenoma supports adenoma-carcinoma sequence hypothesis[12,33,34]. This is further reinforced by the idea of "foci of dysplasia" within hepatic adenoma. A focus of dysplasia once developed in a hepatocellular adenoma is debated to invariably progress into transformation to HCC[14]. This is reasoned through inability to prevent development of HCC in patients with hepatic adenoma secondary to prolonged exogenous hormone therapy inspite of discontinuation of offending agent[33].

Genomic studies have revealed chromosomal aberrations [loss of heterozygosity (LOH) for M6P/IGFRII receptor, LOH in hMLH1] in both HCA and HCC[35,36]. However these aberrations are to a lesser degree in HCA compared to HCC. Scientists are still in search of the initial critical genetic event involved in malignant transformation. There is also little evidence to prove a common origin of HCA and HCC from same stem cells or committed progenitor cells[14].

MANAGEMENT

Owing to inconclusiveness in imaging, possible lethal complications that included bleeding, malignant transformation and the long term disease free guarantee that surgery offers; surgeons of the past classically preferred to operate on all hepatic adenomas. Recent advancements in molecular biology has substantially increased our understanding on pathophysiology of the disease. With greater technological expertise and development of surgical techniques, current surgeons have a more comprehensive outlook towards the management of such tumors (Figure 1). However, the rarity of the tumor has still restricted the quality of evidence available for current recommendations and guidelines.

Management options for HCA include surgery for: (1) all adenomas larger than five cm; (2) adenomas harboring HCC or dysplastic foci; (3) β-catenin activated HCA; (4) increasing size or imaging features of malignant transformation; (5) rising alpha fetoprotein (AFP); (6) HCA in males; and (7) HCA in GSD[14]. Minimally invasive options including laparoscopic liver resection has been proven to safe with equivalent or superior outcome when compared to open procedures[37]. However, this approach in anatomically unfavorable segments 7 and 8 need high technical expertise and control.

Adenomas that are < 5 cm, which present in anatomically challenging locations and those that undergo regression on steroid withdrawal can be managed conservatively[38]. All adenoma patients (surgical and nonsurgical candidates) who consume exogenous hormone therapy should be advised to discontinue their use. Malignant transformation has been reported

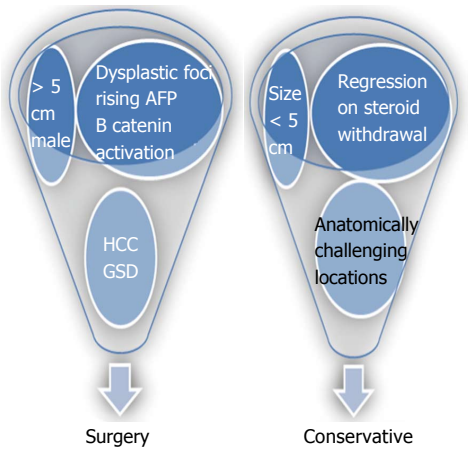

Figure 1 Indications of surgical and conservative approach in management of hepatocellular adenomas. HCC: Hepatocellular carcinoma; GSD: Glycogen storage diseases; AFP: Alpha fetoprotein.

infrequently in adenomas < 3.5 cm and in tumors that have regressed in size over time. Hence such lesions treated conservatively needs to be followed closely with imaging (CT/MRI) at 6- to 12-mo intervals for first two years. Following this, annual imaging may be modulated based on lesion stability and growth patterns[38]. The role of AFP in surveillance is still not established.

The role of selective arterial embolization in management of HCA is not very clear. However, multifocal lesions or those deemed un-resectable due to their location may be managed by such interventional radiology techniques[39]. Further, embolization may enable possibility of elective hepatic resection in cases of bleeding HCAs[34]. The role of preoperative embolization to facilitate safe hepatic resection in very large or hyper vascular adenomas is still controversial. Radiofrequency ablation might be useful in cases not amenable to surgery or in patients who would require major hepatic resection otherwise[40]. However, large sizes of adenomas may demand multiple sessions of the same.

Liver transplantation should be reserved for patients in whom hepatic resection is not technically feasible due to tumor size/location/recurrence/multiplicity and those with adenomatosis or glycogen storage disease. Owing to the complexities associated with liver transplantation, this mode of management should be used as sparingly as possible; when all other modalities of treatment may fail to provide cure. The use of liver transplantation for spontaneous intra-partum rupture of a hepatocellular adenoma has been reported in literature[41].

Pregnancy poses a varied challenge in the management of HCAs. An increased level of endogenous hormones during pregnancy has been postulated towards the increase in size of adenomas during pregnancy. Pregnancy induced hyper dynamic circulation combined with increased liver vascularity may further increase the risk of adenoma rupture, most seen in the third trimester[42]. In the past, owing to high maternal and fetal mortality rates (44% and 38% respectively), fertile females with HCA were discouraged from pregnancy[42]. However, of late due to increased awareness of the disease entity and better imaging modalities, pregnancy associated morbidities and mortalities have been greatly reduced.

Pregnancy is no longer a contraindication in hepatocellular adenoma < 5 cm[43]. All fertile females with adenomas, aspiring pregnancy should discontinue use of exogenous hormone therapy if any. Some still advocate resection for these lesions before pregnancy. However, the present trend is to allow conservative management of these smaller lesions with ultrasound monitoring of the adenoma every 6 wk. However those with adenomas > 5 cm or those who experienced adenoma related complications in previous pregnancies should undergo resection prior to pregnancy. Radiofrequency ablation or embolization might be useful in cases not amenable to surgery[39,40].

Adenomas > 5 cm detected incidentally during pregnancy often calls for an individualized approach. Surgery is most tolerated during second trimester, during which the risks to the mother and the fetus are minimized. Radiofrequency ablation during pregnancy has been described before in literature during first and second trimesters[44]. However their safety and efficacy still needs to be proven. Angioembolisation during pregnancy is questionable owing to the radiation risk to the fetus early in pregnancy. Their use during various trimesters of pregnancy warrants extensive studies before conclusion can be drawn.

LIVER CELL ADENOMATOSIS

Flejou *et al*[45] in 1985 was the first to propose adenomatosis as a varied clinical entity from HCA. Liver cell adenomatosis is characterized by the presence of multiple adenomas (> 10), involving both lobes of the liver, in absence of glycogen storage disease or exogenous hormone therapy. They account for 10%-24% patients with HCA. Histologic and radiologic features of these lesions are identical to that in adenomas. These lesions however have a higher risk of impaired liver function and bleeding. Flejou *et al*[45] reported bleeding in 46% of patients in his series. Malignant degeneration risk however does not correspondingly increase with the number of lesions. Chiche *et al*[46] proposed two different lesion patterns found in liver cell adenomatosis - "massive" and "multifocal". Lesions in massive type tend to be larger (2 to 10 cm) and often result in gross hepatomegaly with deformed liver contour. In comparison, the multifocal type has smaller lesions (≤ 4 cm) and rarely deform liver contour[1]. The lesions in massive type tend to progress fast and are more likely to be symptomatic.

Management for these lesions remains challenging. The extensive distribution of these lesions in both lobes makes anatomical/non-anatomical hepatic resection impractical. All females should discontinue use of exogenous hormone therapy and avoid future pregnancies. The massive variant of this disease with predominant

large lesions (> 5 cm) in a single lobe may warrant hemi hepatectomy. The role for radiofrequency ablation or embolization in these patients is debatable[40].

Liver transplantation presents the lone potential curative measure for these lesions. Evidence of malignant transformation or symptomatic patients with recurrent adenoma complications warrants transplantation[45]. However transplantation in turn increases the risk of *de-novo* tumors in addition to other complications that include peri-operative morbidities, infections, graft rejection and renal failure[47,48]. Owing to the rarity of the disease entity and lack of substantial clinical evidence, the effectiveness of transplantation is still controversial. Considering the complexities associated with transplantation, the trend of late is to treat such lesions conservatively. Such patients are to be followed on a regular basis with imaging to identify growth patterns and malignant transformation.

CONCLUSION

HCA despite being benign liver neoplasms often pose diagnostic and therapeutic challenges. Further characterization of the genetics and pathophysiology of such lesions and their radiological correlations may trigger changes in the existing management guidelines. We believe larger prospective studies that focus on the management of hepatic adenomas can aid overcome the current short-comings on the quality of evidence available.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Histopathological differences utilizing the nonalcoholic fatty liver disease activity score criteria in diabetic (type 2 diabetes mellitus) and non-diabetic patients with nonalcoholic fatty liver disease

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Author contributions: Study concept, design, data collection and supervision by Mohanty SR; analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content shared between Puchakayala BK (major contribution), Verma S and Kanwar P; initial statistical analysis performed by University of Chicago statistician and secondary analysis with review was performed by Matt Briggs, consultant statistician at New York Methodist Hospital; liver biopsy slides analysed by Hart J at University of Chicago; secondary analysis of data, formulation of tables and review of manuscript by Sanivarapu RR.

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Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at srm9006@nyp.org. Consent from participants was not obtained as the presented data is anonymized and risk of identification is nil.

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Abstract

AIM: To study clinical and histopathological features of nonalcoholic fatty liver disease (NAFLD) in patients with and without type 2 diabetes mellitus (T2DM) using updated nonalcoholic steatohepatitis clinical research network (NASH-CRN) grading system.

METHODS: We retrospectively analyzed data of 235 patients with biopsy proven NAFLD with and without T2DM. This database was utilized in the previously published study comparing ethnicity outcomes in NAFLD by the same corresponding author. The pathology database from University of Chicago was utilized for enrolling consecutive patients who met the criteria for NAFLD and their detailed clinical and histopathology findings were obtained for comparison. The relevant clinical profile of patients was collected from the Electronic Medical Records around the time of liver biopsy and the histology was read by a single well-trained histopathologist. The updated criteria for type 2 diabetes have been utilized for analysis. Background data of patients with NASH and NAFLD has been included. The mean differences were compared using χ^2 and *t*-test along with regression analysis to evaluate the predictors of NASH and advanced fibrosis.

