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TOPIC HIGHLIGHT

2015 Advances in Hepatocellular Carcinoma

Long noncoding RNAs in hepatocellular carcinoma: Novel insights into their mechanism

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Abstract

Hepatocellular carcinoma (HCC) is the predominant subject of liver malignancies which arouse global concern. Advanced studies have found that long noncoding RNAs (lncRNAs) are differentially expressed in HCC and implicate they may play distinct roles in the pathogenesis and metastasis of HCC. However, the underlying mechanisms remain largely unclear. In this review, we summarized the functions and mechanisms of those known aberrantly expressed lncRNAs identified in human HCC tissues. We hope to enlighten more comprehensive researches on the detailed mechanisms of lncRNAs and their application in clinic, such as being used as diagnostic and prognostic biomarkers and the targets for potential therapy. Although studies on lncRNAs in HCC are still deficient, an improved understanding of the roles played by lncRNAs in HCC will lead to a much more effective utilization of those lncRNAs as novel candidates in early detection, diagnosis, prevention and treatment of HCC.

Key words: Hepatocellular carcinoma; Long noncoding RNA; Dysregulation; Mechanism; Pathway

Core tip: Hepatocellular carcinoma (HCC) is a global concern. Long noncoding RNAs (lncRNAs) are likely to play crucial roles in various pathogenesis of HCC, including tumor growth, proliferation, invasion, metastasis and recurrence. Here, we focus on recent studies of human HCC associated lncRNAs and highlight their functions, mechanisms, as well as their potential to act as novel candidates for early detection, diagnosis, prevention and treatment of HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most common histologic subtype of primary liver cancers, accounting for 70%-85% of liver cancer cases in most countries, is the third-leading cause of worldwide mortality for various cancers[1-3]. It causes nearly 695900 deaths per year, half of which occur in China, as a result of the high chronic hepatitis B virus (HBV) infection incidence[4]. Besides, infection with hepatitis C virus, hepatosteatosis and chronic exposure to toxic chemical substances are the major HCC risk factors[5,6]. Especially, exposure to aflatoxin plays a key role in inducing HCC in our Guangxi Zhuang Autonomous Region[7]. In addition, many key signal transduction pathways have been verified to be involved in the pathogenesis of HCC, including PI3K/Akt/mTOR pathway, Raf/MAPK/ERK pathway, Jak/Stat pathway, WNT-b-catenin pathway, so on and so forth[8-12]. Usually, the treatments for HCC are finite and are only available in the early stage, while most HCC are only detected in their advanced stage when traditional chemotherapy has marginal effects and leads to poor prognosis. Because of its dismal outcome, etiology and carcinogenesis investigation are in urgent need. Recently, human genome analysis in non-protein coding has made new progress. It discovers massive transcription of large RNA transcripts which lack coding protein function, termed as long noncoding RNAs (lncRNAs).

With recent application of next generation sequencing techniques, significant numbers of non-coding RNAs (ncRNAs) and lncRNAs have been discovered to be associated with HCC. And HCC is characterized by dysregulation of numerous gene networks while both protein-coding genes and *ncRNA* genes are involved, just like many other cancers. It is estimated that protein-coding genes only account for less than 2% of the human genome while nearly 70% of the human genome is transcribed pervasively[13]. Accordingly, ample ncRNAs are transcribed from human genome, for instance lncRNAs, microRNAs (miRNAs), small interfering RNAs and PIWI-interacting RNAs[14-16].

NcRNAs were once thought to be body "garbage" or transcriptional "noise". However, accumulating reports have demonstrated that miRNAs and lncRNAs play valid regulatory roles in cancer[17-22]. Recent studies[23] have also elucidated that lncRNAs possess a significant role in epigenetic regulation. *Via* regulating gene expression by miscellaneous mechanisms, including genomic imprinting, chromatin modification, regulation of protein function, transcription and post-transcriptional processing[24-26], lncRNAs are involved in multitudinous physiological functions and pathological processes.

LncRNAs, larger than 200 nucleotides (nt) in length, are commonly defined as endogenous cellular RNA molecules, which are poorly conserved and not capable of being translated into proteins[21,23,27]. They can be monitored by a high-throughput analysis such as transcriptome analysis and microarrays, or through bioinformatics prediction[28]. LncRNAs can be transcribed by RNA polymerase Ⅱ, and then undergo cotranscriptional modifications including polyadenylation and pre-RNA splicing[29]. Many studies have pointed out that lncRNA transcripts play vital roles in various biological processes as they function in gene imprinting and splicing, chromatin modification, immunesurveillance, cell fate specification, cell cycle control and cell apoptosis, or act as nuclear architecture, subnuclear compartments, RNA processing enhancer and promoter[30,31].

LncRNAs have assorted mechanisms in biological processes. Generally speaking, the role of lncRNA as a gene expressing regulator could be found in transcriptional level and posttranscriptional level. Cis-regulation and trans-regulation are two main transcriptional regulation means, by which lncRNAs can target local and distant genes, respectively. The posttranscriptional regulating mechanism is involved in posttranscriptional process of mRNAs which includes splicing, editing, trafficking, translation and degradation. LncRNAs can also function as competing endogenous RNAs for shared miRNA[32,33]. In brief, there are four known molecular functions of lncRNAs: Signal, decoy, guide, and scaffold[21,34].

There are five species of lncRNAs, listed as follow[31,35,36]: Sense or antisense (when overlapping at least one exon of another transcript on the opposite or same strand), bidirectional (when a neighboring coding transcript or its expression on the opposite strand is initiated in close genomic proximity), intronic (when derived from an intron of a second transcript), and intergenic (when it lies as an independent unit within the genomic interval between two genes). An updated definition was given to lncRNAs by other researchers regardless of their length and non-protein coding capability[37]. It described lncRNAs as RNA molecules who may have the function as primary or spliced transcripts which do not confirm to the known varieties of small RNAs or structural RNAs[38]. In recent years, the number of articles focused on lncRNAs has increased greatly. Recent studies have demonstrated that certain

Table 1 Upregulated long noncoding RNAs in hepatocellular carcinoma

Name	Gene locus	Size (bp)	Dysregulation	Potential role in HCC	Ref.
HULC	6p24.3	1638	Upregulated	Associate with HBV infection or histological grade. Associate with tumor growth	[22,29,44-50]
H19	11p15.5	2660	Upregulated	Suppress progression, metastasis. Promote cell proliferation	[22,29,50-57]
TUC338	12q13.13	590	Upregulated	Increased in liver cirrhosis. Modulate cell growth	[58]
MALAT1	11q13.1	8708	Upregulated	Associate with tumor metastasis, recurrence	[21,22,29,59,60]
HOTAIR	12q13.13	12649	Upregulated	Associate with invasion and metastasis. Increases chemosensitivity	[21,22,29,61-65]
HOTTIP	7p15.2	6839	Upregulated	Associate with tumor progression and disease outcome	[28,66-68]
HEIH	5q35.3	1665	Upregulated	Associated with HBV-HCC. Associate with prognosis	[21,29,69]
MDIG	3q11.2	30635	Upregulated	Associate with DNA repair and prognosis	[70]
PVT1	8q24.21	210626	Upregulated	Associate with HCC progression and predict recurrence	[71,72]
Linc00974	17q21.31	4890	Upregulated	Predict tumor growth and metastasis	[73]
UFC1	1q23.3	5113	Upregulated	Promote HCC cell proliferation, inhibit cell apoptosis and induce cell cycle progression	[10,12,74]
PCNA-AS1	20p12.3	384	Upregulated	Promote tumor growth	[75]
UCA1	19p13.12	7375	Upregulated	Involved in chemotherapeutic resistance. Associatewith TNM stage, metastasis and postoperative survival	[50,76]
CCAT1	8q24.21	11887	Upregulated	Promotes HCC progression	[77-79]
ATB	19q13.3	2895	Upregulated	Associat with poor prognosis	[80,81]
URHC	2q24.2	192173	Upregulated	Promote cell proliferation and inhibit apoptosis	[82]

HCC: Hepatocellular carcinoma; HULC: Highly upregulated in liver cancer; MALAT1: Metastasis-associated lung adenocarcinoma transcript 1; HOTAIR: HOX transcript antisense RNA; HEIH: Long noncoding RNA high expression in HCC; CCAT1: Colon cancer associated transcript-1; ATB: Long noncoding RNA-activated by transforming growth factor-β; URHC: Up-regulated in hepatocellular carcinoma; HBV: Hepatitis B virus; TNM: Tumor node metastasis.

Table 2 Downregulated long noncoding RNAs in hepatocellular carcinoma

Name	Gene locus	Size (bp)	Dysregulation	Potential role in HCC	Ref.
MEG3 (GTL2)	14q32.3	34919	Downregulated	Associate with methylation. Predictive biomarker for monitoring epigenetic therapy	[21,83-87]
LncRNA-LET	15q24.1	2606	Downregulated	Reduces hepatic invasion and abdominal metastases	[88]
PTENP1	9p13.3	3917	Downregulated	Repress the tumorigenic properties of HCC cells	[22,89]
SRHC	5p15.31	6365	Downregulated	Inhibit cancer proliferation	[90]
MT1DP	16q13	1255	Downregulated	Act as a tumor suppressor. Overexpression of MT1DP decreases cell proliferation but increases apoptosis	[91]

HCC: Hepatocellular carcinoma; MT1DP: Metallothionein 1D, pseudogene; MEG3: Maternally expressed gene 3; LncRNA: Long noncoding RNA.

lncRNAs are specifically correlated with certain classes of cancer and the different expression level of lncRNAs may function as an indicator for metastasis and prognosis[39-41]. Genome-wide transcriptomic analyses have found that a number of lncRNAs are dysregulated in HCC cell lines or cancer tissues[21,28,29,42,43]. Given the fact that such large scales of lncRNAs are aberrantly regulated in HCC, it is of highly possibility that lncRNAs are directly associated with carcinogenesis of HCC. In this review, we concentrated on cancer-related lncRNAs which have been validated in human HCC. Furthermore, we summarized their mechanism and signaling pathways in HCC.

REPRESENTATIVE LNCRNAS DYSREGULATED IN HCC

Abnormal expressed lncRNAs have been found to be associated with hepatocarcinogenesis and play a key role in metastasis and prognosis[29,34,42]. Accumulating studies recently have focused on the contributions of lncRNAs in HCC development. Here, we summarized differential expressions of lncRNAs and their potential roles in HCC (Tables 1 and 2).

MECHANISMS AND SIGNALING PATHWAY IN HCC

After attentive study, we divided the researched lncRNAs into 3 groups and made the above conclusive table. And with the purpose of identifying the roles of different lncRNAs who might play in the early detection and diagnosis or prevention and treatment of HCC, we further summarized their function and mechanism as follow.

LNCRNAS ASSOCIATED WITH TUMOR GROWTH AND PROLIFERATION IN HCC

Highly upregulated in liver cancer

Highly upregulated in liver cancer (HULC), a well-researched lncRNA who associates with HBV infection and HCC tumor growth is upregulated in HCC and associated with its grades[45]. By viewing dozens of research articles, we found that HULC can accelerate the growth of HCC *via* downregulating its neighbor gene p18 (known as eukaryotic translation elongation factor 1, EEF1E1 or AIMP3) and can disturb the circadian rhythm of HCC *via* upregulating oscillator CLOCK[47].

The complementary base which pairs between 5'UTR of CLOCK mRNA and HULC takes responsibility for the modulation of CLOCK mediated by HULC[91]. With regard to the mechanism of HULC upregulation, HBx regulates the transcription of CREB-dependent promoters by interacting with CREB, and then, the transcription factor CREB contributes to the activation of HULC promoter[47,48]. The variant genotypes of rs7763881 in HULC contribute to decreasing HCC susceptibility in persistent HBV carriers. And single nucleotide polymorphisms (SNPs) in HULC contribute to the risk of HBV chronic infection and HCC[92]. Besides, HULC acts as an endogenous "sponge", who downregulates a series of miRNAs activities, including miR-372. Studies indicate that inhibition of miR-372 results in decreased translational repression of its target gene, PRKACB, and inducing phosphorylation of CREB in turn[48].

Based on the above mechanisms, HULC may have the potential of predicting prognosis in clinical practice. However, only cell lines research were done by researchers, other confirmatory experiments are requisite.

LncRNA-hPVT1

Two studies investigated closely into lncRNA-hPVT1 and concluded that it has a function of promoting cell proliferation, cell cycling and it also functions as an acquisition of stem-cell like contents in HCC cells. LncRNA-hPVT1 upregulates nucleolar protein p120 (NOP2) *via* enhancing the stability of NOP2 proteins and its above functions depend on the presence of NOP2. Studies show that the transforming growth factor (TGF)-β1/lncRNA-hPVT1/NOP2 pathway is compromised in the progression of HCC. Hence, lncRNA-hPVT1 influences the stem-cell like potential of HCC cells and promotes the growth of HCC. Regulation of the lncRNA-hPVT1/NOP2 pathway has a beneficial effect in the treatment of HCC[71]. More researches are needed to be done in order to find effective therapy targeted at lncRNA-hPVT1.

UFC1

Other than the above three lncRNAs, another well-studied lncRNA associated with HCC proliferation is lncRNA-UFC1 (GenBank Accession No. KJ809564), who promotes HCC cell proliferation, induces cell cycle progression and inhibits cell apoptosis[73]. It induces HuR translocation and by silencing HuR expression can abrogate the function of lncRNA-UFC1 function in HCC. Moreover, lncRNA-UFC1 is targeted by miR-34a and the overexpression of miR-34a significantly suppresses the expression levels of cell cycle related proteins, cellular proliferation and HuR expression in lncRNA-UFC1-overexpressing cells[10]. As molecularly targeted therapies are heated studied, UFC1 offers us a new aspect, clinical research are in the urgent need.

ZNRD1 antisense RNA 1

A large case-control study including 1344 HBV natural-clearance subjects, 1344 HBV persistent carriers and 1300 HBV-positive HCC patients was done[93]. The study found out that ZNRD1 antisense RNA 1 (ZNRD1-AS1) is a crucial regulator of ZNRD1 (human zinc ribbon domain containing 1). In ZNRD1-AS1, several SNPs (nucleotide polymorphisms) is identified as expression quantitative trait loci (eQTLs) SNPs, which are connected with the expression of ZNRD1[94,95]. ZNRD1 is involved in DNA damage and repair *via* regulating the expression of excision repair cross-complementing 1 (ERCC1)[96], to restrain cell proliferation and to regulate the expression of miRNAs in cancers[97,98]. Furthermore, ZNRD1 eQTLs SNPs in lncRNA ZNRD1-AS1 have an increased risk for persistent HBV-carriers HCC but a protective influence against chronic HBV infection[93]. As a result, the different roles which ZNRD1-AS1 play make a difference in the treatment of HBV-positive HCC patients and HBV-negative HCC patients.

Colon cancer associated transcript-1

Dysregulation of colon cancer associated transcript-1 (CCAT1) is in association with tumor size, microvascular invasion, AFP and prognosis in patients with HCC. Besides, it is demonstrated that *in vitro* CCAT1 could promote proliferation and migration in HCC by binding to let-7, which contributes to the up-regulation of HMGA2 and c-Myc[76]. Herein, the complex of CCAT1 and let-7 may have the diagnostic function in early detection of HCC and its migration.

Maternally expressed gene 3

Maternally expressed gene 3 (*MEG3*) regulates tumor cell proliferation and apoptosis in HCC partially through the accumulation of p53[83]. UHRF1, as a new identified oncogene, contributes to the upregulation of *MEG3* in HCC by regulating DNMT1, while upregulation of *MEG3* in HCC cells can partially diminish the promotion of proliferation produced by UHRF1. In addition, UHRF1/DNMT1/MEG3/p53 axis signaling pathway is involved in HCC progression[99]. Furthermore, loss of *MEG3* gene expression is related to hypermethylation of the promoter region in HCC[82]. Impressively, enforced expression of *MEG3* in HCC remarkably decreases both anchorage-independent and anchorage-dependent cell growth, and induces cell apoptosis[83]. Associated with anti-oncogene p53, *MEG3* shows a promising future in being one of the therapeutic targets for HCC treatment.

PTENP1

The over-expression of PTENP1 (a pseudogene of PTEN) represses the oncogenic PI3K/AKT pathway and elicits pro-death autophagy by sequestering miR-20a, miR-19b and miR-17 *in vitro*. It also inhibits tumor growth *in vivo*. These are accompanied by dampened angiogenesis or neovasculature maturation, enhanced apoptosis and autophagy[88]. It's necessary to do further *in vivo* experiments to confirm its detailed mechanism.

The above 7 lncRNAs have been widely studied, and they are considered to be associated with tumor growth and proliferation in HCC. With further clinical trials, their

application in the prediction and diagnosis of HCC would be possible.