RESULTS: Patients with NAFLD and T2DM were significantly older (49.9 *vs* 43.0, $P < 0.01$), predominantly female (71.4 *vs* 56.3, $P < 0.02$), had higher rate of metabolic syndrome (88.7 *vs* 36.4, $P < 0.01$), had significantly higher aspartate transaminase (AST)/alanine transaminase (ALT) ratio (0.94 *vs* 0.78, $P < 0.01$) and Fib-4 index (1.65 *vs* 1.06, $P < 0.01$) as markers of NASH, showed higher mean NAFLD activity score (3.5 *vs* 3.0, $P = 0.03$) and higher mean fibrosis score (1.2 *vs* 0.52, $P < 0.01$) compared to patients with NAFLD without T2DM. Furthermore, advanced fibrosis (32.5 *vs* 12.0, $P < 0.01$) and ballooning (27.3 *vs* 13.3, $P < 0.01$) was significantly higher among patients with NAFLD and T2DM compared to patients with NAFLD without T2DM. On multivariate analysis, T2DM was independently associated with NASH (OR = 3.27, 95%CI: 1.43-7.50, $P < 0.01$) and advanced fibrosis (OR = 3.45, 95%CI: 1.53-7.77, $P < 0.01$) in all patients with NAFLD. There was a higher rate of T2DM (38.1 *vs* 19.4, $P < 0.01$) and cirrhosis (8.3 *vs* 0.0, $P = 0.01$) along with significantly higher mean Bilirubin (0.71 *vs* 0.56, $P = 0.01$) and AST (54.2 *vs* 38.3, $P < 0.01$) and ALT (78.7 *vs* 57.0, $P = 0.01$) level among patients with NASH when compared to patients with steatosis alone. The mean platelet count (247 *vs* 283, $P < 0.01$) and high-density lipoprotein cholesterol level (42.7 *vs* 48.1, $P = 0.01$) was lower among patients with NASH compared to patients with steatosis.

CONCLUSION: Patients with NAFLD and T2DM tend to have more advanced stages of NAFLD, particularly advanced fibrosis and higher rate of ballooning than patients with NAFLD without T2DM.

Key words: Non-alcoholic steatohepatitis; Non-alcoholic fatty liver disease; Advanced fibrosis; Non-alcoholic fatty liver disease activity score; Type 2 diabetes; Liver biopsy

Core tip: This retrospective cohort study shows that type 2 diabetes mellitus (T2DM) is a reliable predictor for both nonalcoholic steatohepatitis (NASH) and advanced fibrosis. Patients with nonalcoholic fatty liver disease (NAFLD) and uncontrolled T2DM tend to have advanced fibrosis and higher rate of ballooning histologically. It is important to recognize the differences between composite NAS and individual histological features while interpreting liver biopsies among NAFLD patients. Early diagnosis of NASH and advanced fibrosis in patients with NAFLD has important clinical significance especially to prevent further progression of liver disease to cirrhosis, hepatocellular carcinoma and other related complications. Thus, optimization of risk factors for NAFLD such as metabolic syndrome, uncontrolled T2DM and dyslipidemia is of paramount importance.

Puchakayala BK, Verma S, Kanwar P, Hart J, Sanivarapu RR, Mohanty SR. Histopathological differences utilizing the nonalcoholic fatty liver disease activity score criteria in diabetic (type 2 diabetes mellitus) and non-diabetic patients with nonalcoholic fatty liver disease. *World J Hepatol* 2015; 7(25): 2610-2618 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i25/2610.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i25.2610

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) includes a histological spectrum of liver diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) and the latter histological entity can progress to cirrhosis in patients without significant alcohol consumption[1]. NAFLD is considered to be the hepatic manifestation of metabolic syndrome (MS)[2]. Type 2 diabetes mellitus (T2DM) is not only associated with NAFLD, but has also been shown to be an independent risk factor for the development of NASH[2-4]. While screening for diabetes and other risk factors for NAFLD is easily performed, evaluation and management of NAFLD is still challenging. In spite of having a well-defined understanding of the stages of NAFLD progression, the exact pathogenesis is still unclear[5].

Despite the availability of various non-invasive tools, liver biopsy is still regarded as the gold standard for accurate measurement of histopathological features of NAFLD[6,7]. Identifying the unique histopathological features of NAFLD among patients with T2DM is not only important to stage the disease progression but also help us understand the impact of diabetes in progression of NAFLD. At present, there are only few studies describing histopathological differences among NAFLD patients with and without T2DM in a multiethnic United States population cohort[5,8]. Moreover, these studies utilized the Brunt scoring system[8,9] and were limited in sample size[8] or included pooled data from various centers[5].

Therefore, the primary aim of our study was to evaluate and compare the clinical, laboratory and detailed histological findings using the updated NASH

clinical research network (CRN) scoring system among biopsy proven NAFLD patients with and without T2DM using a single histopathologist from a major tertiary health care center. We also explored the risk of NASH and advanced fibrosis in both groups in addition to comparing the background data of NAFLD and NASH separately.

MATERIALS AND METHODS

Study design

The pathology database from the University of Chicago Medical Center (UCMC) containing the terms "steatosis", "steatohepatitis" and/or "fat" from June 1, 1995 to June 30, 2005 was retrospectively analyzed and 683 positive biopsy reports were consecutively identified for further analysis. UCMC's computerized medical records were then retrospectively reviewed to obtain patient-related demographic, clinical, and laboratory data. Patients either lacking adequate information and/or having presence of other concomitant liver diseases including hepatitis B and C, iron overload, medication-related steatosis, significant alcohol use (current daily alcohol consumption of 40 g/d or more in males and 20 g/d or more in females) and liver transplant were excluded. Data on T2DM and MS were collected based upon American Diabetes Association[10] and National Cholesterol Education Program ATP Ⅲ criteria[11]. Obesity was defined as body mass index (BMI) $\geqslant$ 30 kg/m^2 since data on waist circumference measures of central obesity were unavailable[12]. Out of the 683 patients with fatty liver histology, only 238 patients were found to be meeting the criteria for NAFLD and on further review, 3 patients had inadequate data regarding presence or absence of T2DM, thereby leaving a total of 235 patients eligible for further analysis. Moreover, all liver biopsies were scored by a single pathologist using the updated NASH CRN scoring system[6]. The pathologist was blinded to the clinical and laboratory data of the patients. The clinical, laboratory and histological data were all computerized and stored securely. The indication for liver biopsy as elicited in previous study[13] among the 238 patients with NAFLD was abnormal liver function tests followed by abnormal intraoperative appearance of liver and subsequently followed by abnormal imaging and associated abdominal pain respectively. The reasons for ineligibility are also demonstrated clearly in the original study[13]. This study was originally reviewed and approved by the institutional review board of the University of Chicago Medical Center. The informed consent was waived as part of the previous study[13] and there were no patient identifiers in the current study.

Case definitions and liver histology

NAFLD was defined histologically by the presence of minimum 5% of steatosis on liver biopsy. Steatosis was scored as 1, 2, or 3 for 5%-33%, 34%-66%, and > 66% steatosis, respectively. Fibrosis was scored as 0, 1, 2, 3 and 4 for no fibrosis, perisinusoidal or periportal, perisinusoidal and portal/periportal, bridging, and cirrhosis, respectively. Lobular inflammation was scored as 0, 1, 2, or 3 based on presence of no inflammation, < 2 foci per 200 × field, 2-4 foci per 200 × field, and > 4 foci per 200 × field, respectively. Ballooning was scored as 0, 1, and 2 for no balloon cell, few balloon cells, and many cells/prominent ballooning cells, respectively. Lastly, Mallory's hyaline was scored as 0 for "none to rare" or 1 for "many". A NAFLD activity score (NAS) of $\geqslant$ 5 was considered NASH while fibrosis score of $\geqslant$ 2 was considered advanced fibrosis[6].

Statistical analysis

Patients with NAFLD were sub-divided according to presence or absence of T2DM. Background data for NASH and NAFLD was analyzed separately. Results are expressed as mean ± SD for continuous variables and as frequencies for categorical variables. A *t*-test for unequal variance was performed to compare the means of continuous variables. Categorical variables were compared by χ^2 test. Separate logistic regression analyses were performed to study the variables associated with presence of NASH and fibrosis. Variables which were significant on univariate analysis were included in the multivariate analysis and independent variables with $P > 0.1$ were excluded sequentially from the models. The odds ratios and associated *P*-values of the remaining variables are reported. Two-sided *P* values < 0.05 were considered statistically significant. Data analyses were performed using Stata (StataCorp, College Station, TX). The statistical methods of this study were reviewed by Matt Briggs, consultant statistician at New York Methodist Hospital.

RESULTS

Demographic, comorbid and biochemical features

Patients with NAFLD and T2DM, were significantly older (49.9 *vs* 43.0, $P < 0.01$), had higher proportion of females (71.4 *vs* 56.3, $P < 0.02$), and showed higher fasting glucose (167.0 *vs* 102.9, $P < 0.01$), higher HbA1c (8.09 *vs* 5.83, $P < 0.01$), higher BMI (41.0 *vs* 35.9, $P < 0.01$), higher international normalized ratio (1.02 *vs* 0.97, $P = 0.03$), higher rates of hypertension (71.4 *vs* 37.3, $P < 0.01$), higher rate of dyslipidemia (83.8 *vs* 61.2, $P < 0.01$), increased rate of metabolic syndrome (88.7 *vs* 36.4, $P < 0.01$) and positive indirect markers of NASH such as aspartate transaminase (AST)/alanine transaminase (ALT) ratio (0.94 ± 0.4 *vs* 0.78 ± 0.4, $P < 0.01$) and Fib-4 index (1.65 *vs* 1.06, $P < 0.01$) compared to patients with NAFLD without T2DM. Conversely, patients with NAFLD without T2DM had significantly higher rates of abnormal liver function tests as a leading cause of liver biopsy (74 *vs* 54.5, $P = 0.02$), higher ALT (79.2 *vs* 59.2, $P = 0.01$) and showed higher serum iron levels (89.3 *vs* 71.3, $P < 0.01$) compared to patients with NAFLD and T2DM (Table 1).

Background data evaluating all patients with NAFLD showed higher rate of T2DM (38.1 *vs* 19.4, $P < 0.01$)

Table 1 Characteristics of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic fatty liver disease without type 2 diabetes mellitus and nonalcoholic fatty liver disease with type 2 diabetes mellitus (mean ± SD)

Parameter	All patients (*n* = 235)	NAFLD (without T2DM) (*n* = 158)	NAFLD (with T2DM) (*n* = 77)	*P* value
Age (yr)	45.3 ± 11.9	43.0 ± 12.2	49.9 ± 9.8	< 0.01
Gender (male/female)	91/144	69/89	22/55	0.02
Race (% Caucasian)	65.1	63.9	67.5	0.9
BMI (kg/m^2)	37.6 ± 11.5	35.9 ± 10.6	41.0 ± 12.6	< 0.01
Hypertension, *n* (%)	114 (48.1)	59 (37.3)	55 (71.4)	< 0.01
Dyslipidemia, *n* (%)	139 (68.8)	82 (61.2)	57 (83.8)	< 0.01
Metabolic syndrome, *n* (%)	115 (53.7)	52 (36.4)	63 (88.7)	< 0.01
Abnormal LFT's as an indication of liver biopsy, *n* (%)	159 (67.6)	117 (74.0)	42 (54.5)	0.02
Platelets	257.5 ± 73.5	262.6 ± 67.7	247.4 ± 83.3	0.15
INR	0.99 ± 0.16	0.97 ± 0.10	1.02 ± 0.23	0.03
Protein (g/dL)	7.4 ± 0.72	7.4 ± 0.73	7.3 ± 0.70	0.12
Albumin (g/dL)	4.3 ± 0.5	4.3 ± 0.5	4.2 ± 0.46	0.06
AST (U/L)	49.8 ± 34.5	50.4 ± 38.4	48.3 ± 24.8	0.6
ALT (U/L)	72.6 ± 58.5	79.2 ± 65.4	59.2 ± 38	0.01
Bilirubin (mg/dL)	0.67 ± 0.4	0.68 ± 0.4	0.62 ± 0.3	0.3
Alkaline Phosphate	96.7 ± 61.8	94.9 ± 68.2	100.1 ± 46.9	0.5
Total cholesterol (mg/dL)	200.2 ± 47.3	200.9 ± 45.4	198.8 ± 50.9	0.8
HDL cholesterol (mg/dL)	44.3 ± 11.8	44.2 ± 12.0	44.6 ± 11.7	0.9
Triglycerides (mg/dL)	211.4 ± 119.9	209.7 ± 127.7	214.4 ± 105.9	0.8
LDL cholesterol (mg/dL)	118.9 ± 41.0	120.5 ± 41.3	116.4 ± 40.8	0.6
Fasting glucose (mg/dL)	125.6 ± 55.5	102.9 ± 28.3	167.0 ± 67.8	< 0.01
HbA1c	6.93 ± 2.09	5.83 ± 1.11	8.09 ± 2.26	< 0.01
Iron (μg/dL)	82.6 ± 38.7	89.3 ± 40.8	71.3 ± 32.0	< 0.01
Total iron binding capacity (μg/dL)	322.4 ± 72.3	317.16 ± 71.8	333.0 ± 72.9	0.2
Ferritin (ng/mL)	258.2 ± 306.0	281 ± 323.1	223.0 ± 276.7	0.3
AST/ALT	0.836 ± 0.432	0.781 ± 0.434	0.947 ± 0.41	< 0.01
FIB-4 index	1.26 ± 1.01	1.06 ± 0.77	1.65 ± 1.28	< 0.01
APRI	0.581 ± 0.471	0.549 ± 0.458	0.643 ± 0.493	0.16

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; AST: Aspartate transaminase; ALT: Alanine transaminase; LDL: Low-density lipoprotein cholesterol; NAS: NAFLD activity score; HDL: High-density lipoprotein cholesterol; BMI: Body mass index; INR: International normalized ratio; APRI: AST to platelet ratio index; LFT: Liver function test.

and presence of cirrhosis (8.3 *vs* 0.0, *P* = 0.01) among patients with NASH compared to those with steatosis (fatty liver) alone. Moreover, there was significant higher values of AST (54.2 *vs* 38.3, *P* < 0.01), ALT (78.7 *vs* 57.0, *P* = 0.01) and Bilirubin (0.71 *vs* 0.56, *P* = 0.01) and lower values of high density lipoprotein (HDL) (42.7 *vs* 48.1, *P* = 0.01) and platelet count (247 *vs* 283, *P* < 0.01) among patients with NASH respectively. There was no difference in age, sex, ethnicity, BMI and various other risk factor variables between the groups (Table 2).

Histological features

Patients with NAFLD and T2DM had significantly higher mean NAS (3.5 *vs* 3.0, *P* = 0.03) and fibrosis scores (1.2 *vs* 0.52, *P* < 0.01) compared to patients with NAFLD without T2DM. In addition, a significantly higher percentage of patients with NAFLD and T2DM showed advanced fibrosis (27.3 *vs* 13.3, *P* < 0.01) and prominent ballooning (27.3 *vs* 13.3, *P* < 0.01) compared to patients with NAFLD without T2DM. However, there were no statistically significant differences between groups regarding NAS ⩾ 5 (29.9 *vs* 20.9, *P* = 0.12), steatosis ⩾ 2 (66.2 *vs* 55.1, *P* = 0.1) and inflammation ⩾ 2 (9.1 *vs* 6.3, *P* = 0.4). On the contrary, there was a trend for presence of higher Mallory bodies in patients with NAFLD and T2DM compared to patients with NAFLD without T2DM (28.6 *vs* 17.7, *P* = 0.06) (Table 3).

NASH

After controlling for age, gender, ethnicity and BMI, a multivariate analysis showed significant association between NASH and presence of diabetes (OR = 3.27, 95%CI: 1.43-7.50, *P* < 0.01) and ALT ⩾ 36 IU (OR = 3.88, 95%CI: 1.15-13.1, *P* = 0.029) in all patients with NAFLD, while only ALT ⩾ 36 IU (OR = 4.21, 95%CI: 1.13-15.6, *P* = 0.03) was significantly associated with NASH in patients with NAFLD and T2DM (Table 4).

Fibrosis

After controlling for age, gender, ethnicity and BMI on multivariate analysis, only T2DM (OR = 3.45, 95%CI: 1.53-7.77, *P* < 0.01) and low platelet (OR = 0.99, 95%CI: 0.99-1.0, *P* = 0.025) showed an independent association for advanced fibrosis among all patients with NAFLD. No separate indicator for advanced fibrosis was noted among patients with NAFLD and T2DM (Table 5).

DISCUSSION

Research over the past few decades has dwelled upon identifying the potential risk factors associated with NASH and advanced fibrosis which indeed has

Table 2 Characteristics of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic steatohepatitis and steatosis (mean + SD)

Parameter	All patients (n = 235)	NASH	Steatosis	P value
Age (yr)	45.3 + 11.9	45.9 + 12.4	43.8 + 10.4	0.2
Gender (male/female)	91/144	67/101	24/43	0.6
Race (% caucasian)	65.1	64.9	65.7	0.1
BMI (kg/m^2)	37.7 + 11.5	37 + 10.6	39.4 + 13.7	0.17
Hypertension, n (%)	114 (48.5)	82 + 48.8	32 + 63.9	1
Dyslipidemia, n (%)	139 (68.8)	100 (70.9)	39 (63.9)	0.3
Metabolic syndrome, n (%)	115 + 53.7	67 (42.7)	32 (56.1)	0.08
Platelets	258 + 73.5	247 + 72.1	283 + 71.1	< 0.01
INR	0.996 + 0.162	1 + 0.178	0.98 + 0.112	0.3
Protein (g/dL)	7.39 + 0.762	7.39 + 0.733	7.37 + 0.714	0.8
Albumin (g/dL)	4.27 + 0.536	4.27 + 0.555	4.26 + 0.487	0.8
AST (U/L)	49.8 + 34.5	54.2 + 36.3	38.3 + 26.1	< 0.01
ALT (U/L)	72.6 + 58.5	78.7 + 63.1	57 + 40.7	0.01
Bilirubin (mg/dL)	0.668 + 0.416	0.71 + 0.456	0.567 + 0.275	0.01
Alkaline Phosphate	96.7 + 61.8	100 + 70.9	88.3 + 28.3	0.1
Total cholesterol (mg/dL)	200 + 47.3	202 + 48.6	197 + 44.5	0.5
HDL cholesterol (mg/dL)	44.4 + 11.9	42.7 + 10.7	48.1 + 13.6	0.01
Triglycerides (mg/dL)	211 + 120	220 + 125	191 + 105	0.18
LDL cholesterol (mg/dL)	1119 + 41	121 + 41.6	114 + 39.8	0.33
Fasting glucose (mg/dL)	126 + 55.5	129 + 55.9	117 + 53.8	0.14
HbA1c	6.93 + 2.09	7.12 + 2.19	6.37 + 1.69	0.11
Iron (μg/dL)	82.6 + 38.7	83.8 + 40.5	78.6 + 31.9	0.52
Total iron binding capacity (μg/dL)	322 + 72.3	319 + 74.6	335 + 63.4	0.31
Ferritin (ng/mL)	258 + 306	276 + 324	201 + 232	0.21
Mallory bodies	50 + 21.3	50 + 29.8	0	< 0.01
CAD	67 + 28.9	50 + 29.9	17 + 26.2	0.63
Gastric bypass	21 + 8.94	14 + 8.33	7 + 10.4	0.61
Cirrhosis	14 + 5.96	14 + 8.33	0	0.01
Steatosis	1.92 + 0.861	2.1 + 0.83	1.46 + 0.765	< 0.01
Inflammation	0.44 + 0.62	0.625 + 0.663	0	< 0.01
Fibrosis	0.753 + 1.11	1.05 + 1.21	0	< 0.01
Ballooning	0.821 + 0.712	1.15 + 0.575	0	< 0.01
NAS score	3.18 + 1.69	3.86 + 1.45	1.46 + 0.765	< 0.01

AST: Aspartate transaminase; ALT: Alanine transaminase; HDL: High-density lipoprotein cholesterol; NAS: Nonalcoholic fatty liver disease activity score; NASH: Nonalcoholic steatohepatitis; LDL: Low-density lipoprotein cholesterol; BMI: Body mass index; INR: International normalized ratio; CAD: Coronary artery disease.

Table 3 Histological findings of patients with nonalcoholic fatty liver disease, divided according to nonalcoholic fatty liver disease without type 2 diabetes mellitus and nonalcoholic fatty liver disease with type 2 diabetes mellitus n (%)

Parameter	All patients (n = 235)	NAFLD (without T2DM) (n = 158)	NAFLD (with T2DM) (n = 77)	P value
NAS score (mean ± SD)	3.2 ± 1.7	3.0 ± 1.6	3.5 ± 1.7	0.03
Fibrosis score (mean ± SD)	0.75 ± 1.1	0.52 ± 0.9	1.2 ± 1.3	< 0.01
Fibrosis score				< 0.01
< 2	191 (81.3)	139 (88.0)	52 (67.5)	
⩾ 2	44 (18.7)	19 (12.0)	25 (32.5)	
NAS score				0.12
< 5	179 (76.2)	125 (79.1)	54 (70.1)	
⩾ 5	56 (23.8)	33 (20.9)	23 (29.9)	
NAS score				0.11
< 3	90 (38.3)	66 (41.7)	24 (31.2)	
⩾ 3	145 (61.7)	92 (58.3)	53 (68.8)	
Steatosis				0.1
< 2	97 (41.3)	71 (44.9)	26 (33.8)	
⩾ 2	138 (58.7)	87 (55.1)	51 (66.2)	
Ballooning				< 0.01
< 2	193 (82.1)	137 (86.7)	56 (72.7)	
2	42 (17.9)	21 (13.3)	21 (27.3)	
Inflammation				0.4
< 2	218 (92.7)	148 (93.7)	70 (90.9)	
⩾ 2	17 (7.3)	10 (6.3)	7 (9.1)	
Mallory bodies	50 (21.3)	28 (17.7)	22 (28.6)	0.06

NAFLD: Nonalcoholic fatty liver disease; NAS: NAFLD activity score; T2DM: Type 2 diabetes mellitus.