LNCRNAS ASSOCIATED WITH METASTASIS AND PROGNOSIS IN HCC

Invasion and metastasis often adumbrate an advanced stage while recurrence often indicates a poor prognosis. It's the same in HCC development. The following lncRNAs were found associated with metastases and recurrence which may predict a dismal outcome.

H19

A dozen of studies have elucidated that H19 serves as a potential prognostic marker as well as potential target for HCC therapy. By decreasing the expression of markers for epithelial-to-mesenchymal transition, such as claudin 1, cytokeratin-8 (KRT-8), KRT-19 and CDH1 (E-cadherin), H19 suppresses the progression of HCC. By mediating hnRNPU/PCAF/RNAPol Ⅱ, H19 suppresses the migration of HCC. By increasing histone acetylation, H19 can epigenetically activates miR-200 family, and thus, it suppresses HCC metastasis[50]. Moreover, the identification of AKT/GSK-3β/Cdc25A signaling pathway as the downstream signaling pathway of H19 explains the molecular mechanism of metastasis and invasion in HCC[100]. And it has also been demonstrated that the deletion of H19 endodermal enhancer can regulate expression of insulin-like growth factor 2 (IGF2) and H19 in the early stage of liver carcinogenesis as well as that paternal inheritance of the deletion of H19 endodermal enhancer can delay tumor formation by increasing apoptosis of the hepatocytes and reducing IGF2 expression[101]. In the main, by various signaling pathway, H19 can be a promising indicator for prognosis and can be targeted in the treatment of HCC.

Metastasis-associated lung adenocarcinoma transcript 1

Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a critical regulator of maintaining the transformative phenotype in HCC, it associates with tumor metastasis and recurrence[102]. MALAT1 is a genuine target gene of the Wnt/TCF/β-catenin and Hippo/yes associated protein (YAP) signaling pathway. It is negatively regulated by SRSF1 *via* the following two pathways. The first pathway is by accelerating the degradation of MALAT1 which is blocked by YAP at the stage of post-transcriptional. When overexpressed, YAP stimulates the translocation of SRSF1 from the nucleus to the cytoplasm, thus the nuclear-retained MALAT1 avoids degradation[103]. The second pathway is by binding to YAP. SRSF1 inhibits the transcriptional activity of YAP and prevents the recruitment of YAP on the MALAT1 promoter at a transcriptional stage[104].

MALAT1 can interact with the arginine/serine SR proteins and modulate their distribution to the nuclear speckles. Furthermore, MALAT1 can regulate alternative splicing of pre-mRNAs *via* controlling active SR proteins' levels[103].

To sum up, by participating in different pathway, MALAT1 shows a crucial role in the carcinogenesis of HCC which lays the foundation of early detection of metastases and assessment of recurrence.

HOX transcript antisense RNA

High expression of HOX transcript antisense RNA (HOTAIR) indicates a notably poorer prognosis with respect to overall survival (OS) and a remarkably larger tumor size in HCC patients[62]. HOTAIR is selectively required to target polycomb repressive complex 2 (PRC2) occupancy, thus, induces histone H3 tri-methylated at lysine 27 (H3K27) trimethylation and silenced transcription of the HOXD locus[105]. The 5' domain of HOTAIR can bind to PRC2, while the 3' domain of HOTAIR can bind to the LSD1 (lysine specific demethylase 1)/CoREST (Co-repressor of RE1-silencing transcription factor)/REST complex; after that, the complexes are targeted and assembled to the HOXD locus; and co-ordinately regulate histone H3K27 methylation and histone histone 3 methylated at lysine 4 demethylation; consequently, the transcription across 40 kb of the HOXD locus is silenced in trans by DNA methylation[106]. These indicate that the HOTAIR-induced assembly and targeting of LSD1 and PRC2 complexes is a general mechanism for gene silencing across the genome, which plays a vital role in the association with invasion and metastasis in HCC[64]. Expression of HOTAIR is also associated with tumor size and lymph node metastasis in HCC patients[62]. Knockdown of HOTAIR reduces the levels of matrix metallopeptidase 9 and vascular endothelial growth factor, which play an important role in metastasis and cell motility[60].

In addition, HOTAIR is found to promote invasion and migration in HCC by inhibiting RBM38. Silencing of RBM38 restores cell motility, while knockdown of HOTAIR conspicuously reduces cell motility as the downregulation of HOTAIR increases the expression of RBM38 both on mRNA levels and protein levels[63].

With its association with tumor size, tumor metastasis and OS, HOTAIR is regarded as one of the prognosis indicator. It's of great potential value to use it in the clinic in the future.

HOTTIP

HOTTIP (the transcript of HOXA at the distal tip with 3.8 kb), transcribed from the 5' tip of the HOXA locus, coordinates the activation of some 5' *HOXA* genes and is negatively regulated by miR-125b. High HOTTIP expression indicates an increased metastasis formation and a decreased overall survival[65]. HOTTIP binds the adaptor protein WDR5 directly and targets WDR5/MLL complexes across HOXA, thereby driving histone H3 lysine 4 trimethylation and the gene transcription and influences HCC cell proliferation rates as a result[27]. Its overexpression contributes to hepatocarcinogenesis through regulating the expression of its neighboring protein-coding genes[67]. With these function, HOTTIP

can be potentially used in clinic as a prognosis predictor.

LncRNA high expression in HCC

LncRNA high expression in HCC (HEIH), associated with recurrence and overall survival in HBV-related HCC, is overexpressed in HCC tissues[68]. HEIH influences the expression of enhancer of zeste homolog 2 (EZH2) target genes by increasing the binding of EZH2 levels. Downregulation of HEIH induces G(0)/G(1) arrest which is caused by the interaction of EZH2 with HEIH. Thus, level of HEIH is significantly associated with recurrence and is an independent prognostic factor for survival in HCC[68]. In summary, HEIH connects with HBV-related HCC and plays a role as the risk factor for HCC recurrence. Therefore, predicting the possibility of HCC recurrence by detecting the relative change of the HBV virus and HEIH level in serum is worth further studying.

ATB

Overexpression of lncRNA-ATB (lncRNA-activated by TGF-β) significantly correlates with *EMT* gene signature expression, macrovascular invasion, microvascular invasion, portal vein tumor thrombus and encapsulation. Moreover, higher expression of lncRNA-ATB is significantly correlated with shorter recurrence-free survival and overall survival, suggesting that lncRNA-ATB contributes to HCC progression[27].

LncRNA-ATB upregulates ZEB1 and ZEB2 *via* competitively binding with the miR-200 family, then it induces EMT and invasion. Besides, lncRNA-ATB promotes organ colonization of disseminated HCC tumor cells through binding with autocrine induction of interleukin 11 (IL-11), IL-11 mRNA and triggering signal transducer and activator of transcription 3 signaling[79,80]. On the whole, lncRNA-ATB facilitates the invasion-metastasis cascade, which makes it a predictor for HCC prognosis.

LncRNA-p21

LncRNA-p21, located upstream of *CDKN1A* gene, who triggers apoptosis in HCC, is a transcriptional target of p53 and *p53* gene is an important tumor suppressor gene[107,108]. Importantly, lncRNA-p21 can bind to heterogeneous nuclear ribonucleoprotein K (hnRNP-K), therefore it contributes to the localization of hnRNP-K and transcriptional repression of p53-regulated genes[109] and as a result, triggers apoptosis. Theoretically, lncRNA-p21 should be down-regulated in HCC, however, corroborating experiments are needed to delineate the exact underlying mechanism. As the transcriptional target of anti-oncogene p53, p21 shows a promising future in being one of the therapeutic targets for inducing cellular apoptosis.

The above 7 lncRNAs are considered to be associated with metastasis and prognosis in HCC. Although many researches have been complicated, studies with more cases and further ward clinical trials would be needed in order to develop novel therapeutics and treatment for HCC patients.

LATEST FIND OF LNCRNAS RELATED TO HCC

Linc00974

Dysregulation of Linc00974 increases KRT19 levels, which results in the activation of both TGF-β and Notch signaling pathways, which causes the invasion and proliferation of HCC both *in vivo* and *in vitro*. Linc00974 influenced KRT19 expression by interacting with miR-642[72]. Being significantly correlated with tumor differentiation grade, size and metastasis makes Linc00974 a feasible predictor in the carcinogenesis of HCC.

Up-regulated in hepatocellular carcinoma

High level of up-regulated in hepatocellular carcinoma (URHC) expression is significantly associated with tumor size, tumor number and shorter overall survival after surgery[81]. URHC can regulate cell proliferation and apoptosis by repressing ZAK expression. ZAK, also known as MLK-like MAP triple kinase-α or ZAK-α, belongs to the mixed lineage kinase family and functions as a tumor-suppressor gene in HCC[110]. Inactivation of the ERK/MAPK pathway is required for the increase in HCC growth, which is induced by URHC-ZAK regulation[81]. However, only microarray analysis was done, which limited its conviction. Experiments *in vivo* and *in vitro* are desperately needed.

SRHC

SRHC (NCBI No: uc003jdr) is an important downstream target gene of HNF-4A and it is correlated with α-fetoprotein (AFP) levels and the degree of differentiated tumors[89]. SRHC is combined with HNF-4A to promote its transcription, thus inhibiting the proliferation of tumor cells and promoting cell differentiation in HCC[89]. Serum AFP level monitoring is well developed and have been used as a standardized index of HCC diagnosis, therefore, detecting SRHC in predicting HCC may have great value in clinical practice as well.

Metallothionein 1D, pseudogene

Metallothionein 1D, pseudogene (MT1DP) inhibits tumor cell growth could be rescued by a combination of overexpression of Runt related transcription factor 2, FoxA1 and YAP. In addition, MT1DP inhibited transformative phenotype of liver cancer cells and cell proliferation by reducing protein synthesis of FoxA1[90]. With this inhibiting function, it's of great research value whether MT1DP can be a potential therapeutic target in the treatment of HCC or not.

LncRNA low expression in tumor

LncRNA low expression in tumor (LncRNA-LET) plays a decisive role in hypoxia-induced metastasis in HCC through a hypoxia-inducible factor 1, alpha subunit

(HIF-1a)/histone deacetylase 3 (HDAC3)/LET/NF90 pathway[110]. Precisely, HDAC3 represses LET by decreasing the LET promoter region's histone acetylation-mediated modulation under hypoxic conditions. And then, downregulation of LET recedes the direct interactions between LET and NF90, then enhances the stabilization of NF90 and increases the expression of HIF-1a (a target mRNA of NF90 involved in hypoxia-induced metastasis). As a result, LET inhibits the metastasis of HCC *via* this positive feedback loop[87]. Therefore, when LET is downregulated, patients usually face a poor prognosis.

For these 5 newly found lncRNAs, investigations with more preclinical models of HCC would be desired in order to further strengthen the conclusions and provide a more rational support for ward clinical application.

CONCLUSION

In conclusion, lncRNAs play a crucial role in various biological processes in HCC, such as initiation, progression, metastasis, treatment and prognosis.

Two latest published articles also reached the same conclusion. On the basis of dozens of studies, Yang *et al*[111] discussed the probable molecular mechanisms depended on lncRNA level change, and drew the conclusion that lncRNAs can be applied in HCC diagnosis and treatment. However, their summary was omissive as they only analyzed the upregulated lncRNAs, and even some upregulated lncRNAs were left out, such as HOTTIP, MVIH, UFC1, UCA1, CCAT1, *etc*. In our article, we not only analyzed the differential expression of lncRNAs in human HCC (upregulated as well as downregulated), but also summarized their specific mechanisms and pathways and gave an outlook in their potential as candidates in diagnosis and treatment of HCC. Another review[111] provided different lncRNAs compared to us, such as RP11-160H22.5, XLOC_014172 and LOC149086. However, these lncRNAs were not embodied in NCBI database, and their mechanisms were unavailable, which brought us new research direction.

Although plenty of studies[28,112-115] cast light on the characteristics and mechanisms of different lncRNAs involved in HCC as we summarized above, till now, research on lncRNA still remains in its infancy and a large portion of lncRNAs surely remains to be further discovered. As systematic identification of lncRNAs and their well-understanding of mechanisms can pave the way for early diagnosis and therapeutics designing for HCC, there is still a long way to go in the research field of HCC-related lncRNAs.

Continuous researches are needed to verify the detailed function and mechanisms of revealed lncRNAs and to find innovative lncRNAs and sequentially, the predictive and diagnostic roles of lncRNAs in HCC can be validated. Thereby, diagnosis of HCC in an early stage and controlling its development and progression will become possible. Further clinical and ward trials are also in the urge, so that we can develop therapeutic roles of lncRNAs in HCC. In a word, future studies should aim at investigating how can a discovered lncRNA be used to identify HCC patients and used as a guidance being applied in treatment to have optimal responses and to reduce the likelihood of relapse.

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TOPIC HIGHLIGHT

2015 Advances in Hepatitis C Virus

Hepatitis C genotype 4: The past, present, and future

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Abstract

Hepatitis C virus (HCV) genotype (GT) 4 represents 12%-15% (15-18 million) of total global HCV infection. It is prevalent in Northern and Equatorial Africa and the Middle East, and is also present in some countries in Europe. GT-4 (and subtype 4a in particular) dominates the HCV epidemic in Egypt. In underdeveloped countries, risk factors associated with HCV infection may be due to unsafe medical practices or other factors such as familial transmission, mother's HCV status, or illiteracy. HCV prevention and control programs should include health education, increased community awareness towards the disease, controlling infection distribution in health-care centers, proper sterilization of medical and dental instruments, and ensuring safe supply of blood and blood-products. Response rates to a 48-wk combined pegylated-interferon (PEG-IFN) and ribavirin (RBV) treatment range from 40%-69%, and HCV-GT-4 has been considered better than GT-1 but worse than GT-2 and GT-3 in treatment with PEG-IFN/RBV. However, with the introduction of the HCV-GT-1 effective protease inhibitors boceprevir and telaprevir in 2011, HCV-GT-4 became the "most difficult (GT) to treat". Recently, the direct-acting antivirals (DAAs) with pan- genotypic activities simeprevir, sofosbuvir, and daclatasvir have been recommended in triple regimens with PEG-IFN/RBV for the treatment of HCV-GT-4. An IFN-free regimen will be available for treatment of all genotypes of HCV in the near future. To date, several DAAs have been developed and are currently being evaluated in various combinations in clinical trials. As new regimens and new agents are being approved by the Food and Drug Administration, we can expect the guidelines for HCV treatment to be changed. The availability of shorter, simpler, and more tolerable treatment regimens can reduce the morbidity and mortality associated with HCV infection. With such a large number of therapeutic agents available, we can end up with a range of choices that we can select from to treat patients.

Key words: Hepatitis C virus; Genotypes; Transmission; Pegylated-interferon; Ribavirin; Direct acting antivirals;

Hepatitis C virus vaccine

Core tip: Hepatitis C virus (HCV) genotype (GT) 4 represents 12%-15% of total global HCV infection. It is higher in limited resource countries. Response rates to a 48-wk peg-interferon/ribavirin combination ranges from 40%-69% for HCV-GT-4. Direct-acting antivirals may significantly improve treatment outcomes in HCV-GT-4, but use of these agents in countries endemic for HCV-GT-4 is currently precluded by the very high costs. A new hepatitis C vaccine from GlaxoSmithKline has shown promise in early clinical tests, prompting strong and broad immune responses. Another Egyptian clinical trial in the field of HCV vaccination: Clinical Trials phases Ⅰ and Ⅱ, started on March 2011. ClinicalTrials.gov Identifier NCT01718834.

Abdel-Ghaffar TY, Sira MM, El Naghi S. Hepatitis C genotype 4: The past, present, and future. *World J Hepatol* 2015; 7(28): 2792-2810 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i28/2792.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i28.2792

INTRODUCTION

Harvey J Alter is an American Virologist, Medical Researcher, and Physician. In mid-1970s, he was the first person to prove a new type of hepatitis virus, initially called non-A, non-B hepatitis (NANBH). During the following years, medical researchers Michael Houghton, George Kuo, and Qui-Lim Choo from Chiron Corporation (an American multinational biotechnology firm) and Dr. Daniel W Bradley from the Centers for Disease Control and Prevention of America (CDC) collaborated in identifying the virus: Using the molecular cloning process to identify an unknown organism, and confirming that the organism is a virus after finding an NANBH specimen in the organism in 1988. Two articles were published on it, and the name was changed from NANBH to hepatitis C virus (HCV) in April 1989. Later on, HCV was shown to be the principal cause of parenterally transmitted NANBH worldwide[1]. After the discovery of this virus, a flurry of international studies was conducted to document its distribution and prevalence in humans[2]. It is now well established that HCV is a global health challenge, with an estimated 2%-3% of the global population having chronic HCV infection[3]. Estimates over the last 15 years show HCV affection to have increased to 2.8%, which means > 185 million infections worldwide[4].