Table 4 Variables associated with nonalcoholic steatohepatitis (nonalcoholic fatty liver disease activity score ⩾ 5) (multivariate analysis)

Variables	*P* value	Odds ratio	95%CI
All patients			
Presence of T2DM	< 0.01	3.27	1.43-7.50
ALT ⩾ 36	0.029	3.88	1.15-13.1
Protein	0.045	1.84	1.01-3.35
Patients with NAFLD without T2DM			
Protein	0.048	3.06	1.01-9.25
Patients with NAFLD with T2DM			
ALT ⩾ 36	0.03	4.21	1.13-15.6

ALT: Alanine transaminase; NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus.

Table 5 Variables associated with advanced fibrosis (multivariate analysis)

Variables	*P* value	Odds ratio	95%CI
All patients			
Presence of T2DM	< 0.01	3.45	1.53-7.77
Platelet	0.025	0.99	0.99-1.0
INR	< 0.01	4151	97.7-176426
Patients with NAFLD without T2DM			
Platelet	0.03	0.99	0.98-0.99
INR	0.001	16950	62.4-4607293
Patients with NAFLD with T2DM			
Platelet	0.045	0.99	0.98-0.99

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; INR: International normalized ratio.

significant prognostic value. T2DM is being increasingly recognized as an important risk factor in NAFLD progression, especially with NAFLD being regarded as an extension of metabolic syndrome[2-4]. However, there is a lack of definite predictors for NASH among NAFLD patients with T2DM[14,15], thereby highlighting the need to obtain liver histopathology for accurate determination of NASH and fibrosis. Recognizing unique histological features among NAFLD patients with T2DM would enhance our understanding of disease progression and aid development of potential remedies. Prior studies evaluating the histopathological features of NAFLD among T2DM patients utilized the Brunt system of histological evaluation[3,8,16-20] and were limited by lack of a comparative control group of patients with NAFLD without T2DM[3,16,17,20,21]. To date, only one multicenter study compared the histopathological differences among patients with NAFLD with and without T2DM[5] utilizing the NAS criteria among United States population with the limitation of potential inter-observer variability due to histology being read by multiple pathologists. Therefore, we conducted a detailed comparative histological evaluation using the NAS criteria in patients with NAFLD with and without T2DM at a single center using a single well-trained histopathologist to identify factors predicting NASH and advanced fibrosis in a multiethnic United States cohort.

Our study highlights the findings that patients with NAFLD and T2DM have higher ballooning (27.3 *vs* 13.3, $P < 0.01$) compared to patients with NAFLD without T2DM. Ballooning is considered to be the most important feature of steatohepatitis and correlates with features of insulin resistance very well[7]. Recently, Leite *et al*[21] examined the histological features of NAFLD in T2DM patients using the updated NASH CRN grading system and also assessed for interpathologist's agreement on histological features. Although they demonstrated presence of significant higher ballooning (42%-55%) among patients with NAFLD and T2DM, they had several limitations. The biopsies were evaluated by two pathologists, and the kappa score for inter-observer agreement for assessment of the degree of ballooning was 0.45 indicating that there was more disagreement between the pathologists than agreement. Moreover, the study sample in Leite *et al*[21] lacked a comparative control group of patients with NAFLD without T2DM. Thus, our study overcomes the limitations of the Leite *et al*[21] study, but also demonstrates that patients with NAFLD and T2DM have higher rates of prominent ballooning compared to patients with NAFLD without T2DM using a larger and more diverse patient population including Asians, Hispanics, African Americans and Caucasians. Interestingly, in a large clinical trial involving 173 biopsy proven pediatric NAFLD patients, Lavine *et al*[22] demonstrated a significant improvement in hepatocyte ballooning at 96 wk of therapy with vitamin E -0.5 (95%CI: -0.8 to -0.3, $P = 0.006$) and metformin -0.3 (95%CI: -0.6 to -0.0, $P = 0.04$) compared to placebo. No other histological features of NAFLD had shown any significant improvement otherwise. Furthermore, Chen *et al*[23] in a large case-control study demonstrated that diabetes is associated with higher risk of HCC (OR = 2.29, 95%CI: 2.25-2.35, $P < 0.001$) and use of metformin resulted in 7% reduction in the risk of HCC in diabetic patients incrementally (adjusted OR = 0.93, 95%CI: 0.91-0.94, $P < 0.0001$) by inhibiting hepatoma proliferation and inducing cell cycle arrest. Thus, hepatocyte ballooning is not only a distinct histological feature of NASH but may play an essential role in the management and prognosis of NASH. Further studies are needed to explore the exact role of hepatocyte ballooning in the progression onto cirrhosis and HCC.

While ballooning is one component of the overall NAS, our results showed that the mean NAS was significantly higher among patients with NAFLD and T2DM (3.5 *vs* 3.0, $P = 0.03$) compared to patients with NAFLD without T2DM. However, there was no significant difference in NAS ⩾ 5 (20.9 *vs* 29.9, $P = 0.12$) between the two groups in our study (Table 2) which could likely be attributed to relatively smaller sample size of the patients with NAFLD and T2DM ($n = 77$). NAS is different from Brunt scoring system, as it includes the active and potentially reversible features of NAFLD, such as steatosis, inflammation and ballooning, and is separate from the potentially less reversible features like fibrosis[6]. Moreover, the numeric value of the composite

NAS is distinct from the qualitative histopathologic diagnosis utilized by Brunt scoring system (*i.e.,* definite NASH, kappa score = 0.57)[7]. Furthermore, Miyaaki *et al*[20] and Adams *et al*[24] have pointed that patients with NAFLD and T2DM tend to have advanced fibrosis and less steatosis, which could be attributed to the natural progression of disease or advanced age of the population with T2DM at the time of evaluation. Thus variation among individual histological features of NASH among patients with NAFLD and T2DM could lower the overall composite NAS. Therefore, it is important to quantify individual histological features separately along with NAS while interpreting liver biopsies among NAFLD patients. Further large population-based studies are needed to explore the additional utility of NAS in patients with NAFLD and T2DM.

In our study, patients with NAFLD and T2DM demonstrated significantly higher mean fibrosis score and advanced fibrosis compared to patients with NAFLD without T2DM which corroborates with prior studies[5,8,20]. On adjusting for age, gender, BMI and ethnicity, T2DM was noted to be an independent predictor for NASH and advanced fibrosis among all patients with NAFLD in our study which is not surprising. Recently, Loomba *et al*[5] conducted a cross-sectional analysis of 1069 patients with NAFLD from the NAFLD Database study and PIVENS trial to examine the association of personal and family history of T2DM to histological features of NASH and advanced fibrosis. On sub-analysis using a comparative group of patients with NAFLD without T2DM and adjusting for age, sex, ethnicity and BMI, patients with NAFLD and T2DM had increased risk of NASH (OR = 2.48, 95%CI: 1.31-4.72, P = 0.01), any fibrosis (OR = 2.94, 95%CI: 1.49-5.81, P < 0.01) and advanced fibrosis (OR = 6.03, 95%CI: 3.16-11.52, P < 0.0001) which is similar to our study results. However, the diagnosis of NASH was based on modified Brunt criteria as opposed to composite NAS criteria in our study and comparative histological analysis for differences in ballooning or NAS $\geqslant$ 5 was lacking. Younossi *et al*[8], in a cohort study of 132 patients with NAFLD, demonstrated that patients with NAFLD and T2DM (n = 44) had significantly higher rates of cirrhosis (25 *vs* 10.2, P = 0.04), liver related mortality (RR = 22.83, 95%CI: 2.97-175.03, P = 0.003) and overall mortality (RR = 3.30, 95%CI: 1.76-6.18, P = 0.002), compared to patients with NAFLD without T2DM (n = 88). However, there was no statistical difference for fibrosis $\geqslant$ 2 between the two groups (17 *vs* 32, P = 0.07) in their study, which is contrary to our study and could be related to their smaller sample size.

Early diagnosis of NASH and advanced fibrosis in patients with NAFLD and T2DM has important clinical significance. In a long term follow up study of biopsy proven NAFLD patients, Ekstedt *et al*[25] reported higher mortality particularly among patients with NASH compared to reference population with respect to liver-related causes (2.8 *vs* 0.2, P = 0.04) and from cardiovascular disease (15.5 *vs* 7.5, P = 0.04). Younossi *et al*[8] in their study reported higher occurrence of cirrhosis (25 *vs* 10.2, P = 0.04), overall mortality (RR = 3.30, 95%CI: 1.76-6.18, P = 0.002) and mortality related to liver disease (RR = 22.83, 95%CI: 2.97-175.03, P = 0.003) among diabetic patients with NAFLD as opposed to non-diabetic patients with NAFLD. Moreover, Welzel *et al*[26], found a 2.9 fold risk for development of HCC among diabetic patients in a recent analysis within the SEER-database. Thus, it is imperative to identify and evaluate patients with NAFLD and T2DM early on for presence of NASH and advanced fibrosis. Currently, no single non-invasive panel has been proven to be a valid substitute for a liver biopsy[27]. In our study, the indirect markers for NASH such as AST/ALT ratio (0.94 ± 0.4 *vs* 0.78 ± 0.4, P < 0.01) and Fib-4 index (1.65 *vs* 1.06, P < 0.01) showed higher significance among patients with NAFLD with T2DM compared to patients without T2DM while APRI score (0.64 *vs* 0.54, P = 0.16) did not. The utility of most non-invasive tests is limited as a screening test to exclude advanced fibrosis due to high negative predictive value (76.9%-90.5%) and a modest positive predictive value (36.1%-61.1%)[28]. Moreover, patients with NAFLD and BMI > 30 (OR = 8.4, 95%CI: 6.6-10.8, P < 0.0001) and T2DM (OR = 2.0, 95%CI: 1.5-2.6, P < 0.0001) pose unique challenge for accurate liver stiffness measurement using Fibroscan, mainly due to attenuation of elastic waves by subcutaneous and pre-hepatic fat thickness[29]. Therefore, a selective liver biopsy could be helpful among obese, elderly patients especially with NAFLD and T2DM to accurately stage NAFLD. Furthermore, our results (Table 5) comparing the background features among patients with NASH and steatosis showed higher number of patients with NASH to have T2DM and cirrhosis in addition to indirect evidence for advanced disease and fibrosis reflected by significantly higher Bilirubin, AST and ALT and lower platelet and lower HDL values. Therefore optimization of risk factors of NAFLD such as metabolic syndrome, T2DM and dyslipidemia to prevent further progression of liver disease to cirrhosis, HCC and other related complications is warranted.

Our study has some limitations. Data on duration of T2DM and insulin resistance were lacking, hence we could not assess for the association of these variables with NAFLD progression. However, the mean HbA1c among patients with NAFLD and T2DM was 8.09 which reflects uncontrolled T2DM thereby minimizing the confounding effect of diabetic medication and highlighting the role of uncontrolled T2DM in NAFLD progression. Furthermore, the data being obtained at a major tertiary care referral center, could have led to selection bias. Lastly, the smaller sample size of patients with NAFLD and T2DM limited our ability to show statistically significant rate of cirrhosis. Therefore, larger prospective studies on patients with NAFLD with and without T2DM are needed to further our understanding of the relationship between T2DM and NAFLD progression.

In conclusion, patients with NAFLD and T2DM tend to have higher ballooning and advanced fibrosis compared to patients with NAFLD without T2DM. Our study also demonstrated that T2DM is an independent predictor

for both NASH and advanced fibrosis, while utilizing the more current NAS criteria. Patients with NAFLD and T2DM in general should be advised and educated regarding optimal diabetes control, hyperlipidemia management and vascular disease screening which may not only prevent cardiovascular complications but also could prevent further progression to NASH and/or advanced fibrosis/cirrhosis. Future prospective studies are needed to explore the role of NASH (especially hepatocyte ballooning) among patients with and without T2DM and development of advanced fibrosis, HCC and cardiovascular disease burden and mortality.

COMMENTS

Background

Identifying the unique histopathological features of nonalcoholic fatty liver disease (NAFLD) among patients with type 2 diabetes mellitus (T2DM) is not only important to stage the disease progression but also help us understand the impact of diabetes in progression of NAFLD. Presently, only few studies have described the histopathological differences among NAFLD patients with and without T2DM in a multiethnic United States population cohort.

Research frontiers

While screening for diabetes and other risk factors for NAFLD is easily performed, management of NAFLD is still evolving. Current interest is in understanding the pathogenesis of NAFLD progression, which will pave way to potential novel treatments.

Innovations and breakthroughs

The authors' study highlights the findings that patients with NAFLD and T2DM have higher ballooning compared to patients with NAFLD without T2DM. Ballooning is considered to be the most important feature of steatohepatitis and correlates with features of insulin resistance very well. Similarly, significantly higher mean fibrosis score and advanced fibrosis is demonstrated among patients with NAFLD and T2DM. It is important to quantify individual histological features separately along with NAS while interpreting liver biopsies among NAFLD patients.

Applications

T2DM is an independent predictor for both NASH and advanced fibrosis. Hepatocyte ballooning is not only a distinct histological feature of NASH but may play an essential role in the management and prognosis of NASH. Patients with NAFLD and T2DM in general should be advised and educated regarding optimal diabetes control, hyperlipidemia management and vascular disease screening thereby preventing progression of liver disease burden and mortality.

Terminology

NAFLD includes a histological spectrum of liver diseases ranging from simple steatosis to NASH and the latter histological entity can progress to cirrhosis. T2DM is shown to be an independent risk factor for the development of NASH. The components that make up NASH include fat in liver cells (steatosis), inflammation, scar tissue (fibrosis) and degeneration of liver cell (ballooning).

Peer-review

This is an excellent work dealing with a very interesting topic, the histopathological alterations in diabetic and non-diabetic patients with NAFLD. There are few studies describing the mentioned differences and this work constitutes a novel approach. The authors showed clearly the impact of T2DM on NAFLD. Comorbidity seems to be very important in converting the disease and determining the severity of the disease either ways.

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Human immunodeficiency virus and viral hepatitis among high-risk groups: Understanding the knowledge gap in the Middle East and North Africa Region

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Abstract

AIM: To identify gaps in the existing knowledge on single, dual and triple infections of human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV) in the Middle East and North Africa (MENA) region among men who have sex with men (MSMs), female sex workers (FSWs), injecting drug users (IDUs) and prisoners.

METHODS: We performed an extensive literature search on articles published on the topic in the 25 countries of the MENA region. PubMed database was used as the main search engine. Case reports, case series, qualitative studies, editorials, commentaries, authors' replies and animal studies were excluded. Original articles and reviews dealing with the prevalence of HIV, HBV and HCV and their co-infection were included. Data on population type, sample size, age and markers of infections were extracted from the relevant studies.

RESULTS: HIV, HBV and HCV are blood-borne viruses with similar modes of transmission. The categories of people at high risk of acquiring HIV-1, HBV and HCV commonly include: MSMs, FSW and IDUs. It is well established that HIV-positive individuals co-infected with HBV or HCV suffer from liver pathology associated with morbidity and mortality. Moreover, HIV-infected individuals do not respond well to treatment for HBV or HCV and hence are at increased risk of hepatic toxicity. Consequently, co-infection of HIV-positive individuals with HBV and/or HCV is a global health problem of

significant magnitude. Our review reveals the paucity of epidemiological data for key populations in many countries of the region. Limited number of studies exists in the MENA region on the status of HIV, HBV and HCV and their co-infections among prisoners, MSMs and FSWs. Evidence support the continued increase of the HIV epidemic among MSMs. In addition to the lack of studies on MSMs and FSWs in the MENA region, our review highlights the lack of data on the practices, characteristics, or the status of HIV infection and viral hepatitis among male sex workers selling or exchanging sex for money.

CONCLUSION: The MENA countries are in urgent need of advanced research and strengthening of the data collection systems and reporting practices of these infections among key populations.

Key words: Human immunodeficiency virus; Hepatitis B virus; Hepatitis C virus; Men who have sex with men; Female sex workers; Injecting drug users; Prisoners

Core tip: Despite the availability of preventive and control measures, co-infection of human immunodeficiency virus (HIV)-positive individuals with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) is a global health problem of significant and increasing magnitude. While the potential of worse HIV outcomes are suggested to be associated with viral hepatitis, it is still yet to identify the populations in the Middle East and North Africa (MENA) region with dual infections (HIV-HBV or HIV-HCV) or triple co-infections with HIV, HBV and HCV. This review highlights the available data on HIV, HBV and HCV and their co-infections in the MENA countries with specific focus on high-risk groups (men who have sex with men, female sex workers, injecting drug users and prisoners).

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INTRODUCTION

In 2013, 35 million people were estimated to be living with human immunodeficiency virus type-1 (HIV-1). Globally, 2.1 million people became newly infected with HIV in 2013, down from 3.4 million in 2001. Acquired immunodeficiency syndrome (AIDS)-related deaths peaked in 2005 and have fallen by the end of 2013 whereby 1.5 million people died from AIDS-related causes worldwide compared to 2.4 million in 2005[1]. The use of combined antiretroviral therapy (cART) has significantly reduced the mortality and morbidity caused by (HIV-1). In the Middle East and North Africa (MENA) countries, the picture of the HIV-1 epidemic is different. 230 000 people living with HIV were reported in 2013, 15000 deaths and a treatment coverage estimated to be 11% (range of 8%-16%)[1].

Recent data show that liver pathology is a significant cause of morbidity and mortality in HIV patients co-infected with hepatitis B virus (HBV) or hepatitis C virus (HCV). Moreover, HIV-infected individuals do not respond well to treatment for HBV or HCV and hence are at increased risk of hepatic toxicity[2,3]. Globally, 240 million people are chronically infected with HBV (defined as hepatitis B surface antigen positive for at least 6 mo) [World Health Organization (WHO)][4]. Data from other sources have reported 350-400 million people infected worldwide with HBV[3]. The majority of these cases are in Asia and Africa where HBV is endemic[5]. One hundred and thirty-170 million people are infected with HCV[6] with 3-4 million people newly infected each year with HCV. These numbers exceed the number of people living HIV-1.

According to the WHO Eastern Mediterranean Regional Office, there are approximately 4.3 million people infected with HBV (1.79% of the global prevalence) and 800000 people infected with HCV in the region (0.57% of the global prevalence) each year[7] HIV, HBV and HCV are blood-borne viruses with similar modes of transmission. HIV-1 is mainly a sexually transmitted virus (vaginal or anal sex). HIV-1 can also be transmitted *via* sharing needles or syringes used to prepare injection drugs with someone who is HIV-positive. Similarly, HCV is most commonly transmitted through the sharing of injecting tools; consequently, people at high risk of acquiring HCV include among others people who inject drugs and HIV-infected people[8]. While transmission of HBV varies depending on a country level of endemicity[9], injecting drug use has been described as an important mode of transmission among young adults[10]. In summary, the categories of people at high risk of acquiring HIV-1, HBV and HCV include commonly: Men who have sex with men (MSMs), female sex workers (FSW), injecting drug users (IDUs) and prisoners engaging in risky behaviors.

Despite the availability of preventive and control measures, co-infection of HIV-positive individuals with HBV and/or HCV is a global health problem of significant and increasing magnitude. Globally, 5%-20% of the HIV-infected individuals are inflicted by chronic hepatitis B and therefore accounting for an estimate of 2 to 4 million[11]. In countries where the viruses are highly endemic, the co-infection can reach 25%[12]. Worldwide, the burden of HIV-HCV co-infection is estimated to be 4 to 5 million persons[13]. While the potential of worse HIV outcomes are suggested to be associated with viral hepatitis, it is still yet to identify the populations in the MENA region with dual infections (HIV-HBV or HIV-HCV) or triple co-

Table 1 Human immunodeficiency virus, hepatitis B virus and hepatitis C virus and their co-infection status among prisoners from Middle East and North Africa countries between 2005 and 2015

Country	*n*	Mean age/age	HIV (%)	HBV (%)	HCV (%)	HIV-HBV co-infection (%)	HIV-HCV co-infection (%)	Triple infection (%)	Ref.
Egypt	500	41.0	0.0	9.8	15.8	0.0	0.0	0.0	[14]
Iran	160	16.6	0.6	0.6	NS	0.0	NS	NS	[15]
	392[a]	35.9	17.0	4.5	80.5	0.8	14.5	0.8	[16]
	358[b]	34.7	0.0	6.1	8.1	0.0	0.0	0.0	[17]
	163	34.5	0.0	7.4	7.4	0.0	0.0	0.0	[18]
	249[a]	35.4	15.1	4.7	64.8	1.1	14.3	1.1	[19]
	150[a]	31.4	42.5	18.9	75.9	NR	NR	NR	[20]
Lebanon	580	31.7	0.2	2.4	3.4	0.0	0.0	0.0	[21]
Libya	6371	> 16	18.2	6.9	23.7	NR	NR	1.5	[22]

[a]Among prisoners who inject drugs; [b]Drug-related convictions; *n*: Sample size; NR: Not reported; NS: Not studied. All numbers were rounded to the nearest 1. HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

infections with HIV, HBV and HCV. This review highlights the available data on HIV, HBV and HCV and their co-infections in the MENA countries with specific focus on high-risk groups. The aim of this review is to identify gaps in the existing knowledge on the interplay between these viruses in the region among MSMs, FSWs, IDUs and prisoners.