EPIDEMIOLOGY

HCV is a small single-stranded ribonucleic acid (RNA) of positive polarity, and is an enveloped virus belonging to the *Hepacivirus* genus within the *Flaviviridae* family[5]. It consists of approximately 9600 nucleotides in length, which encode three structural proteins (core, E1, and E2), the ion channel protein p7, and six nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B)[6]. Because each protein is involved in HCV entry, infection, replication, or maturation, they are potential antiviral targets. Hepatitis C virus replication takes place entirely within the cytoplasm, therefore it does not establish latency making it easier to cure[7].

All RNA viruses show a high degree of genome-sequence heterogeneity. HCV RNA is characterized by three tiers of variability: Genotype (GT), subtype, and quasispecies, *i.e.*, the sequence variation within a single patient. The most recent classification includes seven GTs (numbered 1-7), 67 confirmed and 20 provisional subtypes, which differ in sequence by approximately 30% to 35%, and approximately 20% to 25%, respectively. All GTs except 5 and 7 are subdivided into numerous subtypes (1a, 1b, 1c, 2a, 2b, *etc.*)[8]. There is a clear geographic pattern in the distribution of HCV genetic diversity (Figure 1). Highly divergent "endemic" strains that belong to the same GT are typically found in a restricted geographic area, indicating the presence of the GT in that location for hundreds or even thousands of years[9]. GT-1 dominates worldwide (83.4 million cases, 46.2% of all HCV cases), approximately one-third of which are in East Asia. GT-3 comes second (54.3 million, 30.1%). GT-2, GT-4, and GT-6 compromise a total 22.8% of all cases, and GT-5 comprises the remaining < 1%. Subtypes, specifically 1a, 1b, 2a, and 3a, are distributed worldwide, and are especially dominant in high income countries[10]. Within the United States, 75% of isolates are HCV GT-1a or GT-1b and the remainder are generally GT-2 or GT-3[11]. In Europe, HCV-GT-1a and GT-1b are the commonest subtypes. GT-2 dominates in West Africa, and GT-3 dominates in South Asia and parts of Scandinavia. While GT-1 and GT-3 dominate in most countries, GT-4 and GT-5 are mostly found in lower-income countries[10].

HCV GT-4 is prevalent in Northern and Equatorial Africa and the Middle East, while GT-5 and GT-6 have been identified in South Africa and Hong Kong, respectively[12]. GT-4 represents 12%-15% (15-18 million) of total global HCV infection (Table 1)[13], and its distribution is restricted to Egypt, Central Africa, and the Middle East regions[10], although it has recently been reported in the Caribbean region and in India[14-16]. GT-4 (and subtype 4a in particular) dominates the HCV epidemic in Egypt[10,17].

EPIDEMIOLOGY OF HCV INFECTION IN EGYPT

Prevalence

An anomaly in the distribution of HCV infection, however, was discovered in Egypt, where the prevalence was approximately 10-fold higher than in other countries[18].

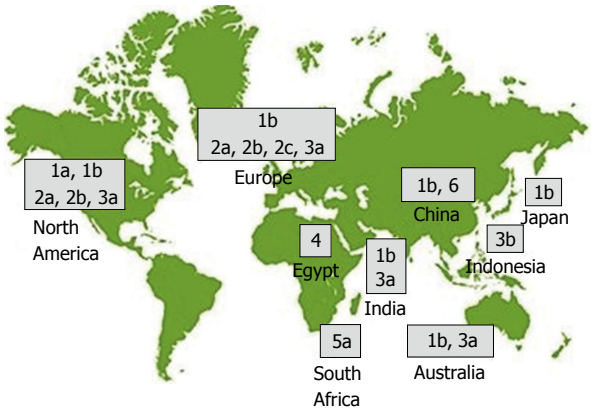

Figure 1 Global hepatitis C virus genotype distribution. Johns Hopkins Hospital Division of Gastroenterology and Hepatology. Available from: URL: https://gi.jhsps.org/Upload/200710291122_15908_000.jpg.

Table 1 Hepatitis C virus genotype-4 % among infected patients in a selection of countries in Europe, the Middle East, Africa, and India[13]

Country or region	GT-4 % of the total HCV infected patients in the country
Southwestern France	7.4%
Germany	3.6%
Southern Italy	1.4%
Northern Italy	3.1%
Southern Spain	14%
Saudi Arabia	60%
Lebanon	30%
Syria	30%
Cameroon	76%
Nigeria	60%
Egypt	91%
Gabon	71%
Southern India	6.2%

HCV: Hepatitis C virus; GT: Genotype.

The prevalence is higher in Egypt than in industrialized countries (ranging from 0.5% to 2.3%)[2], as well as in limited-resource countries, even Pakistan (high prevalence rate of 6.5)[19].

In 2008, an Egyptian Demographic Health Survey (EDHS) was carried out in Egypt on 11126 women and men aged 15-59 years, and interviews were obtained for each individual. It was the first nationwide representative sample for HCV antibody testing done in Egypt. The blood samples were tested by a third generation enzyme-linked immunosorbent assay to detect the HCV antibody (Adaltis EIAgen HCV Ab, Adaltis Italia, Casalecchio di Reno, Italy) at the Central Laboratory in Cairo. HCV antibodies that were Sera positive were tested for HCV RNA[20]. Results showed HCV antibody prevalence was 14.7% (95%CI: 13.9%-15.5%). Most (> 90%) HCV isolates were found to belong to GT-4 with the remaining belonging to GT-1[21].

Prevalence was highest in Lower Egypt (Nile Delta), followed by Upper Egypt, then by Urban governorates (Cairo, Alexandria, Port-Said, and Suez), and then by frontier governorates (17.5%, 14.7%, 9.5% and 3.8% respectively). Increased HCV antibody prevalence was shown with increasing age, in males ($P < 0.001$), and in rural areas ($P < 0.001$). Two thirds of the participants positive for HCV antibody were viremic, and viremia was more prevalent in males, but there was no difference in prevalence according to age or type of exposure. Decreased HCV antibody prevalence was shown with increasing educational level and wealth, but the prevalence was increased with increasing number of people in the same household. Previous history of blood transfusion, parenteral anti-schistosomiasis treatment (PAT), contaminated syringes, and female circumcision were all associated with HCV infection in univariate analysis[20].

The prevalence of HCV antibody positivity in 2008 (after adjusting for the younger than 15 years and the older than 59 years individuals) was estimated at 12%. However, only two thirds of the infected population were viremic in the EDHS, resulting in an all age group viremic prevalence of 8.5% in 2008[22]. Mass campaigns of PAT were blamed for the HCV epidemic in Egypt[23,24]. Between 1964 and 1982, 2 million Egyptians, most of whom were children above five years of age and young adults who lived in areas where schistosomiasis was prevalent, received intravenous weekly injections of antimony salts for 12-16 wk. Insufficient sterilization of the syringes was considered the cause of the HCV transmission at that time[23]. EDHS, performed 30 years after the treatment campaigns, showed PAT to be related to 7.8% and 11.0% of infected people in rural and urban areas, respectively, whereas other means of transmission attributed to the rest of the patients. The introduction of Praziquantel in 1982, an oral drug to treat schistosomiasis[24], did not stop the transmission of HCV due to multifactorial mechanisms including blood transfusion[25-27], contaminated syringes[26,28], dental intervention[26-28], surgical and invasive medical procedures[26-30].

An attempt to determine HCV prevalence in special populations estimated a vertical transmission rate among 1224 pregnant women. Presence of maternal positive HCV antibodies was in 105 of the women (8.6%, 95%CI: 7.05-10.17) with only 83 (6.8%) positive for HCV-RNA. Tests on infants during their first month showed that 43 out of 53 infants (81%) were positive for HCV antibodies and 7 of them (13%) were HCV-RNA positive. Six months later, only two infants (3.8%) remained HCV-RNA positive[31].

In another cohort study[32] to detect mother-to-infant infection, 1863 pregnant women were included and tested for HCV infection. Among those pregnant women, 15.7% and 10.9% tested positive for HCV antibodies and HCV-RNA, respectively. Out of 33 (10%) infants positive for both antibodies and RNA, 29 had RNA-positive mothers and four had only antibody positive mothers who underwent clearing of infection. Fifteen (4.6%) had detectable HCV-RNA at 12 mo and 14 had cleared infection early. At 2-3 years of age, only eight (2.4%) had persistent HCV-RNA while

seven had late clearance of infection. The rate of HCV vertical transmission can be increased in the presence of human immunodeficiency virus (HIV) coinfection or elevated maternal HCV viral load, but it is not affected by breastfeeding or HCV GTs[33].

Prevalence ranged among blood donors between 5%-25%, among blood-transfusion dependent patients between 10%-55%, and among dialyzed patients between 50%-90%[34]. Many studies were conducted in the pediatric population. Among hemophilic children, HCV-antibodies were positive in 40% of patients[35], 47.5% of them were positive for HCV-RNA. In 2011, Barakat *et al*[36] screened 500 school children from 10 schools and found 5.8% HCV seroprevalence with viremia in 75% of them. In 2010, El-Karaksy *et al*[37] reported that the prevalence of HCV in diabetic and non-diabetic children aged below nine years was 2.5% *vs* 1.4% ($P = 0.25$).

Incidence

In the last estimate of HCV incidence in Egypt it was found that the number of new infections annually is < 150000[38]. In 2008, the EDHS estimated the incidence in Egypt at a rate of 6.9/1000 (95%CI: 5.5-7.4 per person per year), indicative of possibly ongoing hyper epidemic transmission[18].

Another study was conducted on 2852 uninfected infants who were followed from birth up to 5.5 years, to detect incidence and risk factors for acquired HCV in rural Egyptian children. Fifteen infants (0.53%) seroconverted to either HCV antibodies and/or HCV-RNA: 10 of them had both antibody and HCV-RNA positive, 4 of them had only antibody positive, whereas the last one had only HCV-RNA positive. The incidence rate at all ages was 2.7/1000 person-years (PY): Higher during infancy than between 1-5 years of age (3.8/1000 PY and 2.0/1000 PY respectively). It was shown that prolonged hospitalization and low birth weight increased the risk of infection, whereas maternal HCV was the source of infection in only two older children. By the end of the follow-up period, six children (40%) had natural clearance[39]. A four-year population-based cohort study was conducted on seronegative villagers to calculate the incidence of new HCV cases. Out of 10578 participants, 25 (11 females and 14 males) caught the infection (incidence rate of 2.4/1000 PY; 95%CI: 1.6-3.5)[40].

Risk factors associated with HCV infection in Egypt

Using systematic literature review methods, Reker *et al*[41] selected 11 articles published between 2008 and 2013 which met the study selection criteria aiming to determine risk factors responsible for the high incidence and prevalence of HCV in Egypt. They categorized the risk factors into two major groups: "Unsafe medical practices and other risk factors". Unsafe medical practices included surgery, intravenous injections, PAT, dental intervention, stitches, and catheterization. The other risk factors included illiteracy, maternal HCV, and familial transmission[41].

PREVENTION

Primary prevention is the best strategy. Health education and increased community awareness towards the disease is needed. Development of new approaches to learn more about HCV transmission is required. Effort should be invested towards making HCV vaccine and direct-acting antivirals (DAAs) accessible for patients during infancy and early childhood[33].

Prevention inside Egypt

The Egyptian Ministry of Health and Population implemented a program in 2001 to decrease HCV transmission through medical practices, and launched a comprehensive national viral hepatitis control program in 2008 for treatment. This led to a decrease in the incidence of infection among dialyzed patients from 28% to 6%[42]. But Egypt still continues to suffer from an ongoing HCV epidemic. Therefore, a comprehensive plan is required to control the infection, which includes increase in community awareness and health education, proper sterilization of medical and dental instruments, a safe blood supply, monitoring the effect of programs, and providing proper treatment[41-48].

CLINICAL PRESENTATIONS

Acute HCV

Primary infection is mostly asymptomatic which makes early detection of the disease difficult, and this leads to underestimation of its true incidence rate. Its diagnosis may be confirmed *via* a documented seroconversion to anti-HCV in a person who was previously negative. Primary HCV infection has no specific markers, and it may be diagnosed with or without an increase in alanine aminotransferase (ALT) levels[49,50].

In primary exposure, serum HCV RNA cannot be detected before a window of 1-3 wk. Symptoms are mild and non-specific, so patients often do not seek medical assistance. Elevated ALT levels indicating the first signs of liver injury can be detected 4-12 wk after infection, and wide fluctuations are common. Severe liver inflammation is uncommon, and fulminant hepatitis is rare. Seroconversion may occur between 4 and 10 wk after exposure[51].

When HCV viremia persists more than 6 mo, it is defined as chronic. The risk of chronic HCV developing from acute infection is high, ranging between 55% and 85%[52,53]. Natural clearance is more likely to occur in symptomatic cases[54]. On the other hand, asymptomatic cases are more likely to progress to chronicity. Spontaneous clearance in adults with chronic HCV is rarely seen[55], but is more observed in children (8% to 45%). Spontaneous viral clearance is unlikely beyond four years of age, and viral infection that does not get cleared in the first years will progress to chronicity[56-59]. We can expect natural clearance mostly to occur within the first three months after exposure[60]. Other factors

may also increase the chance of viral clearance include interferon *L3* gene, formerly known as interleukin 28B (IL28B polymorphisms)[61] and the intensity of the cellular immune response[51]. El-Awady *et al*[62] conducted a study in 2012 on a total of 404 subjects divided into patients infected with HCV-GT-4a (n = 304) and a healthy group (n = 100). They found a significant increase (P < 0.0005) in frequencies of IL28B rs12979860 C/C GTs in the healthy population than in the other group. On the other hand, the C/C GT was significantly higher (P < 0.0005) in spontaneous resolvers cases (n = 84) than in healthy subjects, so they reported that GT C/C was associated with viral clearance during acute infection and suggested a central role of this GT against HCV disease progression[62].

Higher rates of spontaneous resolution have been found in infants with the rs12979860 CC GT for the IL28B polymorphism. Infants, in particular, may have defense mechanisms that explain the inefficiency of HCV perinatal transmission, including the placenta, which has an immunoprotective role, and human leukocyte antigen DR13, where they are less likely to experience chronic HCV from vertical transmission[63]. Co-infection with HIV seems to hinder resolution. The role of type of exposure, viral load, age, sex, and previous recovery following HCV exposure is debatable. Patients, especially those with higher risk of transmission, should be educated that HCV re-infection is possible even after viral eradication[64,65].

Chronic HCV - disease progression

HCV can progress to cirrhosis after decades. It can also lead to liver cell failure or hepatocellular carcinoma (HCC) (approximately 2% to 4% yearly). Several factors influence the progression of the disease, the most important of which is the extent of intrahepatic inflammation elicited by HCV[66]. Persistent normal ALT level indicates slow progression of the disease[67]. Unlike the case with other viral infections, serum HCV RNA is not an indicator for disease progression[68]. GT-3 HCV is associated with accelerated fibrogenesis and an increased risk of developing HCC relative to other GTs[69-71]. Host factors are important in influencing disease progression, and those include gender, race and age. Liver fibrogenesis and HCC incidence are more seen in males[68,72], whereas mild liver fibrosis and normal liver enzymes are more observed in females. Spontaneous eradication of the virus is also more seen in females[73]. A study on black and white Americans comparing disease progression showed no significant difference, making the impact of race unclear[74]. Egyptians infected with HCV GT-4 have high rates of advanced fibrosis. An Egyptian study reported strong correlation between subtypes 4a and 4o with HCC[75].

Age at infection affects prognosis[76,77]. Slower progression of the disease, at least during the first 1-2 decades, is observed in children[78] and females when infected at a young age[79]. A study by Yosry *et al*[80] was conducted on Egyptian children aged 3-17 years with chronic HCV on the relationship between HLA class Ⅱ with clinical chemical and histopathological state in that special population. The most frequent alleles were DRB1*03, DRB1*04, and DRB1*13 (45.6%, 39.1% and 26.1%), respectively. Nearly half of the patients had DRB1*03, and it was associated with a minimal amount of liver affection. Low serum albumin (P = 0.04) was shown in patients with DRB1*04, while high aspartate aminotransferase (AST) level (P = 0.05) was shown in patients with DRB1*13. In comparison to controls, DRB1*15 was significantly reduced among cases.

Environmental factors are important in influencing disease progression, and those include alcohol consumption, tobacco inhalation and coffee consumption. Excessive alcohol intake affects disease progression and HCC risk[77,81]. Tobacco inhalation is associated with liver affection, and consequently with increased fibrosis score[82], whereas coffee has a protective role against fibrosis[83] and HCC[84]. Steatosis[66,85], insulin resistance[86,87], and type 2 diabetes[88,89] are associated with disease progression and the possibility of HCC. Iron overload is related to severe fibrosis[90]. HCV and HBV coinfection increases the incidence of HCC[81]. Large meta-analyses on patients co-infected with HIV have also shown accelerated disease progression[91,92]. Liver transplantation almost always leads to HCV re-infection, as well as accelerated disease progression (compared to non-transplant patients) which may be related to a variety of factors: Chronic HCV infection is observed in liver biopsy after 1 year post-transplantation in 50%-90% of patients, whereas cirrhosis is seen in around 20% within 5 years[93,94].

Extrahepatic manifestations

Several extrahepatic manifestations have been associated with HCV infection that may significantly affect its morbidity and mortality. Between 38% and 76% of chronic HCV patients develop at least one extrahepatic manifestation, the presence of which when clinically significant may sometimes represent a sufficient indication for treatment, even in the presence of mild liver disease[95]. HCV infection can lead to various extrahepatic manifestations, including diseases that affect the small vessels, skin, kidneys, salivary gland, eyes, thyroid, and immune system. The majority of these manifestations are immune mediated[96].

The most common extrahepatic manifestation associated with HCV infection is mixed cryoglobulinemia, or type Ⅱ or Ⅲ cryoglobulinemia, a lymphoproliferative disorder characterized by the production and tissue deposition of immune complexes formed by monoclonal and polyclonal immunoglobulins[97,98]. Anti-HCV antibodies and HCV RNA tend to concentrate in the cryoprecipitate[97]. The vast majority (up to approximately 95% in some studies) of patients with essential mixed cryoglobulinemia have HCV infection[98]. Approximately one half of HCV-infected patients have circulating cryoglobulins. Complexes tend to accumulate in small- to medium-sized blood vessels: Leukocytoclastic vasculitis is

the typical histopathologic finding and can be found in the skin as well as in various organs and tissues, including the brain, gut, and peripheral nerves[99]. Deposition of immune complexes may affect the kidneys, resulting in glomerulonephritis, mostly of the membranoproliferative type, that may lead to renal insufficiency. A long-term consequence of the syndrome is the establishment of B-cell non-Hodgkin's lymphoma[100]. Successful treatment of HCV infection with antivirals leads to a decrease of cryoglobulin levels in serum and to the remission of cryoglobulin - related symptoms and pathologic lesions[101].

Type 2 diabetes is more frequent in HCV infection than in HBV[102], affecting patients who are already at risk for glucose metabolism disturbances. In such patients, overt diabetes may develop earlier than in HCV-negative persons[103]. Glucose metabolism is altered by HCV at early stages of the infection, leading to insulin resistance[86] which, when combined with type 2 diabetes, accelerates liver disease progression[86,88] and reduces the response to combined pegylated interferon (PEG-IFN) with Ribavirin (RBV)[104,105], although the response to regimens containing direct-acting antiviral (DAA) does not seem to be affected by glucose metabolism alterations[106]. Other extrahepatic-associated diseases include porphyria cutanea tarda, lichen planus, necrolytic acral erythema, sialadenitis, sicca syndrome, and autoimmune thyroiditis[96].

SCREENING - DIAGNOSIS

HCV screening is necessary for all individuals at high risk of HCV infection due to a history of illicit injection drug use, history of hemodialysis, history of tattooing, healthcare workers upon accidental exposure, infants born to HCV-positive mothers, history of transfusion with blood or organ transplantation, HIV infection, or chronic liver disease/hepatitis with unknown cause including elevated liver enzymes[107,108].

Serologic assays

Two types of assays for detecting the presence of HCV infection include serologic assays to test for antibody to HCV and molecular assays to test for HCV RNA. Identifying patient GT is important to establish prognostic risk and to guide management. HCV GT helps predict the degree of response to treatment: Patients with GT-1 or GT-4 are less likely to be cured than those with GT-2 or GT-3 with combined therapy[109].

The American Association for the Study of Liver Diseases (AASLD) and the Infectious Diseases Society of America (IDSA) recommend that persons for whom HCV testing is advised should initially be tested for HCV antibodies (Management Guidelines)[108]. HCV antibody testing is sensitive and inexpensive, and it detects antibodies against the core and the NS3, NS4, and NS5 proteins. Current serologic assays are highly specific (> 99%). In children born to HCV-positive mothers, testing for anti-HCV antibodies is unreliable before 18 mo of age because their detection may be related to the passive transfer of maternal antibodies and not active infection[110].

In 2011, the Food and Drug Administration (FDA) granted approval to the OraQuick HCV Rapid Antibody test (OraSure Technologies, Bethlehem, PA), for detection of HCV antibody in finger stick capillary blood and venipuncture whole blood. Its sensitivity and specificity are similar to those of FDA-approved, laboratory-conducted HCV antibody assays[111]. Because the test is rapid, it can be performed for a larger population at risk[110].

Molecular tests (HCV-RNA and genotyping)

The CDC has recommended that positive HCV antibody results can be confirmed with a HCV RNA test that detects HCV viremia[112]. It is recommended to perform quantitative HCV RNA testing on all patients candidate for HCV treatment. HCV RNA testing is also recommended for patients with negative HCV antibody test results if they are immunocompromised. In special situations in which acute HCV infection is suspected, it is important to remember that HCV antibodies may not be present. Although the seronegative window of acute infection has diminished with improved sensitivity of HCV antibody testing, HCV RNA testing is recommended as early as 1-2 wk after the initial exposure in individuals in whom early detection is desired[108,112,113].

Liver histology

The AASLD, IDSA, and European Association for the Study of the Liver (EASL) guidelines recommend that the fibrosis stage be initially determined either by liver biopsy (LB) or validated noninvasive techniques in all patients with HCV infection[108,113]. A LB provides the grade and stage of liver disease and may reveal unsuspected cirrhosis, necessitating surveillance for HCC. In children with HCV infection there is great variability in the degree of inflammation and fibrosis reported. Badizadegan *et al*[114] found periportal fibrosis in 78% and cirrhosis in 8% of 40 children younger than 18 years of age treated at Boston Children's Hospital. A study in Cairo, Egypt, found fibrosis in 72.1% of children with HCV GT-4 (46.5% with mild and 25.6% with moderate to severe fibrosis stages), with a median age for progression of fibrosis at 5.5 years[115].

A major effort has been devoted to identifying alternative noninvasive or minimally invasive procedures for assessing liver disease by detecting the liver fibrosis stage and establishing antiviral therapy. Several algorithms have been created using clinical information or serologic markers commonly gathered at the time of a routine diagnostic workup, such as AST, ALT, and platelet count[116] or more specific substances, such as α-2-macroglobulin or hyaluronate. Combining more than one noninvasive assay appears to increase the diagnostic accuracy and may eliminate the need for LB. Transient elastography is another noninvasive approach that defines liver fibrosis using ultrasound and low-frequency

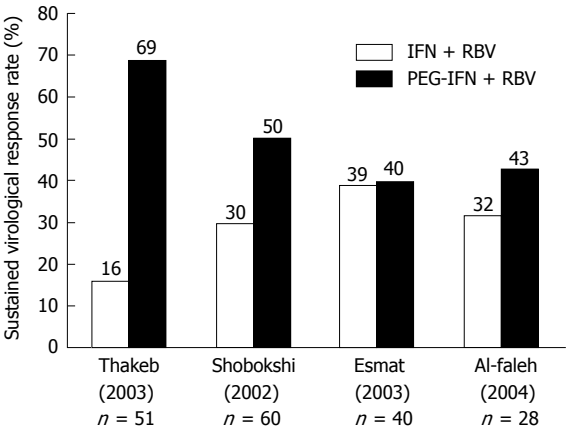

Figure 2 Sustained virologic response to 48 wk of combination therapy in patients with hepatitis C virus genotype-4 using standard-dose pegylated-interferon and ribavirin (pegylated-interferon-α2a 180 μg or pegylated-interferon-α2b 1.5 mg/kg and RBV 1-1.2 g/d). All studies were randomized control trials with intention-to-treat analysis: (P < 0.001) (P < 0.01) (P = not supplied) (P = 0.43)[128-131]. PEG-IFN: Pegylated-interferon; RBV: Ribavirin.

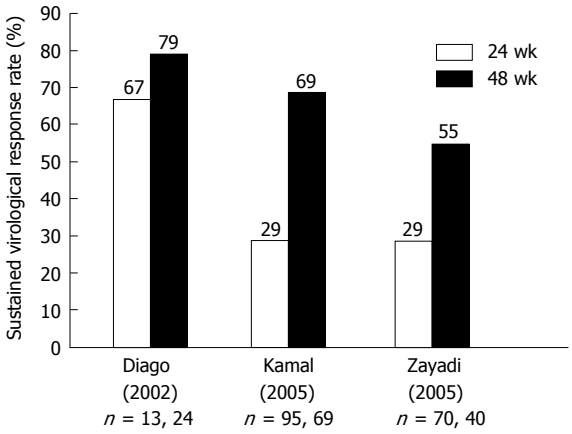

Figure 3 Results of hepatitis C virus genotype-4 treatments with pegylated-interferon and ribavirin: 24 wk *vs* 48 wk. (P = not reported) (P = 0.001) (P = 0.006) showing far less sustained virologic response rates with 24 wk of therapy[132-134].

elastic waves[117]. A study was conducted on chronic HCV Egyptian patients to evaluate different new noninvasive methods for assessment of liver fibrosis. The aim of that study was to evaluate whether GT-4, increased body mass index, and co-infection with schistosomiasis can interfere with liver fibrosis assessment. Egyptian HCV chronic patients (n = 312) with GT-4 underwent a LB, an elastometry measurement (Fibroscan©), and serum markers: AST-to-platelet ratio index, fibrosis-4 score (Fib4), and Fibrotest©. The researchers found that the algorithm using the Fib4 for identifying patients with F2 stage or more reduced by nearly 90% the number of LBs, and reported that noninvasive techniques were feasible in Egypt for HCV GT-4-infected patients and that Fib4 may be used to assess the F2 threshold, which decides whether treatment should be proposed or delayed[118].

BACKGROUND IN HCV TREATMENT

At the start of the millennium, two major advances in the management of HCV took place: one, the approval by the FDA of PEG-IFN for the treatment of HCV infection, which allowed weekly subcutaneous injections instead of the previous daily or thrice weekly injections with standard IFN; and the use of weight-based RBV. By the mid-2000s, it was established that PEG-IFN-α2a or PEG-IFN-α2b could be combined with weight-based RBV for GT-1 infected patients, or with flat-dosed RBV for GT-2 or GT-3 infected patients, and that combination was better than treatment with standard IFN and RBV[119]. HCV-GT-4 response to treatment with PEG-IFN/RBV has been considered better than GT-1, and worse than GT-2 and GT-3[120,121].

With the introduction of the HCV-GT-1 effective protease inhibitors boceprevir (BOC)[122,123] and telaprevir[122,124-126] in 2011, HCV-GT-4 became the "most difficult (GT) to treat", as both protease inhibitors are not indicated for treatment of GT-4[127]. Many studies used 48 wk duration of combined therapy to treat HCV-GT-4, and a few of them compared responses between 24-wk treatment and 48-wk treatment response rates. Figures 2 and 3 summarize the results of those studies after using standard-dose PEG-IFN and RBV. The results show sustained virologic response (SVR) rates with 24 wk of therapy to be far less than rates with 48-wk, making the longer duration the standard of care[13].

Drawbacks for the IFN/RBV treatment include its long course duration, severe side effects, and cost, as it is not as affordable for patients in limited-resource countries[135-137].

A major indicator of good response to PEG-IFN/RBV therapy in patients with HCV GT-4 is the IL28B GT[138-140]. The favorable CC phenotype is found in 20%-30% of Egyptian patients with chronic HCV[141,142]. In Europe, SVR rates for HCV GT-4 infected patients who are IL28B CC is > 80%[139,140,143]. In Egypt, CC patients had response rates between 67% and 87%[141,142]. Mutations in the NS5A region, particularly in patients with more than 6 aa mutations in the Interferon RBV resistance - determining region (IRRDR) are highly associated with good response to PEG-IFN and RBV combination therapy, while a less diverse (⩽ 5) IRRDR sequence is associated with non-response[144-146]. Another predictor of response to PEG-IFN/RBV therapy was found to be insulin resistance, which impairs response rates to PEG-IFN/RBV therapy in HCV GT-4 patients, and patients with Homeostasis Model Assessment for Insulin Resistance scores > 2 had lower SVR rates than those with scores < 2 (36% *vs* 72%)[147].

Abu-Mouch *et al*[148] found that adding vitamin-D to the standard of care with PEG-IFN/RBV therapy for HCV-GT-1 infected patients increases SVR rates from 42% to 82%. Esmat *et al*[149] reported that vitamin D supplementation, despite its role in other GTs, has no positive impact on treatment outcome in HCV-GT-4 patients where SVR was achieved in 51.2% in group 2, [who received the standard of care therapy (SOC

therapy) plus vitamin D3 (Cholecalciferol) in a dose of 15000 IU/wk during the treatment course] and 71.4% in group 1 (who received the SOC therapy consisting of PEG-IFN-α2b plus RBV) by per-protocol analysis and in 44% in group 2 and in 68.6% in group 1 by intention to treat analysis (*P* value 0.22 and 0.220, respectively)[149].

The Ministry of Health in Egypt has embarked on a national treatment program since 2006, where all eligible patients are treated with PEG-IFN/RBV for 48 wk. Annually, 40000-50000 patients have been treated, and 350000 patients had received therapy in this program by 2013[150]. SVR rates for patients treated with the original PEG-IFN-α2a and alfa-2b were 54%-59%[151,152] and response rates to a locally produced biosimilar PEG-IFN were reported at 52%[153].

TREATMENT CONSIDERATIONS IN CHILDREN

Infected children are generally asymptomatic and often have normal ALT levels. However, children are likely to clear the virus. Given the typically slow histologic progression of liver disease in children, some argue that treatment should be postponed until adulthood. In addition, response rates with currently available antiviral therapies approved by the FDA for the pediatric population remain suboptimal, and are associated with high costs and potential toxicities[154].

On the other hand, early eradication of HCV is likely to reduce the social stigma associated with viral infection, a source of significant caregiver stress[155], and improve the psychosocial status of patients and their families. Children also possess multiple characteristics that make them ideal candidates for treatment. Shorter duration of infection and a lesser degree of hepatic fibrosis are associated with improved response to antiviral therapy for HCV. In addition, children have fewer co-morbidities than adults and parental motivation enhances adherence to treatment. Children also tolerate currently available therapies better than adults, with mild adverse effects[156]. Economically, the cost of treatment is less than that in adults (fewer drugs used). It is also expected that eradicating the infection sooner will decrease the risk of transmission to the population at large[157-164].

The decision to initiate treatment should be individualized to each patient. However, for children with GT-1 and GT-4 who have mild disease at the time of biopsy, a watch and wait approach is acceptable, anticipating the availability of more efficacious drugs. According to the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition and the AASLD guidelines for management of HCV infection, considerations for treating children aged 3-17 years follow the same guidelines as adults[109].

The results of the first randomized, placebo-controlled trial of chronic pediatric HCV treatment in the United States have been published. They show that 21% of children treated with PEG-IFN alone in contrast with 53% of those treated with PEG-IFN in combination with RBV achieved a SVR, demonstrating the superiority of the combination over single therapy[165]. An international multicenter study of 107 children published by Wirth *et al*[166] demonstrated a SVR in 53% of patients with GT-1, 93% of those with GT-2 and GT-3, and 80% of those with GT-4.

In 2008, the results of these two trials led to FDA approval of combination therapy with PEG-IFN-α2b and RBV for use in children three years of age or older infected with HCV. The established duration of treatment at present is 48 wk for GT-1 and GT-4[154]. A study was conducted on 118 Egyptian children with chronic HCV, including both naïve patients and previous non-responders. All received PEG-IFN-α2b plus RBV. Early virological response was achieved in 69.5%, end of treatment response (ETR) was 51.7%, and SVR was achieved in 50% of the patients. Children with previously failed treatment achieved a SVR rate of 28.6%[167]. A pilot study was conducted on a small sample of Egyptian chronic HCV children (seven cases). ETR was 42.9% and SVR was 28.6%. Children with SVR were the youngest; their mean duration of infection was 4.5 years *vs* 12.7 years in the others. Side effects of both IFN and RBV were mild, and required no reduction in doses[168].