MATERIALS AND METHODS

In an attempt to understand the lack of full spectrum knowledge of the epidemiology of HIV, HBV and HCV and their existing co-infections in MENA countries and specifically in high risk-groups, we performed an extensive literature search on articles studying these viral infections in the 25 countries of the MENA region. These countries include: Algeria, Bahrain, Cyprus, Djibouti, Egypt, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Libya, Mauritania, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Somalia, Sudan, Syria, Tunisia, Turkey, United Arab Emirates (UAE) and Yemen. PubMed database was used as the main search engine. The WHO, Centers for Disease Control and Prevention, Joint United Nations Programme on HIV/AIDS (UNAIDS), World Bank and MENA National AIDS Programs websites were also checked for any updated data on the distribution of any of these infections. Case reports, case series, qualitative studies, editorials, commentaries, authors' replies and animal studies were excluded. Original articles and reviews dealing with the prevalence of HIV, HBV and HCV and their co-infection were included. Data on population type, sample size, age and markers of infections were extracted from the relevant studies.

The search was conducted using predefined combination of keywords or terms to select manuscripts and reviews published during the past decade, *i.e.*, between 2005 and 2015. The following keywords were used in order to identify articles studying HIV and HBV or HCV co-infection: "HIV" and "HBV" or "HCV" in combination with each of the 25 different countries of the MENA region. In order to identify the articles on high risk groups and their impact on the epidemiology of HIV, HBV and HCV in the MENA region, the systematic PubMed search included the combination of the following keywords and each of the MENA countries listed above: (1) "men who have sex with men" or "MSM" or "homosexual" or "gay"; (2) "female sex workers" or "FSW" or "prostitute"; (3) "injecting drug users" or "IDU" or "drug injectors" or "intravenous drug users"; and (4) "prisoners".

RESULTS

To our knowledge, 52 articles were deemed eligible using our search technique. These articles included published data on markers of HIV, HBV and HCV and their co-infections in the countries of the MENA region during the past decade. Data were available from 12 countries: Cyprus, Egypt, Iran, Israel, Lebanon, Libya, Morocco, Palestine, Saudi Arabia, Sudan, Turkey and Yemen. The data available on HIV, HBV, HCV and their co-infections from these countries are summarized among prisoners (Table 1), FSWs and MSMs (Table 2), IDUs (Table 3) and other defined populations (Table 4). The latter include blood donors, soldiers, HBV patients, non-injecting drug users, waste handlers and hospital patients.

Distribution of HIV, HBV, HCV and their co-infection prevalence among prisoners

Limited data were published on HIV and HBV/HCV co-infections among prisoners in the MENA region. Data from Egypt, Iran, Lebanon and Libya are summarized in Table 1. The majority of the studies originated from Iran and were geographically distributed as follows: three from Northern Iran prisons[16,17,20], two from Center Iran[15,18] and one from Southern Iran[19]. The number of participants included in these studies ranged between 150 prisoners in one facility in Iran[20] to 6371 prisoners participating in the Libyan study[22]. Most of these studies were conducted on male prisoners[14-17,20-22] and only one study included female prisoners in Iran[18]. The mean age of the study participants ranged between 31.4 and 41 years except for one study conducted on juvenile prisoners in Isfahan, Iran (mean age 16.6 years)[15]. None of these studies mentioned the reason of imprisonment. These studies could be referred to as

Table 2 Human immunodeficiency virus, hepatitis B virus and hepatitis C virus and their co-infection status among female sex workers and men who have sex with men in Middle East and North Africa countries between 2005 and 2015

Country	*n*	Mean age/age	HIV (%)	HBV (%)	HCV (%)	HIV-HBV co-infection (%)	HIV-HCV co-infection (%)	Triple infection (%)	Ref.
FSWs									
Lebanon	103[a]	⩾ 18	0.0	0.0	0.0	0.0	0.0	0.0	[23]
Libya	69[a]	⩾ 15	10.1	2.9	7.2	0.0	4.3	0.0	[24]
Turkey	130	38.9	0.0	3.1	0.8	0.0	0.0	0.0	[25]
MSM									
Lebanon	101[a]	⩾ 18	1.0	1.0	0.0	0.0	0.0	0.0	[23]
Libya	227[a]	⩾ 15	5.3	3.1	8.4	0.0	4.4	0.0	[24]

[a]Non-adjusted prevalence using respondent-driven sampling method. All numbers were rounded to the nearest 1. HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 3 Human immunodeficiency virus, hepatitis B virus and hepatitis C virus and their co-infection status among injecting drug users in Middle East and North Africa countries between 2005 and 2015

Country	*n*	Mean age/age	HIV (%)	HBV (%)	HCV (%)	HIV-HBV co-infection (%)	HIV-HCV co-infection (%)	Triple infection (%)	Ref.
Cyprus	40	25-31	0.0	0.0	50.0	0.0	0.0	0.0	[27]
Iran	202	-	NR	NS	52.0	NS	9.4	NS	[28]
	417	⩾ 17	24.4	NS	80.0	NS	24.0	NS	[29]
	258	28.8	18.8	NS	65.9	NS	NR	NS	[30]
	233	32.3	7.7	22.7	40.3	4.7	6.4	4.7	[31]
	117[a]	< 30	0.7	0.7	59.0	0.0	0.0	0.0	[32]
	899	33.9	10.7	50.7	34.5	7.8	8.7	6.5	[33]
	100	17-58	19.0	6.0	56.0	NR	15.0	5.0	[34]
	268	37.0	10.8	6.0	39.2	NR	NR	NR	[35]
	153	30.7	5.9	22.9	59.5	2.0	5.2	1.3	[36]
	539	35.3	NR	NR	NR	0.0	1.1	NR	[37]
	200	36.5	1.5	4.5	12.0	0.0	0.0	0.0	[38]
	1327	26.5	20.2	NS	13.5	NS	NR	NS	[39]
	518	35.2	15.5	3.7	69.5	0.6	11.2	0.6	[16]
Israel	743	33.8	1.9	8.6	69.3	NR	NR	NR	[40]
Lebanon	106[a]	⩾ 18	0.9	2.8	52.8	0.0	0.0	0.0	[44]
Libya	328[a]	⩾ 15	87.1	4.5	94.2	4.2	83.2	NR	[41]
Palestine	192	41.3	0.0	2.6	43.8	0.0	0.0	0.0	[42]
Saudi Arabia	297	31.0	0.7	6.1	37.7	NR	NR	NR	[43]

[a]Estimated prevalence using respondent-driven sampling method. All numbers were rounded to the nearest 1. *n*: Sample size; NR: Not reported; NS: Not studied; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

seroprevalence studies since enzyme-linked immunosorbent assay and enzyme immunoassay were used to detect antibodies to HIV, HBV and HCV. Few studies reported in addition to anti-hepatitis B surface antigen (HBsAg) titers the levels of HBs Ag as a marker of HBV infection[17,18]. We don't have data on the hepatitis B vaccine status of these prisoners, except for one study held in Isfahan (Iran) clearly stating that the prisoners were not vaccinated against HBV[18]. It has been noted in four studies that the populations included were not only prisoners but also individuals who inject drugs; those studies were all done in different areas from Iran[16,17,19,20].

The prevalence of HIV among prisoners as reported during the last decade in these countries ranged from 0% to 42.5% with Iran reporting the highest prevalence rate[20] followed by Libya (18.2%)[22]. It is rather interesting that HIV was not reported in the study held in the Minia governorate, Egypt among a cohort of 500 prisoners in[14]. Compared to Egypt, Lebanon and Libya, Iran also reported highest prevalence rates of HBV and HCV at 18.9%[20] and 80.5%[16], respectively. Other studies from Iran reported significantly lower rates of HBV with 0.6% being the lowest[15] and 4%-7% reported in other areas[16-19]. In general, Table 1 shows that HBV prevalence rates among prisoners ranges between 0.6%[15] to 18.9%[20] whereas HCV prevalence ranged between 3.4% in Lebanon[21] to 80.5% in Iran[16]. We similarly assessed the status of co-infection and found that two studies didn't report on the HIV-HBV/HCV co-infection, one from Iran[20] and one Libya[22]. However, two studies from Iran reported HIV-HBV co-infection rates to be 0.8%[16] and 1.1%[19]. The other five studies reported a zero co-infection rate. The co-infection prevalence of HIV and HCV was reported in two Iranian studies with an average rate of 14%[16,19]. Triple infections of 0.8%, 1.1% and 1.5% were recorded in Iran[16,19] and Libya[22]. The other studies either reported a 0% rate or did not assess these co-infections (Table 1).

Distribution of HIV, HBV, HCV and their co-infection status among FSWs and MSMs

During the past decade, Lebanon, Libya and Turkey

Table 4 Human immunodeficiency virus, hepatitis B virus and hepatitis C virus and their co-infection status among different populations from Middle East and North Africa countries between 2005 and 2015

Country	*n*	Mean age/age	HIV (%)	HBV (%)	HCV (%)	HIV-HBV co-infection (%)	HIV-HCV co-infection (%)	Triple infection (%)	Ref.
HIV infected individuals									
Iran	64	-	-	-	-	18.8	NS	NS	[49]
	168	38.7	-	-	-	NS	87.5	NS	[50]
	1338	32-42	-	-	-	NS	78.0	NS	[51]
	106	36.6	-	-	-	20.8	67.0	NR	[52]
	80	37.0	-	-	-	11.3	33.8	25.0	[53]
	130	50.2	-	-	-	11.5	77.0	9.2	[54]
	391	-	-	-	-	14.5	72.0	7.9	[55]
	201	36.0	-	-	-	44.3	67.2	36.3	[56]
	1444	38.4	-	-	-	NS	78.4	NS	[57]
Morocco	503	39.0	-	-	-	29.4	5.4	NR	[58]
Sudan	358	35.0	-	-	-	26.8	NS	NS	[59]
Turkey	949	37.9	-	-	-	0.0	0.9	0.0	[60]
Blood donors									
Cyprus	5057	34.5	0.0	3.0	0.5	0.0	0.0	0.0	[61]
Iran	6499851	-	< 0.1	0.6	0.1	NR	NR	NR	[62]
	2026628	38.0	< 0.1	0.4	0.1	NR	NR	NR	[63]
UAE	592	-	1.2	67.2	31.6	NR	NR	NR	[64]
Others									
Cyprus									
Soldiers	12488	34.5	0.0	2.2	0.5	0.0	0.0	0.0	[61]
Iran									
HBV patients	264	41.6	0.4	-	4.5	0.4	NR	NR	[65]
HIV infected patients' partners	168	33.2	NR	NS	NR	NS	9.5	NS	[50]
Non-injecting drug users	336	28.5	1.5	5.6	4.5	1.2	0.9	0.9	[31]
Referral from behavioral counseling center	379	29.7	4.0	2.9	35.6	0.8	3.4	0.3	[66]
Libya									
General Population	9170	34.0	0.2	3.7	0.9	< 0.1	0.1	< 0.1	[67]
Medical waste handlers	300	-	0.0	2.3	2.7	0.0	0.0	0.0	[68]
Non-Medical waste handlers	300	-	0.0	0.3	0.0	0.0	0.0	0.0	[68]
Turkey									
ER patients	1000	51.7	0.0	5.0	1.8	0.0	0.0	0.0	[69]
In and out-patients	97000-225000	-	0.3	33.9	1.2	NR	NR	NR	[70]

All number were rounded to the nearest 1. *n*: Sample size; NR: Not reported; NS: Not studied; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

reported on the prevalence of HIV, HBV, HCV and their co-infection in MSMs and FSWs (Table 2). The age of the participants was reported to be above 15 or 18 years old for FSWs and MSMs in Libya and Lebanon whereas a mean age of 39 years was reported for the participants in the Turkish study on FSWs. The respondent-driven sampling (RDS) method was used to recruit participants (MSMs and FSWs) for the studies from Lebanon and Libya[23,24]. The RDS method was used to increase the chances of recruiting hard-to-reach vulnerable populations[26]. On the other hand, a cross-sectional study design was reported by Gul *et al*[25] on FSWs in Turkey and recruitment of participants was not addressed in details (a questionnaire was administered). HIV and/or viral hepatitis were not detected in FSWs in Lebanon; whereas 1% out of 101 Lebanese MSM participating in the study reported HIV-1 or HBV but not HCV nor any co-infection, dual or triple[23]. This study reported on the prevalence of HIV and HCV through detection of antibodies and that of HBV through the detection of anti-hepatitis B virus core antigen.

When comparing the rates reported in Libya among FSWs and MSMs, it was clear that HIV-1 was more prevalent among FSWs (10.1%) as compared to MSMs (5.3%). Similar rates were reported for HBV infection (HBsAg) in Libya with 2.9% among FSWs and 3.1% among MSMs (Table 2). HCV was detected among 7.2% of participating FSWs whereas 8.4% was reported among MSMs. Dual HIV-HCV infections were detected among FSWs and MSMs in Libya (4.3% and 4.4%, respectively)[24] but not in Lebanon[23] and Turkey[25]. Triple infections of HIV, HBV and HCV were not detected in FSWs and MSMs in the three countries reporting on these high-risk groups in the region.

Distribution of single, dual and triple HIV, HBV and HCV infections among IDUs

During the last 10 years, seven out of the 25 MENA countries published data on HIV and viral hepatitis among IDUs (Table 3). The majority of these studies (n = 13) were from Iran and were published between 2007 and 2015[16,28-39]. The remaining studies originated from Cyprus, Israel, Lebanon, Libya, Palestine and Saudi Arabia[27,40-44]. A common feature between the MENA

countries reporting on IDUs (Table 3) is the predominance of male participants[16,27-44]. The Iranian studies covered different geographical areas from Iran, with 5 studies performed in Tehran[16,28-30,33], 2 in Isfahan[32,37], 2 in Shiraz[31,39], 2 in Arak[34,36], 1 in Kashan[38] and 1 study included Tehran, Mashhad and Shiraz[35], simultaneously. The pool of participants included in these studies had a mean age ranging between 17 and 58 years[16,28-39]. The majority of the studies included IDUs between 29 and 37 years of age. The participants were recruited through interviews (structured questionnaires)[16,28-39] and rarely through the RDS method to approach potential participants[32]. Furthermore, two studies were performed in Drop in Centers[37,39]. Drop in centers are institutions providing health related services to reduce harm[45]. Zamani *et al*[32] observed that the development of "social networks" is allowing for an increase in sharing of needles in parts of Iran. Seroprevalance data were reported for HIV, HBV and HCV in 11 studies from Iran[16,28,31,34,36-39] with the remaining studies reporting HBsAg as a marker of HBV infection along with the seroprevalances of HIV and HCV among the participants[32,35]. Interestingly, Honarvar *et al*[31] reported on different behaviors adopted by the IDU population participating in their study. Those behaviors were categorized as follows: Sexual behaviors, condom use in sexual contracts, cigarette smoking, tattooing and cupping[31].

According to Mathers *et al*[46], 8 out of the 25 MENA countries (Cyprus, Egypt, Israel, Lebanon, Morocco, occupied Palestinian territory, Oman and Tunisia) have needle and syringe programs (NSPs), however the number of IDUs accessing those programs is not up-to date. While Iran was not part of that study, Des Jarlais *et al*[47], reported that the first NSP established in Iran was in 2003[48]. Des Jarlais *et al*[47] showed that the increase of NSPs was associated with a notable decrease in the number of IDUs in Iran. Recent data show that by 2010, Iran reached a number of 637 NSP sites[47].

For ease of the interpretation of the results, we will report the range of single, dual and triple infections as reported in the 7 countries of the MENA region (Table 3). The seroprevalence of HIV ranged between 0% to 87.1%. Cyprus[27] and Palestine[42] reported the lowest percentages and Libya the highest[41]. The RDS method was applied by Mirzoyan *et al*[41] in Tripoli, the capital of Libya. Thus, there is lack of information from other areas. Authors did not also report on the laboratory assays performed to report on HIV, HBV and HCV. Consequently, we could not report especially for hepatitis B whether the reported data are seroprevalence or prevalence/infection data. With regard to the study done in Israel, authors could not conclude on specific results because the majority of the participants were immigrants[40]. Similarly, a 0% rate was reported by the Palestinian study (East Jerusalem Governorate); the authors stated that they aimed at estimating HIV related risky behaviors and could not report on the magnitude of the epidemic[42]. In Iran, a variable range of HIV-positives was reported with a low of 0.7%[32] to a high of 24.4%[29]. The majority of these studies reported though a range between 10% and 24%[16,30,33-35,39]. HBV was reported to be less than 10% in Cyprus, Israel, Lebanon, Libya and Palestine (Table 3). In Iran, the range of HBV seroprevalence and infection was variable in different areas, with values of less than 10%[16,32,34,35,38], 23%[31,36] and 51%[33]. HCV rates were high in Cyprus (50%), Israel (69%), Lebanon (53%), Libya (94%) and Palestine (40%). Variable results were reported in different parts of Iran with the lowest being 12% and 13%; HCV single infections among IDUs in Iran jumped in other studies to above 35% and reached at time 80%.

Few studies reported the co-existence of HIV and HBV among IDUs; these include a rate of 4% in Libya[41] as compared to 2%[36], 4.7%[31] and 7.8%[33] in Iran. Dual HIV-HCV co-infection was not detected in Cyprus[27], Lebanon[44] and Palestine[42]. The highest HIV-HCV co-infection rate (83.2%) was reported in Libya[41] as compared to those of Iran where the highest rate was found to be 24%[29].

Finally, triple infection with HIV, HBV and HCV were either rarely reported or even studied (Israel, Libya, Saudi Arabia and many parts of Iran) or did not exist among IDUs as the case in Cyprus, Lebanon and Palestine (0% triple infections) (Table 3). In Iran, a range of zero[32,38] to 6.5% is reported. The latter study included IDUs from Tehran[33] where the highest percentage of HIV-HBV co-infection was reported as compared to the rest of the studies in MENA.

HIV, HBV, and HCV among populations other than high-risk groups

Table 4 includes the categories studied for HIV, HBV, HCV and their co-infections in few MENA countries other than the high-risk groups (*i.e*., MSMs, FSWs, IDUs and prisoners). HBV and HCV were detected in already known HIV positive patients in Iran[49-57], Morocco[58], Sudan[59] and Turkey[60]. The age of these patients ranged between 35 and 50 years[51-60]. The purpose of these studies was stated to be either to study HIV co-infection with HCV[50], HBV[49] or triple infection[51-56]. Only three intended to assess the risk factors of these dual and triple infections[54,55,57]. Iran was again the leading country in the number of published studies on HBV and HCV co-infections. These studies took place in different areas from Iran: Shiraz[50,51,57], Isfahan[49,54], Tehran[52,56], Lorestan[55] and Mazandaran[53]. Another study had its population come from Shiraz counseling center for behavioral diseases, and the study was a long cohort taking place from 2003-2011[57]. The majority of these studies (9 out of the 12) reported results on the HIV infected population were seroprevalance data[49-53,56-58,60] while a study from Isfahan and another from Lorestan reported on anti-HIV, anti-HCV and HBsAg[54,55]. The purpose of these studies and the recruitment of participants were variably reported (for example HIV-positive patients in one study attended a consultation center for behavioral diseases)[49]. The aim of the study by Alipour *et al*[50] was to identify and quantify the risk

factors of HCV transmission in HIV infected persons and their partners. Alipour *et al*[50] documented that HIV was mainly transmitted by intravenous (IV) drugs and blood transfusion. This comes in agreement with two other reports from Iran suggesting that the prominent mode of transmission for HIV was IV drug use[54,56], and the rest came from engaging in risky sexual behaviors, in addition to the majority being associated with prisons[54]. In contrast, the Turkish study reported the most common route of HIV and HCV transmission to be heterosexual behavior followed by IV drugs and homosexual practices[60]. The rate of HBV infection among HIV-infected individuals ranged from 0% in Turkey[60] to 44.3% in Iran[56], whereas HCV prevalence rate ranged from 0.9%[60] and 87.5%[50]. Triple infection was either not studied or not reported in the majority of the studies. However in Iran, when studied, the rate of triple infection ranged between 9.2% and 36.3%[54,57].

Data on blood donors were extracted from the few published studies performed in Cyprus[61], Tehran-Iran[62,63] and UAE[64]. The majority of the participants were males whose age ranged between ⩽ 35 and 38 years[61-63]. The study performed in the UAE did not report on the mean age of study participants[64]. The data from these countries reported on markers of HIV, HBV and HCV infections. The studies on blood donors included large samples; the studies held in Iran[63] and the UAE[64] extended over 6 years. Importantly, HIV prevalence was 1.2% in UAE[64], and less than 0.1% in Cyprus and Iran[61-63]. Compared to the other studies among blood donors, only the study performed in UAE reported high HBV and HCV infections, 67.2% and 31.6% respectively[64]. In Cyprus, HBV infection rate reached 3%[61], while in Iran the rate was less than 1%[62,63]. Similarly the HCV infection rate was less than 1% in Cyprus and Iran[61-63]. Dual and triple infections were either not reported (Iran, UAE) or were not looked for among participating blood donors (Cyprus)[61]. Protection against HBV as detected by anti-HBsAg among blood donors of the UAE was high (67%). Similarly, the rate of HCV among these participants was 31.6% (Table 4).