In another study on chronic HCV children, the novel *Hansenula*-derived PEG-IFN: IFN-α2a (Reiferon Retard) was used in the infected patients, whether naïve or previously treated. At week 12, patients were divided according to polymerase chain reaction (PCR) results into two groups: The first group included patients who continued treatment on a weekly basis (7-d schedule) and the second group included patients who continued treatment on a 5-d schedule. Patients from either group who were PCR-negative at week 48, but had at least one PCR positive test during therapy, were assigned to have an extended treatment course of up to 72 wk. The study was registered at www.ClinicalTrials.gov (NCT02027493). The 5-d schedule did not affect the response rate. Treatment duration (whether 48 wk or extended 72 wk) gave similar response rates ($P = 0.49$). Type of previous treatment (short acting IFN *vs* PEG-IFN) did not affect the response to retreatment. On the other hand, SVR was significantly higher in previous relapsers than in previous non-responders ($P = 0.039$). It was safe, but the customized regimen did not improve response rate as SVR was detected in 23.9% of children, breakthrough was seen in 39.1% of patients, and 30.4% were non-responders. Despite recent success after the introduction of combination therapy with IFN-alpha and RBV, GT-4 is considered difficult to treat. Approximately 60% of patients fail to respond. Resistance to antiviral therapy remains a serious problem in the management of chronic HCV[169].

EVOLUTION OF TREATMENT FOR HCV

Development of future treatments: DAAs

Currently, research is orientated to the development

and approval of DAA agents regulated within the Center for Drug Evaluation and Research at the FDA for the treatment of chronic HCV. DAA agents inhibit specific stages in the HCV replication cycle through targeting the HCV polyprotein and its cleavage products[170].

The HCV life cycle: The HCV genome is composed of approximately 9600 nucleotides and generates structural and non structural (NS) proteins. The structural proteins are used to assemble new viral particles and the NS proteins support viral RNA replication. The NS3/4A, a serine protease (NS3) and cofactor (NS4A) catalyze the post-translational processing of NS proteins from the polyprotein. The products released go on to form a replicative complex (NS5A) responsible for producing viral RNA using the RNA dependent/RNA polymerase (NS5B). Finally, virions are assembled, packaged, and released[118].

HCV NS3/4A serine protease inhibitors

Targeting this protease may inhibit viral replication. Telaprevir (TPV), BOC, and simeprevir (SMV) are all examples of HCV protease inhibitors[120,122,171].

HCV NS5B inhibitors

The HCV polymerase inhibitors are another promising DAA class. These molecules are divided into nucleoside/nucleotide competitive polymerase [nucleoside inhibitors (NIs)] and allosteric inhibitors of RNA polymerase [non-NIs (NNIs)]. NS5B NIs as sofosbuvir (SOF) are structural analogues to the natural substrates of the polymerase and are incorporated into the RNA chain. This causes direct chain termination[172]. Since the active site of NS5B is highly conserved, NIs are effective against all GTs, and resistance to NIs is usually very low. Allosteric polymerase inhibitors inhibit the NS5B by binding to one of several discrete sites on the HCV polymerase, resulting in a conformational protein change. They are less potent than NIs, resistance occurs more frequently[173] and they appear to be GT specific. SOF is a nucleotide analogue inhibitor of the HCV NS5B RNA-dependent RNA polymerase[174].

HCV NS5A inhibitors

Inhibition of NS5A is associated with significant reductions in HCV RNA levels, which makes these agents among the most potent antiviral molecules yet developed. NS5A inhibitors have pan-genotypic activity, *i.e.*, they suppress replication of all HCV GTs. Daclatasvir (DCV) and ledipasvir (LDV) are examples of NS5A inhibitors[171].

With the advent and improvement of DAAs, drugs now can target almost all steps of the HCV life cycle, including entry, translation, RNA replication, assembly, and export of progeny viruses[175]. This has facilitated the designing of highly potent oral drugs characterized by shorter treatment duration, simplified dosing, a high genetic barrier to resistance, and improved safety profiles[176].

COMBINATION THERAPY WITH PEG-IFN, RBV AND A DAA

Combination therapy with PEG-IFN, RBV and a HCV NS3/4A serine protease inhibitor

Selective inhibitors of HCV NS3/4 serine protease, BOC and TPV were developed and found to be effective in treating HCV-infected GT-1 patients in combination with PEG-IFN and RBV. Both agents are not indicated for use in GT-2 and GT-3 patients. Combination therapy using TPV or BOC with PEG-IFN and RBV resulted in higher rates of SVR for the treatment of naïve HCV GT-1 patients (range 61%-75%) compared with treatment with PEG-IFN and RBV (range 38%-49%). However, the addition of these drugs resulted in increased adverse effects such as anemia, neutropenia, fatigue, pyrexia, and insomnia[120,122]. Telaprevir may cause skin rash, in up to 5% of cases it can be severe, and it may cause Stevens-Johnson Syndrome (SJS)[177]. On December 2012; FDA Drug Safety Communication reported severe skin affection after combination treatment of PEG-IFN and RBV with Incivek (Telaprevir). These types of skin reactions (toxic epidermal necrolysis, drug rash with eosinophilia and systemic symptoms, and SJS) lie along a continuum of serious skin reactions that can be difficult to distinguish from each other. FDA added instructions on the drug label to stop Incivek combination treatment whenever symptoms of severe skin rash appear[178].

The rates of stoppage of treatment containing TPV or BOC were higher than treatment of PEG-IFN and RBV alone. This affected compliance and the ability to complete treatment duration[179].

Combination therapy with PEG-IFN, RBV and a second-generation protease inhibitor, SMV

SMV, a HCV NS3/4 serine protease inhibitor, was recently developed, approved by the FDA in November 2013, and is now used to treat HCV GT-1 patients in combination with PEG-IFN and RBV[171,180]. The response rate is much higher than what was obtained with the use of first-generation protease inhibitors, BOC and TPV. Recently, DAAs with pan-genotypic activities SMV, SOF and DCV have been recommended in triple regimens with PEG-IFN/RBV for the treatment of HCV genotypes 4[181]. SMV is active against genotypes 1, 2, 4, 5 and 6. It is administered as a once-daily tablet orally and has demonstrated a favorable safety profile and limited drug-drug interactions[182]. RESTORE, a phase Ⅲ, multicenter, single-arm, open-label study, conducted in France and Belgium, evaluated SMV (150 mg once-daily for 12 wk in combination with PegIFN/RBV, followed by 12-36 wk of Peg-IFN/RBV only) in 107 patients with HCV 4, either naïve or treatment-experienced[183]. Recent European guidelines have included a 24-48 wk SMV plus PEG-IFN/RBV combination as an option for HCV 4-related compensated liver disease (including cirrhotics), suggesting interruption of treatment if HCV-RNA levels are ⩾ 25 IU/mL at week 4, 12 or 24[123].

Combination therapy with PEG-IFN, RBV and HCV NS5B polymerase inhibitor, SOF

Recently (in December 2013), the FDA approved SOF as a new component of combined HCV antiviral treatment. Phase Ⅲ clinical trial using combination therapy with PEG-IFN, weight-based RBV, and SOF for 12 wk resulted in high SVR rates in GT-1, GT-4, GT-5, and GT-6 patients (89%-90%)[174,184,185]. The addition of SOF to PEG-IFN and RBV reduces the total duration of treatment.

The recent approval of SOF for treatment of HCV-GT-4 promises significant improvement in the outcome of therapy. When given as part of triple therapy with PEG-IFN/RBV for 12 wk in the phase Ⅲ Neutrino trial, SOF resulted in a SVR rate of 96% in HCV-GT-4 patients[185]. When it was given for 12 wk without IFN as a dual therapy with RBV to HCV-GT-4 infected patients of Egyptian origin living in the United States, SVR rates of 79% and 59% were found in naïve and treatment experienced patients, respectively. Extending the treatment for 24 wk yielded higher SVR rates: 100% and 93%[186].

Combination therapy with PEG-IFN, RBV and HCV NS5A inhibitors, DCV

Another compound with promising efficacy against HCV-GT-4 is DCV (a NS5A inhibitor) in combination with PEG-IFN-α2a and RBV. Hézode *et al*[187] reported that this was generally well tolerated and achieved higher SVR rates at week 24 compared with placebo/PEG-IFN alfa/RBV among patients infected with HCV-GT-1 or GT-4.

IFN-FREE DAA THERAPY (IFN-SPARING REGIMEN)

Over the past year, IFN-sparing regimens for treating chronic HCV have become available and more superior, as those are characterized by high rates of SVR, short duration of treatment, increased tolerability, as well as room to tailor treatment according to the patients' individual needs due to the presence of multiple agents that can interrupt several stages of the HCV lifecycle. IFN-sparing treatment is currently the new approach for both treatment-naïve and treatment-experienced patients, including even cirrhotic patients. Most treatment-experienced patients can now achieve high SVR rates (above 90%). Cirrhotic patients can reach high SVR with longer duration of treatment and/or addition of RBV therapy[188].

Single DAA therapy (SOF and RBV)

The first attempt to use an IFN-free regimen for HCV involved a combination of SOF and RBV. SOF is active against all GTs[185] and has an excellent safety profile and high barrier to resistance. All oral combination therapies with SOF and RBV for 12-24 wk in HCV GT-1 were evaluated[189,190]. SVR rates using weight-based dosing of RBV with SOF were higher in GT-1 treatment-naïve patients (SVR 68%-84%)[184,190]. In patients with HCV GT-2 or GT-3, a study showed higher rates of SVR after SOF plus RBV for 12 wk in comparison with PEG plus RBV for 24 wk. For HCV GT-2-infected patients, SVR rates were 97% *vs* 78% for each treatment group, respectively. However, for HCV GT-3-infected patients, the improvement in SVR rates in the SOF group was not observed (SVR 56% *vs* 63%, respectively)[185]. Another study confirmed the high SVR rate for SOF plus RBV in treatment-naïve and treatment-experienced GT-2 patients (SVR 93%) and showed an improved SVR rate when this combination is used for 24 wk in patients with HCV GT-3 (SVR 80%)[191].

A randomized, open-label study was conducted at three centers in Egypt (ClinicalTrials, NCT01838590) using SOF plus RBV for 12 wk (52 patients) or SOF plus RBV for 24 wk (51 patients). Treatment-naïve (TN) and treatment-experienced (TE) patients with chronic HCV-GT-4 (up to 20% with compensated cirrhosis) were included, 74 were GT-4a while 11 were GT- 4l, 4n, 4o, 4p, and 4u. SOF plus RBV for 24 wk resulted in a 90% SVR12 rate in patients regardless of prior treatment experience. SVR12 rates with SOF plus RBV for 12 wk were higher in TN *vs* TE patients. No SOF-resistance mutation S282T was found in any patient with virologic failure. Combined SOF plus RBV for 12 or 24 wk were well tolerated. The authors concluded that SOF plus RBV for 24 wk provides a simple, effective, IFN-free regimen for patients with HCV-GT-4[192].

Phase 2 trials with SOF and RBV in adolescents and children (3-17 years) with GT-2 and GT-3 began in 2014 with an estimated study completion date in May 2018 (ClinicalTrials, Identifier: NCT02175758). HCV infected children may soon realize the benefits from the tremendous research in anti-HCV therapy in the last five years[185,190,193].

Dual DDA combination therapy

Dual therapy tends to be more effective than monotherapy in regards to viral eradication and decreasing the risk of viral resistance[194]. These targets include NS3/4A protease inhibitors (PI), NS5A inhibitors, and NS5B polymerase inhibitors [both NI/tide inhibitors and NNI]. More recently, combinations of these DAAs have been effectively used without the use of IFN and RBV to achieve high rates of SVR. In an open-label study, oral SOF and DCV taken once daily were associated with high SVR in patients infected with HCV GT-1, GT-2, and GT-3, including patients with no response to prior therapy with TPV or BOC[195].

Phase 3 is a multicenter, open-label, single-arm study conducted at multiple sites in Spain, to investigate the efficacy and safety of a 12-wk regimen of SMV in combination with SOF in TN or TE subjects (age: 18-70 years) with chronic GT-4 HCV infection (ClinicalTrials, Identifier: NCT02250807, started January 2015 with estimated study completion date January 2016). The FDA approved SMV to be used with SOF as a combination

therapy in GT-1 in November 2014. Another Egyptian study (NCT 02278419) to investigate the efficacy and safety of an 8- or 12-wk treatment regimen of SMV in combination with SOF in TN and TE adult participants with chronic HCV-GT-4 is ongoing.

For children and adolescents with chronic HCV infection, a Phase 2 study will be conducted to investigate the safety and efficacy of LDV/SOF fixed dose combination in this particular age group (ClinicalTrials, Identifier: NCT02249182). The FDA approved the first combination pill-LDV/SOF (Harvoni) Gilead, for treatment of HCV GT-1 in October 2014.

Triple DDA combination therapy

The FDA approved a three drug regimen called the AbbVie Viekira Pak (Ombitasvir, Paritaprevir, and Ritonavir tablets co-packed with Dasabuvir tablets) to treat patients with chronic HCV-GT-1 infection including cirrhotic cases in December 2014. In a randomized, open-label trial of Faldaprevir (a NS3/4A protease inhibitor) and Deleobuvir (a nonnucleoside NS5B polymerase inhibitor) with or without RBV (phase ⅡB), used by 362 TN patients infected with HCV GT-1, the SVR at 12 wk was 52%-59% among patients who received IFN-free treatment with Faldaprevir plus Deleobuvir plus RBV[196].

The AASLD announced detailed results from The PEARL-Ⅰ study [ABT-450/R (Protease inhibitor and Ritonavir) + ABT-267 (NS5A inhibitor) ± RBV] (open-label Phase 2b), which demonstrated that 100% of GT-4 patients who were new to therapy (n = 42/42) or who had failed previous treatment with PEG-IFN and RBV (n = 49/49) achieved SVR rate at 12 wk post-treatment after taking the AbbVie investigational treatment with RBV. Additionally, 90.9% of patients who were new to therapy achieved SVR 12 (n = 40/44) after taking the treatment without RBV[197].

Another open-label Egyptian study (phase 3) began in November 2014 to evaluate the safety and efficacy of the co-administration of ABT-450/Ritonavir/ABT-267 (ABT-450/r/ABT-267) with RBV in adults with chronic HCV-GT-4 in Egypt. It includes 160 patients and has an estimated study completion date of August 2016 (ClinicalTrials, Identifier: NCT02247401). Currently, Egypt is conducting a national mass treatment program for HCV GT-4 patients based on the EASL's 2014 practice guidelines for HCV-GT-4, which (based on two studies: Neutrino study for IFN-based therapy and Ruane study for IFN-free regimens) involves triple therapy using PEG-IFN/weight-based RBV/SOF 400 mg for 12 wk for IFN-eligible patients or RBV/SOF 400 mg for 24 wk for patients who are unable to tolerate IFN. Advanced liver fibrosis patients are included in the IFN-free regimen.

Due to advancements in HCV therapy, the AASLD and IDSA designed a new protocol for the use of new agents[108]. The EASL also announced new protocols on HCV therapy in 2014, including the use of all-oral combinations of SOF/SMV and SOF/DCV[113]. The AASLD, IDSA, and EASL will release updated guidelines as new therapeutics and regimens become approved by the FDA and European Medicines Agency.

The field of IFN-free HCV therapy is under constant development. Currently, DAA's provide patients with high SVR rates (above 90%) with short duration treatment and increased tolerable adverse effect profiles. Various oral therapy combinations can be used to improve SVR outcomes in the TE patient, according to HCV GT/subtype, type of prior regimen, and presence of cirrhosis[188].

The next steps in the clinical development of anti-HCV therapy are expected in late 2015 and early 2016 with the availability of pangenotypic ultrarapid (4-8 wk) single pill regimens such as Grazoprevir/MK8742, SOF/GS5816, and BMS791325/DAC/Asunaprevir[198].

The most common and tolerable adverse effects of DAA combination therapy are nasopharyngitis, headache and malaise[199]. However The FDA warned on March 2015 that serious slowing of the heart rate can occur when the antiarrhythmic drug amiodarone is taken together with either Harvoni (Ledipasvir/SOF) or with SOF taken in combination with another direct acting antiviral for the treatment of hepatitis C infection. They recommended that health care professionals should not prescribe either Harvoni or SOF combined with another direct acting antiviral, such as DCV or Olysio (SMV), with amiodarone[200].

HCV VACCINE SHOWS PROMISE

A new HCV vaccine from GlaxoSmithKline has shown promise in early clinical tests, prompting strong and broad immune responses. Researchers have evaluated the vaccine in humans, and it is now ready for phase 2 efficacy studies[201].

Another Egyptian clinical trial in the field of HCV vaccination is promising: Safety and efficacy of a novel candidate peptide vaccine against HCV infection in healthy volunteers and in treated (Non-responders/responders) chronic HCV patients. Clinical Trials (phases Ⅰ and Ⅱ) started in March 2011 (Clinical Trial, Identifier: NCT01718834; National Liver Institute, Menofyia University, Egypt).

CONCLUSION

HCV therapy is steadily moving to an all oral, well-tolerated, DAA, short-term, and more efficacious regimen. An IFN-free regimen will be available for treatment of all GTs of HCV in the near future. To date, several DAAs have been developed and are currently being evaluated in various combinations in clinical trials (for current management strategies of chronic HCV see http://www.aasld.org). We can expect changes in treatment recommendations of HCV as new regimens are developed and new agents are approved by the FDA. The availability of shorter, simpler, well-tolerated treatment regimens can have a major impact in reducing the morbidity and mortality associated with HCV infec-

tion. With such a large number of therapeutic agents we can end up with a world of choices that we can select from to treat patients. We hope not just to treat some patients with HCV infection but also treat all patients to achieve a cure regardless of their fibrosis state.

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TOPIC HIGHLIGHT

2015 Advances in Nonalcoholic Fatty Liver Disease

Bile acid receptors and nonalcoholic fatty liver disease

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Abstract

With the high prevalence of obesity, diabetes, and other features of the metabolic syndrome in United States, nonalcoholic fatty liver disease (NAFLD) has inevitably become a very prevalent chronic liver disease and is now emerging as one of the leading indications for liver transplantation. Insulin resistance and derangement of lipid metabolism, accompanied by activation of the pro-inflammatory response and fibrogenesis, are essential pathways in the development of the more clinically significant form of NAFLD, known as non-alcoholic steatohepatitis (NASH). Recent advances in the functional characterization of bile acid receptors, such as farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR) 5, have provided further insight in the pathophysiology of NASH and have led to the development of potential therapeutic targets for NAFLD and NASH. Beyond maintaining bile acid metabolism, FXR and TGR5 also regulate lipid metabolism, maintain glucose homeostasis, increase energy expenditure, and ameliorate hepatic inflammation. These intriguing features have been exploited to develop bile acid analogues to target pathways in NAFLD and NASH pathogenesis. This review provides a brief overview of the pathogenesis of NAFLD and NASH, and then delves into the biological functions of bile acid receptors, particularly with respect to NASH pathogenesis, with a description of the associated experimental data, and, finally, we discuss the prospects of bile acid analogues in the treatment of NAFLD and NASH.

Key words: Bile acids; Bile acid receptors; Nonalcoholic steatohepatitis; Farnesoid X receptor; Transmembrane G protein-coupled receptor 5; Nonalcoholic fatty liver disease; Hepatic steatosis

Core tip: Bile acids and bile acid receptors play important roles in modulation of feature of the metabolic syndrome, hepatic steatosis, and hepatic inflammation. Development of bile acid analogues specifically targeting farnesoid X receptor and transmembrane G protein-

coupled receptor 5 provide potential novel classes of drugs for the treatment of nonalcoholic steatohepatitis.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is now a very prevalent liver disease in the United States. It affects up to 20% of the United States population[1], with an estimated prevalence of 43%-60% in patients with diabetes[2], and 90% in patients with hyperlipidemia[3]. NAFLD, by definition, is macrovesicular fat accumulation in more than 5% of hepatocytes in patients who drink less than 20 g/d. NAFLD represent a spectrum of diseases ranging from simple hepatic steatosis to steatohepatitis. Simple steatosis rarely progresses to advanced fibrosis and thus does not carry an increased liver-related mortality. Nonalcoholic steatohepatitis (NASH) instead describes hepatic inflammation and hepatocyte damage within liver including lobular inflammation and hepatic ballooning in addition to macrovesicular fat. Fifteen percent to 30% of patients with NASH progresses to fibrosis, cirrhosis and cancer[4,5], leading to the need for a liver transplant. Based on the data from the United Network for Organ Sharing and Organ Procurement and Transplantation Network Registry, the percentage of patients who underwent a liver transplant for NASH has increased to 9.7% in 2009 compared to 1.2% in 2001[6]. The number of adults with NASH awaiting liver transplant has almost tripled in 2013, compared to the year 2004[7]. NASH is projected to become the leading etiology for liver transplant in the United States.

The "two hit hypothesis" and the "multiple hits hypothesis" have been proposed to explain the underlying pathogenesis of NAFLD[8,9] (Figure 1). Simple hepatic steatosis reflects the accumulation of triglyceride in the liver, as result of influx of lipids and *de novo* lipogenesis exceeding the export of lipids in the forms of lipoproteins. Insulin resistance has been considered a primary driving force for lipid influx by promoting the lipolysis of peripheral adipose tissue, and increasing the liver uptake of free fatty acids for *de novo* lipogenesis[10]. Hyperinsulinemia and hyperglycemia also inhibit fatty acid oxidation and accelerate lipogenesis[11]. Triglyceride is exported out of liver to peripheral tissues by incorporation into very-low-density lipoprotein (VLDL) carriers, and impairment of VLDL synthesis/export has been implicated in NAFLD pathogenesis[12]. Accumulation of lipids, including triglycerides and free fatty acids, as a first hit, primes the liver - making it susceptible to additional hepatotoxic insults (second or multiple hits), which then lead to hepatocyte injury, inflammation, and fibrosis. The second hit or multiple hits involve pro-inflammatory processes mediated by the gut-liver-axis with microbiota imbalance, mitochondrial dysfunction, oxidative stress pathway activation, and activation of intracellular signal such as nuclear factor κB and c-Jun N-terminal kinase (JNK) pathways[13-17].

Unraveling the pathogenesis of NAFLD has established several important drug targets in recent years. Among them are bile acid receptors including farnesoid X receptor (FXR) and transmembrane G protein-coupled receptor (TGR) 5, which play pivotal roles in regulation of metabolism, inflammation and cell proliferation. These receptors have emerged as attractive targets for drug development for the treatment of NAFLD, and are the focus of this review.

BILE ACID METABOLISM

Bile acid synthesis

Bile acids are generated from cholesterol oxidation in the liver through two major pathways: "classic pathway" also called the neutral pathway, and the "alternative pathway" also called the acidic pathway. Cholesterol 7α-hydroxylase (CYP7A1) is a rate-limiting enzyme in the classic pathway. Both of the primary bile acids, cholic acid (CA) and chenodeoxycholic acids (CDCA), are end products of the classic pathway. The alternative pathway of bile acid synthesis is initiated by sterol 27-hydroxylase (CYP27A1), an enzyme located on the inner membrane of mitochondria and widely expressed in various tissues. The alternative pathway produces oxysterols, notably 25-hydroxycholesterol and 27-hydroxycholesterol, which are important ligands in regulating inflammation, lipid metabolism, and cell proliferation.

Bile acid recycling

Once synthesized in the liver, bile acids are conjugated to glycine or taurine, excreted out of liver, and stored in the gallbladder. In response to a meal, the contraction of the gallbladder delivers bile salts to the small intestine, facilitating the digestion of dietary fat. In the gastrointestinal tract, CA and CDCA are further metabolized by intestinal microbiota to secondary bile acids: Lithocholic acid (LCA) and deoxycholic acid (DCA) by de-conjugation and dehydroxylation. DCA is unable to convert back to CA in the liver, and thus the proportion of DCA in the bile acid pool varies from 1% to 50%, depending on the level and activity of bile acid 7α-dehydroxylating gut bacteria and intestinal transient time. LCA is reabsorbed and reduced to CA in the liver. Overall, approximately 95% of bile acids are reabsorbed in the ileum and transported back to the liver *via* the enterohepatic circulation. Approximately 5% of bile acids are lost in the feces daily. But bile acids do not just aid in digestion and participate in the enterohepatic circulation, they also function as signaling molecules both within and outside of the liver.

FXR AND NAFLD

FXR belongs to the family of nuclear hormone receptors

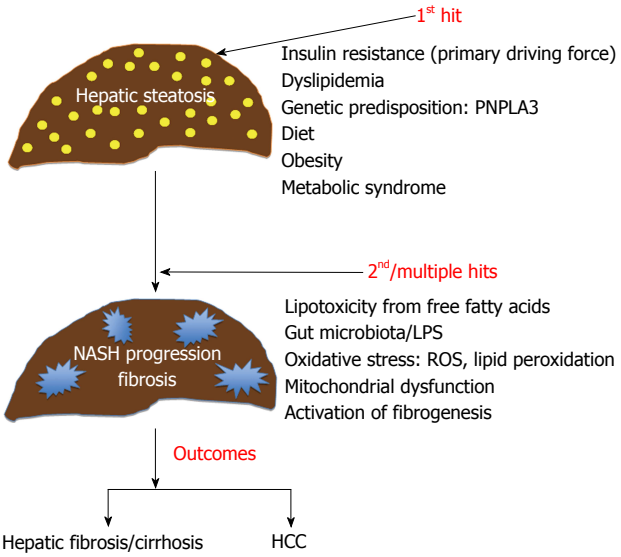

Figure 1 Pathogenesis of nonalcoholic steatohepatitis. Insulin resistance is considered a primary driving force for hepatic steatosis by promoting lipolysis of peripheral adipose tissue, and increasing hepatic uptake of free fatty acids for *de novo* lipogenesis. The second hit, or multiple hits, involve genetic predisposition such as PNPLA3, pro-inflammatory processes mediated by the gut-liver-axis with microbiota imbalance, mitochondrial dysfunction, activation of oxidative stress pathways, and induction of lipotoxicity from free fatty acids. HCC: Hepatocellular carcinoma; NASH: Nonalcoholic steatohepatitis; PNPLA3: Patatin-like phospholipase domain containing 3; LPS: Lipopolysaccharides; ROS: Reactive oxygen species.

that regulate expression of genes involved in a wide array of biologic processes including, development, reproduction, and metabolism, and was first described in 1995[18-20]. Bile acids were subsequently identified as unique endogenous ligands for FXR at physiologic levels[21,22] in 1999. FXR is richly expressed at the ileum, and in liver parenchymal cells. It is also expressed in liver non-parenchymal cells such as endothelial cells, Kupffer cells and stellate cells at very low level. Various bile acids activate FXR in the following order of activity: CDCA > DCA > CA > LCA. The targets and effects of FXR are outlined in detail below and summarized in Figure 2.

FXR biologic functions

Bile acid synthesis: FXR plays an essential role in the feedback regulation of bile acid biosynthesis by repression of CYP7A1, and CYP8B1, two key enzymes in bile acids synthesis. Repression of CYP7A1 is mediated by activation of the orphan nuclear receptor small heterodimer partner (SHP)[22-24], which in turn interact with liver receptor homolog (LRH-1). SHP protein blocks activities of LRH-1 that is known to positively regulate CYP7A1 expression. Mice lacking SHP ($SHP^{-/-}$) failed to repress CYP7A1 in response to a specific agonist for FXR. Yet, Bile acid feeding can restore expression of CYP7A1 in SHP-null mice, indicating the existence of SHP-independent regulation pathways, as well. One of these pathways involves JNK mitogen-activated protein kinase activation[24,25] and fibroblast growth factor 19 (human FGF19, mouse FGF15)[26]. Additionally, FXR induces the expression of ATP-binding cassette transporters such as bile salt export pump, multidrug resistance protein 3 (MDR3) and multidrug resistance-associated protein 2. These transporters export bile acids from hepatocytes into bile canaliculi. Activation of FXR was also found to stimulate the expression of intestinal bile acid-binding protein at ileum, which facilitates enterohepatic recycling of bile acids[21,27].

Lipid and glucose metabolism: FXR also regulates lipid metabolism and gluconeogenesis[28,29]. FXR-null mice develop severe fatty liv er with elevated circulating plasma cholesterol, elevated triglycerides and free fatty acids. Several lipoproteins such as phospholipid transfer protein, apoC-Ⅱ, apoC-Ⅲ, and apoA-1 are FXR targets[30-32] and their decreased expression likely accounts for lipid derangements in FXR-null mice. FXR also regulates lipid synthesis by involving acetyl-CoA carboxylase 1 (Acc1), Acc2, Cd36, and sterol regulatory element-binding protein 1C, with the latter being a major regulator of lipogenesis *via* stimulation of *de novo* lipogenesis, and these FXR effects likely occur through activation of SHP[33] and FGF19[34]. Beyond regulation of lipid metabolism, FXR also plays an important role in glucose homeostasis. Loss of FXR in mice lead to development of impaired glucose tolerance and insulin resistance both in liver and skeletal muscles which is associated hepatic steatosis and elevated circulating free fatty acids[35]. Bile acids alter the expression of genes involved in gluconeogenesis, including phosphoenolpyruvate carboxykinase (PEPCK), glucose-6-phosphatase (G-6-Pase), and fructose-1,6-biphosphatase[36-38]. SHP activation may modulate gluconeogenesis through repression of PEPCK and G-6-Pase[38,39]. FGF15/19 appear also to be critical in glucose regulation. In the postprandial state, FGF15/19 are released from the small intestine and inhibit hepatic glucogenesis, like insulin, through dephosphorylation and inactivation of cAMP regulatory element-binding protein[40].

Hepatic inflammation and fibrosis: FXR also regulates hepatic inflammation and fibrosis[29]. FXR is expressed at very low levels on hepatic Kupffer, stellate, and endothelial cells. Porcine serum treatment or bile duct ligation (BDL) are commonly used experimental methods to induce cirrhosis in rats. Treatment of these rats with 6-ethyl chenodeoxycholic acid (6-ECDCA), an FXR ligand, prevents liver fibrosis in porcine serum-treated rats or BDL-treated rats, and decreases expression of matrix proteins including, α1-collagen, transforming growth factor β-1, αSMA, and tissue inhibitors of metalloproteinase 1 and 2[41]. Interestingly, Fickert *et al*[42] showed that FXR loss reduced fibrosis of the hepatic biliary tree. In the study, hepatic fibrosis was induced in wild type and FXR knock-out mice ($FXR^{-/-}$) by a variety of methods, including carbon tetrachloride (CCl_4) intoxication, 3,5-diethoxycarbonyl-1,4-dihydrocollidine feeding, BDL, or Schistosoma mansoni (S.m.)-infection.

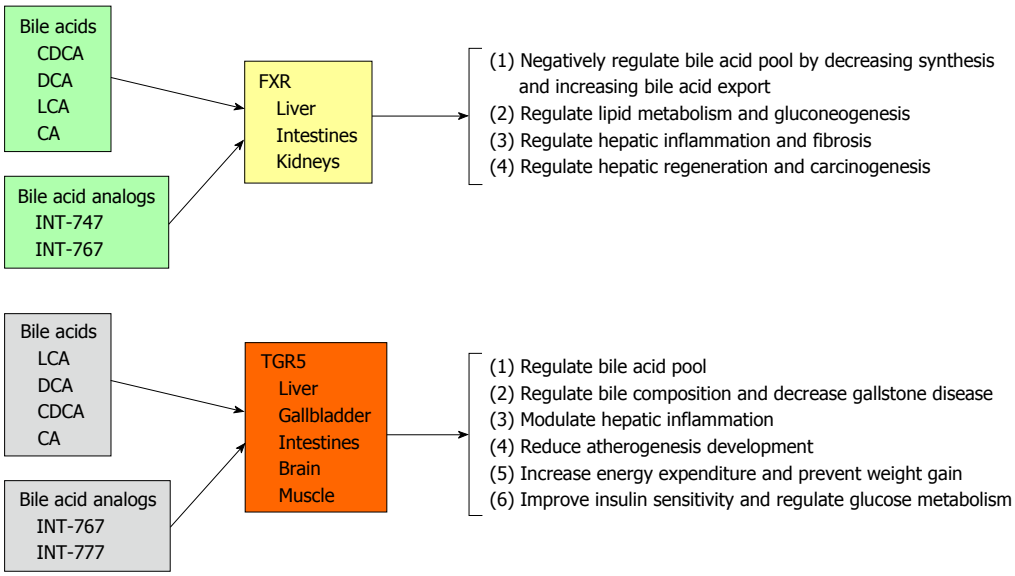

Figure 2 Characteristics of farnesoid X receptor and transmembrane G protein-coupled receptor 5, and their functions. CA: Cholic acid; LCA: Lithocholic acid; DCA: Deoxycholic acid; FXR: Farnesoid X receptor; TGR5: Transmembrane G protein-coupled receptor 5; CDCA: Chenodeoxycholic acids.

Only biliary-type hepatic fibrosis was reduced in FXR (-/-) mice with BDL and 3,5-diethoxycarbonyl-1,4-dihydrocollidine. FXR loss had no effect on the prevention of non-cholestatic liver fibrosis in the study.