Low rates of HBV (2.2%) and HCV (0.5%) were reported among 12488 soldiers in Cyprus, no dual or triple infections since no HIV cases were reported[61]. Two studies were conducted in Iran where the HIV, HBV and HCV rates were respectively 1.5%, 5.6% and 4.5% among non-injecting drug users[31] and 4%, 2.9% and 35.6% among referrals from behavioral counseling centers[66]. When HBV-positive patients were tested for HIV, 0.4% of the participants were HIV-positive and 4.5% HCV-positive[65]. A 9.5% HIV-HCV co-infection prevalence rate was determined among HIV-infected individuals' partners in an Iranian study[50].

In Libya, a population-based study including nine districts in Tripoli showed that the average prevalence rates of HIV reached 0.2%, whereas HBV and HCV rates reached 3.7% and 0.9% respectively[67]. A case-control study in Libya also studied the risk of acquiring these infections among medical and non-medical waste handlers[68]. HIV was not detected among the cases and controls and thus no dual or triple infections[68]. Moreover, 2.3% of the medical waste handlers had HBV compared to 0.3% among non-medical waste handlers[68]. In the latter, HCV cases were not reported; however 2.7% of the medical waste handlers had HCV[68].

Hospital patients were also part of large size studies reported from Turkey extending for 5 mo[69] and 7 years respectively[70]. HIV was not detected among patients visiting the emergency room cases; HBV and HCV rates were 5% and 1.8%, respectively[69]. In- and outpatients participating in a similar study in Turkey were also assessed for HIV, HBV and HCV infections. 0.3%, 33.9% and 1.2% were reported, respectively. Dual or triple infections were not reported.

DISCUSSION

Despite the availability of successful prevention and treatment strategies, co-infection of HIV-positive individuals with HBV and HCV remains a global public health problem. This review highlights the data available on HIV, HBV and HCV co-infections among high-risk groups in the MENA region. Globally, the reported prevalence of HIV-HBV and HIV-HCV co-infections vary among studies. This is expected especially due to the changing epidemiology of these viruses across time. It is estimated that the rate of HIV-infected individuals with concurrent chronic HBV infection can reach up to 25%, especially in high endemic areas for both viruses[12]. In less endemic areas, *i.e*., North America, Europe and Australia, HBV and HIV are acquired through sexual transmission or injection-drug use (prevalence rates are less than 10% in these areas) and half of the IDUs are reported to be co-infected with HIV and HBV[5]. The prevalence of HIV and HCV co-infections is estimated to be 2%-2.9% (moderate) in many parts of Sub-Saharan Africa and less than 2% in Europe and other developed areas[13]. HIV/HCV co-infections among HIV-positives with history of injection drug use is reported to be between 82% and 93%[71] and previously reported to range between 72% and 95%[13]. Authors observed that MSMs were at lower risks of co-infection despite the fact that HCV epidemics have been described among HIV-infected MSMs; 1%-12% and 9%-27% were reported between MSM and heterosexuals, respectively.

HIV infection is known to negatively affect all phases of the natural history of hepatitis B infection leading to persistent infection, increased cirrhosis, higher liver-associated mortality and increased risk of hepatocellular carcinoma[12]. Similarly, viral persistence, increased viral load, rapid progression to end-stage liver disease and fibrosis caused by HCV are negatively impacted by HIV infection[72].

Our review reveals the paucity of epidemiological data for key populations in many countries of the region. Limited number of studies exists in the MENA region on the status of HIV, HBV and HCV and their co-infections

among prisoners, MSMs and FSWs. We also highlight the lack of studies on the prevalence of HIV, HCV and HBV among transgenders. This is alarming especially when MSMs continue to show high burden of HIV prevalence and incidence globally[73,74]. Evidence supports the continued increase of the HIV epidemic among MSMs. In high-income countries and despite the success of cART, HIV epidemic trends are decreasing except in MSMs. HIV is estimated to be increasing at 8% among MSMs in the United States per year since 2001. Similarly, the highest rates of HIV infection are in MSMs in much of Africa, Asia and Latin America. Importantly, and by the end of 2011 only 93 out of 196 countries did not report on the prevalence of HIV in MSMs in the past 5 years[75]. The UNAIDS reported a pooled HIV prevalence of as low as 3% in the MENA region to as high as 25% in the Caribbean[76]. However, Data on MSMs and HIV infections from the Middle East, North Africa and Sub-Saharan Africa were reported to be emerging.

Consequently, data gaps in surveillance are added challenges to understanding the epidemiology of MSMs in the region. This is especially true due to the stigma, discrimination and homophobia associated with this group. An increased risk of unprotected anal intercourse and higher levels of HIV misinformation were associated with homophobia. Discrimination and stigma were also identified as possible barriers for HIV testing and adherence to treatment[77]. In addition, criminalization of HIV transmission and male-male sex make it dangerous for affected individuals to release their status and thus reduce the implementation of recommended services[73].

In addition to the lack of studies on MSMs and FSWs in the MENA region, we don't have any study on the practices, characteristics, or the status of HIV infection and viral hepatitis among male sex workers selling or exchanging sex for money. Male sex workers have been ignored globally in the context of HIV/AIDS. Evidence exists that the HIV burden is either sustained or increasing in this population[78]. We believe, along with others[78], that this is also a key population at high risk of acquiring and transmitting HIV and viral hepatitis. Global and regional studies on biological, behavioral and structural factors affecting HIV, HBV and HCV are clearly needed.

In 2008, a range of 11-21 million people has been estimated to inject drug in the world[79] where data from the Middle East and many countries in Africa were absent. In a more recent review on hepatitis B and C among IDUs, Nelson *et al*[80] reported a range of 6-15 million worldwide with 1.2 million IDUs being HBsAg positive and 6 million being anti-HBC positive. These populations were mainly clustered in the United States, China and Russia. Data from few countries of the MENA region were included in this study (prior to 2009); these include Cyprus, Egypt, Israel, Lebanon, Palestine, Saudi Arabia, Syria and Turkey. Our review shows that not many countries of the MENA region are advocating for research on these viruses among IDUs during the past 10 years (Table 4). Mumtaz *et al*[81] estimated the number of people who inject drugs to be approximately 626000 (33000-1635000) in the MENA with HIV evidence of epidemics in one third of the countries among IDUs. This review highlighted the low levels of condom use, high level of having sex with sex workers as well as the high level of MSMs and sex selling; all of which indicate a high injecting and sexual risk environment. The prevalence of HCV was estimated to range between 31% and 64%.

Our review shows again the scarce number of estimates of single, dual and triple infection among IDUs in the region except for Iran. Iran is the main country in the region with the highest number of IDUs. NSPs are reported to be present in Egypt, Iran, Jordan Israel, Lebanon, Morocco, Oman, Palestine and Tunisia[82,83]. Moreover, the lack of opioid substitution therapy was also reported in the MENA countries except for Israel, Iran, Lebanon, Morocco and the UAE. Harm reduction has been described in prisons in Iran where prisoners can access clean injecting equipment. Evidence suggests that injecting equipment are shared in prisons of Jordan, Kuwait and Lebanon.

We believe that it is also important that HIV, HBV and HCV prevention strategies address the vulnerability among women who use or inject drugs. This is critical especially with the reported increase of HIV prevalence among female IDUs as compared to their male counterparts as well as the increased prevalence of HCV among female IDUs and FSWs[84]. Policies to reduce discrimination and sex-based violence, police mistreatment, registration of female drug-users and protection of their rights, access to NSPs and access to HIV and viral hepatitis treatment are all needed and not addressed in the MENA region for FSWs.

It is critical to acknowledge that HIV and co-infections with HBV and HCV are major threats among high-risk groups in the MENA region. Several limitations exist in this review while trying to interpret the variable data: (1) the different types of sampling methods and approaches used to recruit participants in different studies; (2) the definition of HIV, HBV, HCV infections and their co-infections; this depends on the sensitivity of the surveillance documenting the mode of acquisition of these viruses; and (3) the lack of data on size estimation of MSMs, FSWs, IDUs and other populations in the region. It is clear that urgent reforms are needed to take place in order to push for extensive and comprehensive research agenda for countries to be informed about the impact of HIV transmission among high-risk groups and the change in the dynamics of the pandemic. Estimates of key populations at high risk including IDUS, FSWs, MSMs and other lesbian, gay, bisexual, transgenders are needed to guide policy makers to understand the magnitude of the problem. Vaccination against hepatitis B to all IDUs must be a priority especially those that are already HCV-positive. HIV testing of these high-risk populations should also be part of the strategic plan in MENA countries. Understanding these gaps is key to strategize surveillance, bio-behavioral surveillance,

interventions and treatment plans.

This review highlights the paucity and the variability of existing data on high-risk groups and the status of HIV, HBV, HCV infections and co-infection in the MENA region. Without addressing the risks of expanding epidemics among high-risk groups, an AIDS free society will remain an illusion. It is obvious that resources need to be allocated to inform strategic planning and policy of the silently creeping waves of HIV and viral hepatitis epidemics among these groups. The MENA countries are in urgent need of advanced research and strengthening of the data collection systems and reporting practices of these infections among key populations. These efforts are critical to ultimately incorporate findings in setting national health policy priorities in the region.

COMMENTS

Background

Despite the availability of successful prevention and treatment strategies, co-infection of human immunodeficiency virus (HIV)-positive individuals with hepatitis B virus (HBV) and hepatitis C virus (HCV) remains a global public health problem. This review highlights the data available on HIV, HBV and HCV co-infections among high-risk groups in the Middle East and North Africa (MENA) region. The high-risk groups include men who have sex with men (MSMs), female sex workers (FSW), injecting drug users (IDUs) and prisoners.

Research frontiers

To the authors' knowledge, this is the first review on the status of co-infection (dual and triple) of HIV-positive individuals with HBV and/or HCV among high-risk groups in the countries of the MENA region.

Innovations and breakthroughs

The authors' review highlights the paucity and the variability of existing data from the MENA region on HIV, HBV and HCV co-infections among MSMs, FSWs, IDUs and prisoners. This review draws the attention of researchers to the critical need of addressing the gaps in surveillance, the latter being a challenge to understanding the epidemiology of these co-infections among high-risk groups. Advanced research, strengthening of the data collection systems as well as reporting practices and behaviors among high-risk groups are urgently needed.

Applications

Resources are needed to be allocated to inform strategic planning and policy on HIV and viral hepatitis epidemics among key populations in the MENA region. These efforts are important especially in setting national health policy priorities in the region.

Terminology

This review summarizes the existing data on the status of co-infections of HIV-1, the causative agent of acquired immunodeficiency syndrome, and viral hepatitis among high-risk groups in the MENA region.

Peer-review

Melhem *et al* have reviewed the existing data on the prevalence of coinfection of HIV, HBV and HCV in the MENA region, this is really interesting revision of the existing data.

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