Hepatic regeneration and carcinogenesis: FXR appears to regulate liver regeneration and carcinogenesis. CA feeding has been shown to induce liver growth and decrease mortality in mice that have undergone partial hepatectomy. This effect may involve activation of FGF15/19. Studies have shown that the protective effects with CA feeding after partial hepatectomy were significantly abolished in FGF15 (-/-) mice[43], and proliferation of hepatocytes and cholangiocytes was also noticeably reduced in CA-fed FGF15 (-/-) mice[43]. FXR (-/-) mice developed spontaneous hepatocellular carcinoma (HCC) at age > 12 mo[44,45]. And FXR had a direct effect in down-regulating a number of tumor suppressor genes such as N-myc downstream-regulated gene 2[46] and gankyrin, a proteasomal subunit that assists in degradation of a number of tumor suppressor proteins[46,47]. Interestingly, selective reactivation of intestinal FXR can restore bile acid enterohepatic circulation and protect FXR (-/-) mice from spontaneous HCC development[48].

FXR agonists in the treatment of NAFLD

6-ECDCA, also known as INT-747 or obeticholic acid (OCA), is a lipophilic bile acid derivative and a potent selective FXR activator[49]. In animal studies, it improves hepatic steatosis[50], fibrosis[42], and portal hypertension[51]. The FLINT trial[52], a phase ⅡB randomized, placebo-controlled trial of OCA in human NASH, demonstrated that OCA significantly improved the NAFLD activity score in all components, including steatosis, lobular inflammation, and hepatocellular ballooning, compared to placebo, establishing a clear benefit of in alleviating liver injury and inflammation in NAFLD. There was also some improvement in fibrosis score in the OCA group in the FLINT trial, as well, but the trial was not powered to detect the statistical significance in fibrosis changes. It remains to be determined whether or not OCA will resolve NASH and ameliorate advanced fibrosis, but further trials are ongoing.

TGR5 and NAFLD

TGR5 is a classic G-protein coupled cell surface receptor[53,54] that is activated by bile acids in the order of LCA > DCA > CDCA > CA[53]. In the absence of bile acid binding, TGR5 is tightly associated with a G-protein complex consisting of α, β and γ subunits. Upon binding to bile acids, TGR5 allows the release of α subunit, which in turn activates adenylyl cyclase, leading to the accumulation of cAMP and activation of protein kinase A. TGR5 is widely expressed in various tissues including the liver, gallbladder, bile ducts, adipose tissue, spleen, intestines, and kidneys. Within the liver, TGR5 is abundantly expressed in Kupffer cells and endothelial cells, but not in hepatocytes. The targets and effects of TGR5 are outlined in detail below and summarized in Figure 2.

TGR5 functions and NAFLD

Regulation of the bile acid pool: TGR5 regulates the bile acid pool. The bile acid pool is significantly reduced in TGR (-/-) mice compared to wild-type mice. TGR5 also regulates bile composition as demonstrated by an experiment showing that, when fed with lithogenic diet, TGR (-/-) mice were protected from gallstone diseases. The expression of TGR5 was shown to be present in gallbladder epithelial cells and cholangiocytes, and TGR5 activation induced bicarbonate and chloride secretion from cholangiocytes, which may account for the alteration of bile composition.

Modulation of the immune response: TGR5 also modulates immune responses of immune cells *via* increasing intracellular cAMP[53], and this function appears relevant to the regulation of hepatic inflammation and atherosclerosis development. TGR5 is highly expressed in monocytes and macrophages. Activation of TGR5 increases cAMP in rat alveolar macrophages and, as a result, it reduces the phagocytic activity of the macrophage and inhibits lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin-1 (IL-1), IL-6 and IL-8. This finding was also demonstrated in resident hepatic macrophage Kupffer cells. Activation of TGR5 in isolated Kupffer cells causes an increase in cAMP and reduced expression of pro-inflammatory cytokines, including: TNF-α, IL-1, IL-6 and IL-8, following LPS treatment. Notably, TGR5-deficient mice are more susceptible to LPS-induced liver injury[55]. TGR5 activation also attenuates the formation of atheromatous plaque in low-density lipoprotein receptor knockout mice ($LDL^{-/-}$), a commonly used murine model for atherosclerosis studies that have functioning TGR5 ($LDL^{-/-}$; $TGR5^{+/+}$). This attenuation of plaque formation was achieved through decreases in intra-plaque inflammation and macrophage activation[56]. Recent study has also showed that a TGR5 agonist increased the production of nitric oxide (NO) in endothelial cells, a key anti-atherogenic molecule. This suggests that NO might be one of downstream effectors of TGR5 signaling.

Energy expenditure and metabolism: TGR5 enhances energy expenditure and mitigates obesity and insulin resistance in obese mice. TGR5 is expressed in human brown adipocytes and skeletal myocytes. In brown adipose and skeletal muscle, interactions between bile acids and TGR5 promote the expression of cAMP-dependent 2-iodothyronine de-iodinase (D2), which converts inactive thyroxine (T4) to active 3,5,3-triiodothyronine (T3), a major hormone in increasing basal metabolism and thus, inducing energy expenditure[57]. TGR5 signaling also regulates glucose homeostasis[58]. TGR5 is expressed on enteroendocrine L-cells, and activation of TGR5 on L-cells modulates mitochondrial oxidative phosphorylation and alters ATP/ADP ratio. This leads to the release of glucagon like peptide-1 (GLP-1) from L-cells. GLP-1 further stimulates insulin secretion from pancreas and maintains glucose homeostasis.

TGR5 agonists in the treatment of NALFD

Given the aforementioned biological effects of TGR5, TGR5 becomes an enticing potential target for NASH therapeutics. A specific CA derivative, 6α-ethyl-23(S)-methylcholic acid (INT-777) has been developed as a selective TGR5 agonist[59]. Treatment of high-fat fed mice with INT-777 increased energy expenditure and attenuated both weight gain and expansion of fat pad mass[58]. In addition, INT-777 treatment reduced hepatic steatosis and improved liver enzyme levels without evidence of hepatic fibrosis[58]. INT-777 treatment has also been shown to improve insulin sensitivity, likely through the release of GLP-1 in the intestines, in both diet-induced obese mice and in genetically obese mice that have a leptin receptor gene mutation (*db/db*), which is a well-established model of obesity and diabetes[58]. This effect was blunted in $TGR5^{-/-}$ mice, indicating the specificity of INT-777 treatment in targeting TGR5. With all these features, TGR5 agonists such as INT-777 are very attractive treatment candidates for NASH and other features of the metabolic syndrome[60].

TARGETING BOTH FXR AND TGR5 IN THE TREATMENT OF NAFLD

INT-767, the 23-sulphate derivative of OCA, is a dual FXR/TGR5 agonist[61]. INT-767 has been demonstrated to induce FXR-dependent lipid uptake by adipocytes, mobilizing lipid from the circulation and the liver to peripheral adipose tissue. INT-767 also promotes TGR5-dependant GLP-1 release. Treatment of obese mice with INT-767 significantly decreased total plasma cholesterol and triglyceride levels[61], and improved the histological features of NASH in these mice[62]. These effects have been postulated to be due to INT-767-mediated alterations in the phenotypes of intrahepatic macrophage populations and modulation of cytokine production[62]. Uniquely, INT-767, but not INT-777 or INT-747, ameliorates hepatic injury in MDR2 (-/-) mice, a model for chronic cholangiopathy. This hepatoprotective effect is manifested by a reduction in bile acid synthesis and an increase in bile flow and biliary HCO_3^- output[63].

CONCLUSION

Bile acids and bile acid receptors have pluripotent functions in energy expenditure, regulation of lipids and glucose metabolism, modulation of hepatic inflammation, fibrosis, regeneration, and carcinogenesis. These effects translate into attractive therapeutic targets for NASH and to improve metabolic profiles, ameliorate hepatic injury, and halt hepatic fibrosis. One currently very promising drug is OCA, but additional new drugs are expected in the not-too-distant future that will target the pathways in NASH pathogenesis.

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REVIEW

Treating morbid obesity in cirrhosis: A quest of holy grail

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Abstract

The problem of obesity is increasing worldwide in epidemic proportions; the situation is similarly becoming more common in patients with cirrhosis which negatively affect the prognosis of disease and also makes liver transplantation difficult especially in the living donor liver transplantation setting where low graft to recipient weight ratio negatively affects survival. Treatment of obesity is difficult in cirrhosis due to difficulty in implementation of lifestyle measures, limited data on safety of anti-obesity drugs and high risk of surgery. Currently approved anti-obesity drugs have limited data in patients with cirrhosis. Bariatric surgery remains an option in selected compensated cirrhotic patients. Endoscopic interventions for obesity are emerging and are quite promising in patients with cirrhosis as these are minimally invasive. In present review, we briefly discuss various modalities of weight reduction in obese patients and their applicability in patients with cirrhosis.

Key words: Obesity; Intragastric balloon; Antiobesity drugs; Bariatric surgery; Cirrhosis

Core tip: The rising obesity problem is also associated with increased incidence of simultaneous obesity and cirrhosis. This is a particularly difficult subset of obese patients to treat as there is difficulty in implementation of lifestyle measures, limited data on safety of anti-obesity drugs and high risk of surgery. In present review, we briefly discuss various modalities of weight reduction in obese patients and their applicability in patients with cirrhosis.

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INTRODUCTION

Obesity has been defined as abnormal or excessive fat accumulation that can lead to impairment of health. It's one of the most significant public health problems faced by people of industrialized countries and is rapidly

catching up in developing countries also. Worldwide obesity prevalence has almost doubled since 1980[1]. Obesity has reached epidemic proportions over the world and it is simultaneously associated with various comorbidities, namely diabetes mellitus, hypertension, and cardiac diseases[2]. In 2014, according to estimate more than 1.9 billion adults were overweight with 600 million likely obese. Approximately 39% of adults aged 18 years and above were overweight in 2014, and 13% were obese[3]. Due to various co-morbidities, obesity represents a very serious health problem worldwide. Obesity management is a unique challenge due to the rapid evolution of unfavourable lifestyles[4].

Obesity can be associated with cirrhosis as a virtue of non-alcoholic steatohepatitis (NASH), an important cause of cirrhosis, being a component of the metabolic syndrome and it can also exacerbate co-existing liver injury due to other causes and is associated with more risk of decompensation of cirrhosis[5]. The pathophysiology of NASH has been considered a "two hit" process[6]. The "first hit", hepatic steatosis, makes the liver susceptible to injury mediated by "second hits", like inflammatory cytokines/adipokines, oxidative stress, and mitochondrial dysfunction, leading to steatohepatitis and/or fibrosis[7]. Impaired hepatocyte proliferation progenitors due to cell death has been proposed as "third hit" in pathogenesis of non-alcoholic fatty liver disease[8]. Various cytokines/adipokines involved in NASH pathogenesis includes tumor necrosis factor-alpha, leptin, adiponectin, interleukin-6 (IL-6), *etc.*[9]. Fibrosis/cirrhosis represents the final common endpoint of pathway of almost all chronic liver diseases including NASH. Mechanisms for fibrosis include the secretion of profibrogenic cytokines (tumor growth factor-β, IL-6, IL-8, *etc.*) by the ductular reaction, as well as epithelial to mesenchymal transition of stellate cells to myofibroblasts[10,11]. Steatosis is very commonly associated with hepatitis C, particularly with genotype 3. In chronic hepatitis C, obesity is associated with inflammation, steatosis, insulin resistance, faster progression of fibrosis, and nonresponse to treatment with interferon[12].

OBESITY AND CIRRHOSIS: THE CHALLENGES AND RATIONALE FOR MANAGEMENT

Obesity with cirrhosis is a complex problem. Once cirrhosis is decompensated, lifestyle measures are very difficult to implement and bariatric surgery becomes risky due to increased morbidity and mortality[13,14]. Pharmacological measures (drugs) have a very limited role in management of obesity, are not as effective as surgery and there is rebound weight gain once stopped. The limited drug arsenal available to treat obesity is not well studied in patients with liver disease. No safe anti-obesity drug in cirrhosis is available at the moment. Proportion of patients with NASH associated end stage liver disease as an indication for liver transplantation is increasing gradually[15,16] and these patients are more prone for co-morbidities associated with NASH like coronary artery disease, diabetes, hypertension, dyslipidemia, metabolic syndrome and chronic kidney disease[17]. Obesity in cirrhosis becomes a multi-headed monster leading to more rapid worsening of liver disease and also makes liver transplantation difficult. There is difficulty in finding a suitable donor for morbidly obese patients due to risk of low graft to recipient ratio and subsequent risk of poor graft function and higher mortality in living donor liver transplantation (LDLT) programs which predominant from of liver transplantation in Asia[18]. Increased rates of complications and mortality, as well as decreased graft survival, have been reported in morbidly obese patients often discouraging transplantation in this population and have resulted in the exclusion of morbidly obese patients from liver transplantation at some centres[19]. With increasing number of non-alcoholic steatohepatitis associated end stage liver disease as an indication for liver transplantation, problem of morbid obesity before liver transplantation is going to rise[20].

There are multiple benefits of treating obesity in patients with cirrhosis. Firstly there is a reduction in risk of decompensation as studies have shown higher decompensation over time in overweight and obese cirrhotic[5]. Some patients may improve from compensated stage to lesser degree of fibrosis as shown by bariatric surgery studies[21]. This may avoid need for liver transplantation in many patients. Secondly reduction in weight may improve their candidacy for liver transplantation by improving co-morbidities (like diabetes control), decreasing risk in surgery and improving their graft recipient weight ratio especially in LDLT settings. Thirdly there can be reduced incidence of hepatocellular carcinoma (HCC) as obesity is considered to be an independent risk factor for development of HCC[22].

In current review article we will discuss the various weight reductions strategies in brief and their applicability in patients with cirrhosis.

DIET, LIFE STYLE MODIFICATION AND EXERCISE IN THE MANAGEMENT OF OBESITY IN CIRRHOSIS

The recent worldwide increase in the population of obese individual is also seen in liver cirrhosis patients[23]. At present, in liver cirrhosis due to alcohol and chronic hepatitis C infection, nutritional intake is a spectrum ranging from being either sufficient or excessive[24,25]. Excessive nutrients has to be assessed in every patient, and the various nutritional parameters, like serum albumin and lean body mass, should be evaluated for the appropriate nutritional therapy. However, the amount of body weight reduction has not been evaluated properly in the obese liver cirrhosis patients. The addition of oral Branched chain amino acids (BCAA) granules to diet has been shown to reduce the incidence of HCC[23]. It has also been shown that oral BCAA supplementation

increases serum albumin levels[26]. The mechanism involved may be improved insulin sensitivity in muscle, increase in and reduced oxidative stress[27]. Thus, in obese liver cirrhosis patients, oral BCAA treatment is recommended in addition to correction of nutritional intake. The current epidemic of global obesity has created a new entity: The unique combination of sarcopenia and obesity, now commonly described as sarcopenic obesity[28]. A recent study has shown that sarcopenic obesity is more closely linked with insulin resistance than either sarcopenia or obesity alone[29].

Physical activity levels and also exercise capacity are generally lower in liver cirrhosis patients than in healthy controls[30,31]. Exercise is a key component of management of liver cirrhosis patients because it leads to increased calorie burning, increased skeletal muscle mass, along with exercise capacity, leading to improved quality of life. The advice regarding exercise is made complex in patients with cirrhosis as portal pressure has been shown to increase with moderate exercise (up to 30% of the maximum), which poses a risk for variceal bleeding[32]. The optimal exercise regimen in liver cirrhosis patients remains uncertain. Researcher's recommendation is walking 5000 or more steps every day with a caloric intake of 30 kcal/kg based on a survey done on compensated cirrhosis patients[31]. A randomized pilot study involving liver cirrhosis patients, mostly Child-Pugh A cirrhosis, examined the effect of exercise combined with leucine supplementation (10 g/d). The program included three sessions every week of one hour treadmill along-with cycle ergometry at 60%-70% of the maximum heart rate, over a total period of 12 wk. The intervention group had improved exercise capacity, shown by the 6-min walk test and the 2-min step test with associated improvement in quality of life parameters with no adverse events[33]. Aerobic exercise is expected to improve insulin resistance in patients with cirrhosis which is particularly important for obese patients[34,35]. Future studies will establish efficacious along with safe exercise regimen needed for liver cirrhosis patients.

DRUG THERAPY

Orlistat

Orlistat at a dose of 120 mg was approved by the Food and Drug Administration (FDA) in 1999 for the management of obesity in association with reduced calorie diet, and also to reduce the risk of regaining weight after previous weight loss. Orlistat was the first treatment for obesity that was not an appetite suppressant, but acted by interfering with the action of hormone lipase involved in fat digestion[36]. In one of the longest trials comprising of 3304 patients, 21% also having impaired glucose tolerance, were randomized to receive either placebo or orlistat. During the first year, weight loss was greater in the orlistat-treated group (11% compared with 6% in the placebo group)[37]. Despite being FDA-approved fewer than 10% patients take it for 1 year and less than 2% of patients for 2 years due to poor compliance secondarily to side effects[38,39]. It is advisable to give vitamin supplements to patients treated with this drug. Severe liver injury has been reported rarely with a United States FDA review identifying 13 reports of severe liver damage[40]. Given the side effect profile it is unlikely to become a commonly prescribed drug in cirrhosis patients who may have malnutrition despite obesity.

Lorcaserin

Lorcaserin is a selective agonist of 5-hydroxytryptamine receptor 2C (5-HT2C), which is expressed in hypothalamic pro-opiomelanocortin (POMC)-producing neurons of central nervous system, the centre controlling appetite and satiety[41]. Lorcaserin causes activation of the 5-HT2C receptors which stimulates release of melanotropin-α, subsequently decreasing appetite through stimulation of melanocortin receptor 4[41]. Of significance is the low affinity lorcaserin has for other 5-hydroxytryptamine receptor subtypes, especially 5-HT2B, which has previously been associated with the development of valvular heart disease. Lorcaserin approval by FDA was largely based on two placebo-controlled trials in nondiabetic patients (BLOOM and BLOSSOM) along with a third smaller trial in adults with diabetes (BLOOM-DM)[42-44]. Lorcaserin caused a modest weight reduction of approximately 3.2 kg more than placebo. Adverse effects include headache, nausea, fatigue, and dizziness[45]. Lorcaserin should be discontinued if there is less than 5% weight reduction in 12 wk. No dose adjustment is required in patients with mild to moderate hepatic impairment. It has not been studied in patients with severe hepatic impairment and is not recommended in these groups of patients.

Phentermine/topiramate-extended-release

In 2012, the United States FDA approved a preparation of phentermine and extended-release topiramate for use in adults with a body mass index (BMI) $\geqslant$ 30 kg/m^2 or with BMI $\geqslant$ 27 kg/m^2 with associated comorbidity (hypertension, diabetes, dyslipidemia). Phentermine plus topiramate-extended-release (ER) was recommended for approval based largely on two phase 3 clinical trials (EQUIP and CONQUER)[46,47]. In the EQUIP trial (n = 1267) participants given the top dose *vs* placebo, the mean 1-year weight loss was 10.9% *vs* 1.6%[46]. In CONQUER trial (n = 2487) one-year mean weight loss was 8.1 kg (7.8%) with the recommended dose and 10.2 kg (9.8%) with the top dose *vs* 1.4 kg (1.2%) with placebo[47].

The labelling recommends against prescription in patients with recent or unstable cardiac or cerebrovascular disease, and suggests regular monitoring of resting heart rate. No dose adjustment is needed in patients with mild hepatic impairment. In patients with moderate hepatic impairment, the maximum dose is Phentermine/topiramate-ER 7.5 mg/46 mg once daily. Phentermine/topiramate-ER is not studied in patients with severe hepatic impairment where it has to be

Table 1 Newer weight reduction drugs in pipeline

Drug	Mechanism of action
Cetilistat	Gastrointestinal and pancreatic lipase inhibitor
Velneperit	Neuropeptide Y5 receptor inhibitor, appetite suppression
Tesofensine	Inhibition of serotonin, dopamine, and noradrenaline reuptake
Metreleptin	Leptin receptor agonist
Obinepitide	Dual neuropeptide Y2/Y4 receptor agonist
Beloranib	MetAP2 inhibition

MetAP2: Methionine aminopeptidase 2.

avoided[48].

Bupropion-naltrexone

In September 2014, a sustained release formulation of bupropion-naltrexone was approved by FDA[49]. Bupropion activates POMC neurons in the hypothalamus which gives downstream effects of appetite reduction and increased energy output. The POMC is regulated by endogenous opioids *via* opioid-mediated negative feedback. Naltrexone is a pure opioid antagonist, which further augments bupropion's activation of the POMC.

In a randomized trial of bupropion and naltrexone (varying doses) *vs* double placebo, weight loss was greater in those assigned to active treatment (mean change in body weight in low dose naltrexone and high dose naltrexone was -5% and -6.1% *vs* -1.3% in placebo arm)[50]. Compared with placebo, the combination of bupropion-naltrexone has been shown to reduce weight by approximately 4% to 5%[50-53]. Contraindications include uncontrolled hypertension, seizure history, eating disorders, simultaneously using other bupropion-containing products, chronic opioid use, and monoamine oxidase inhibitors use within last 14 d. Cases of hepatitis and clinically significant liver dysfunction have been seen in association with naltrexone use during naltrexone clinical trials and in post marketing reports of naltrexone use[54]. Thus the combination of bupropion-naltrexone doesn't looks too exciting for the patient of cirrhosis and in the absence of strong data for liver disease patients, shouldn't be prescribed.

Liraglutide

Liraglutide, is a long-acting glucagon-like peptide-1 analog, and a promising option for obese patients with type 2 diabetes. It is the most recent drug to be approved for obesity by FDA in December 2014. In diabetes trials, liraglutide (1.8 mg daily) was associated with a greater reduction in weight (2.0 to 2.5 kg) when compared with placebo or glimepiride[55]. In a randomized trial comparing liraglutide (1.2 to 3 mg), placebo, and open-label orlistat (120 mg orally three times daily) in 564 patients with a mean BMI of 35, weight loss increased with increasing doses of liraglutide, with the mean weight loss ranging from 4.8 to 7.2 kg[56]. Patients who were randomly assigned to receive any dose of liraglutide were found to lose significantly more weight compared to placebo (mean weight loss 2.8 kg). Patients taking the two highest doses of liraglutide (2.4 and 3.0 mg) lost significantly more weight than those assigned to orlistat (6.3, 7.2 and 4.1 kg, respectively)[56]. In a 56-wk SCALE Maintenance randomized study trial comparing liraglutide 3 mg once daily with placebo injection in 422 patients a greater proportion of patients maintained weight loss in the liraglutide group (81.4%) *vs* 48.9% in placebo group)[57]. Common side effects included nausea (37%-47%), vomiting (12%-14%), diarrhoea, reduction of blood sugar levels, and loss of appetite. Less common side effects included pancreatitis, renal impairment, and suicidal tendenciew. In rodent studies, liraglutide has been associated with benign and malignant thyroid C-cell tumors. Liraglutide is not recommended in patients with a personal or family history of medullary thyroid cancer or multiple endocrine neoplasia 2A or 2B. Because of limited experience in patients with hepatic impairment, it must be used cautiously in patients with liver impairment. If data regarding safety becomes available it would an interesting drug given its potential to improve various cardio metabolic factors[58].

Antiobesity drugs: In the pipeline

Apart from the current approved drugs for treatment of obesity many other are in various stages of development. Endogenous cannabinoids which are ubiquitous lipid signalling molecules having both central and peripheral effects mediated by the specific receptors CB1 and CB2[59]. Compounds targeting the peripheral CB1 receptors selectively are under evaluation[60,61]. Various drugs which may hold promise in near future are listed in the table. Other drugs in various stages of development are summarized in the Table 1[62-67].

Overall there is hope of some drugs being available in near future. The role of combination polytherapy needs further evaluation due to paucity of efficacy and safety data in cirrhosis patients currently.

SURGERY

The number of obese patients awaiting organ transplantation is increasing in parallel with the increasing prevalence of obesity. For patients with advanced fibrosis and cirrhosis, historically, bariatric surgery was not advised or offered. Complications of bariatric surgery, including bleeding, gastrointestinal symptoms, nutritional or electrolyte abnormalities, and stomal stenosis, can be seen in 10% to 17% of patients without cirrhosis[68]. Bariatric surgery may be useful in cirrhosis patients needing LT who were denied evaluation primarily because of weight. Furthermore reduction in weight can lead to improvement in liver parameters reducing chances of decompensation over time.

Patients with cirrhosis undergoing surgery of any kind are placed at an increased risk of mortality from liver failure, renal failure, or even postoperative bleeding due to impaired coagulation. This risk depends on the

degree of liver dysfunction or model for end-stage liver disease scores[69]. The mortality rates from bariatric operations have been reported to be in the range of 0.28%-0.35%[14,70]. A large population-based study (n = 674900) has reported that patients with cirrhosis had higher in-hospital mortality rates than those without cirrhosis after bariatric surgery (1.2% *vs* 0.3%)[14].

Takata *et al*[71] reviewed 15 patients of end-stage organ failure, of which 6 with cirrhosis underwent laparoscopic sleeve gastrectomy (LSG). Complications were noted in 2 patients with cirrhosis but there was no mortality. The mean follow-up was 12.4 mo, and the mean excess weight loss noted was 33% for cirrhosis patients at 9 mo[71]. The LSG was selected instead of Roux-en-Y gastric bypass (LRYGB) in this study for the following reasons: (1) some evidence has shown previously that the operative time and overall morbidity are reduced compared with those with LRYGB[13]; (2) the remaining gastric tube remains endoscopically accessible in the case of variceal bleeding; (3) endoscopic access to the biliary system after liver transplantation is preserved; and (4) it is expected that intake and absorption of critical medications will not be significantly altered.

Lin *et al*[72] studied 26 pretransplant patients who underwent LSG. The mean age of patients was 57 years with 17 (65%) of patients being female. Six patients had end-stage renal disease, and 20 patients had end-stage liver disease. There were 6 postoperative complications but no death, the complications being two superficial wound infections, one staple line leak, one postoperative bleed, one transient encephalopathy, and one renal insufficiency that resolved. The mean excess weight loss at 1, 3 and 12 mo was 17%, 26% and 50% respectively[72]. This lead to liver transplantation in seven patients showing LSG is well tolerated, is technically feasible, and improves candidacy for transplantation.

Shimizu *et al*[73] prospectively reviewed 23 patients (12 with known cirrhosis and 11 with unknown cirrhosis). There were 14 females and 9 males with a mean age of 51.5 ± 8.3 and a mean body mass index of 48.2 ± 8.6 kg/m^2. Child-Pugh classes were A (n = 22) and B (n = 1). Procedures performed were LRYGB (n = 14), LSG (n = 8), and laparoscopic adjustable gastric banding (LAGB) (n = 1). No patients had liver decompensation after surgery. The patients lost 67.4% ± 30.9% of their excess weight at 12 mo follow-up and 67.7% ± 24.8% at 37 mo follow-up[73].

Most recently Pestana *et al*[74] reviewed 14 patients [11 patients underwent sleeve gastrectomy (78.6%) and 3 gastric bypass (21.4%)] with Child's A cirrhosis with or without portal hypertension. The mean patient age was 55.5 years, and 10 of 14 patients were women. At 1-year post surgery, only 1 of 8 patients who underwent follow-up ultrasound imaging showed steatosis. The bilirubin level above 2 mg/dL was seen in a patient one year post surgery. One patient developed encephalopathy at 2-year post-surgery. Bariatric surgery in patients with compensated cirrhosis even with mild portal hypertension seems well tolerated[74].

Woodford *et al*[75] studied 14 patients intraoperatively detected with cirrhosis undergoing LAGB. No patients had preoperative clinical evidence of decompensated liver disease. There was no operative mortality.

Table 2 reviews the various studies done on bariatric surgery in cirrhosis patients[71,73-78]. Overall the literature suggests that bariatric surgery is tolerated in compensated cirrhosis although with slightly higher but acceptable complication rate and should be offered to obese cirrhosis patients. This will delay progression of liver disease to decompensation and also increase the candidacy for transplantation in both living donor liver transplantation and dead donor liver transplantation setting.

ENDOSCOPIC INTERVENTIONS FOR MORBID OBESITY IN CIRRHOSIS

Endoluminal interventions performed through the gastrointestinal (GI) tract using endoscope offers potential for a weight loss procedure which is safer and more cost-effective than the current laparoscopic approaches[79]. Endoscopic techniques try to mimic the anatomical features produced by bariatric surgery. There are mainly two types of endoscopic weight loss modalities - restrictive and malabsorptive. Restrictive procedures causes reduction of gastric volume through use of space-occupying prosthesis or through suturing/stapling devices, while malabsorptive procedures causes reduced absorption by preventing contact of food with the duodenum and proximal jejunum. Restrictive procedures include intragastric balloon insertion, endoluminal vertical gastroplasty, transoral gastroplasty and transoral endoscopic restrictive implant system, while malabsorptive procedure include duodenojejunal bypass sleeve. Gastroduodenojejunal bypass sleeve is combines both restrictive and malabsorptive features. Except for intragastric balloon, all the mentioned procedures are comparatively new, with no data on cirrhosis patients.

Intra-gastric balloon placement is minimally invasive modality for weight loss. While this procedure has a well-established role in patients without liver disease, data on cirrhosis is not there. A meta-analysis of intragastric balloon placement in general patients including 15 articles (3608 patients) showed weight loss of 14.7 kg, 12.2% of initial weight, 5.7 kg/m^2, and 32.1% of excess weight at 6 mo. Complications of intra-gastric balloon placement are uncommon and most common side effect is nausea and vomiting (8.6%)[80]. Other side effects included intolerance to the balloon which resulted in early removal, gastric ulcers and erosions, esophagitis, spontaneous deflation, persistent vomiting, gastroesophageal reflux and abdominal pain. However, severe complications are rare with a large Italian series of 2525 cases showing the following complications; 0.08% acute gastric dilatation, gastric perforation in 5 (0.19%, 4 of these had gastric surgery earlier), gastric obstruction in 0.76%, balloon rupture in 0.36%, esopha-

Table 2 Studies of bariatric surgery in cirrhotic patients

Ref.	Study characteristics	Cirrhosis diagnosis	Child pugh	Procedures	Complications	Liver decompensation	Mortality
Pestana *et al*[74] *n* = 14 F:M = 10:4	Mean age = 55.5 yr	Known cirrhosis	A = 14	SG = 11 RYGB = 3	0	1 (late HE)	0
Shimizu *et al*[73] *n* = 23 F:M = 14:9	Mean age = 51.5 yr Mean BMI = 48.2 kg/m^2 Mean stay = 4.3 d	12 preoperatively 11 intraoperatively	A = 22 B = 1	RYGB = 14 SG = 8 AGB = 1	8	0	0
Rebibo *et al*[78] *n* = 13 F:M = 7:6	Median age = 52 yr Median BMI = 46.3 kg/m^2	All intraoperatively	A = 13	SG = 13	2	1 (ascites)	0
Takata *et al*[71] *n* = 6 F:M = 4:2	Mean age = 52 yr Mean BMI = 49 kg/m^2	All preoperatively	A = 4 B = 2	SG = 6	2	1 (ascites) 1 (HE)	0
Dallal *et al*[76] *n* = 30 F:M = 20:20	Mean age = 50 yr Mean BMI = 52.6 kg/m^2 Mean hospital stay = 4 d	Diagnosed intraoperatively in 27 (90%)	A = 30	RYGB = 27 SG = 3	9	0	0
Kral *et al*[77] *n* = 14 F:M = 10:4	Mean age = 40 yr Mean BMI = 54 kg/m^2	All intraoperatively	NA	BPD = 14	2	2	2 (one late hepatic failure)
Woodford *et al*[75] *n* = 14 F:M = 10:4	Mean age = 52.5 yr Mean BMI = 38.9 kg/m^2	All intraoperatively	A or B	AGB = 14	2	0	0

RYGB: Roux-en-Y gastric bypass; SG: Sleeve gastrectomy; BPD: Bilio-pancreatic diversion; AGB: Adjustable gastric banding; HE: Hepatic encephalopathy; M: Male; F: Female; BMI: Body mass index.

gitis in 1.27% and gastric ulcer in 0.2%. They noted significant improvement of co-morbidities[81]. It should be noted that above meta-analysis and Italian study used BioEnterics intra-gastric balloon, the newer Spatz balloon provides option of gradual increase (or decrease) in balloon volume, thus should be associated with less complications and it can be kept for 1 year as compared to 6 mo duration for earlier. If dietary and lifestyle measures continued after balloon removal, these patients sustain initial weight loss. A Brazilian multicenter study of 483 patients showed that significant number of patients maintained their weight loss after balloon removal with a multidisciplinary program which involved clinical, psychiatric, exercise, and dietary therapy[82].

We published use of intragastric ballon in decompensated cirrhosis for the first time in 2012 as letter to editor. The 61-year-old patient had decompensated alcoholic liver disease (CTP score 9). His BMI decreased from 48.3 kg/m^2 to 39.2 kg/m^2 (resulting in a total of 24 kg weight loss) at 6 mo after intragastric balloon placement. His diabetic control also improved, HbA1c level decreasing from 9.2 to 5.4[83].

We have placed a total of 8 intragastric balloons (7 had decompensated cirrhosis) and five of them had successful liver transplantation (3 deceased donor liver transplantation and 2 LDLT), this data is submitted for publication elsewhere. None of these patients had any severe complication other than vomiting in initial few days. One patient didn't lose weight out of these 8 patients and in one patient we had to decrease initial volume of Spatz balloon due to persistent vomiting at day 7. Although intra-gastric balloon appears to be a promising modality for weight loss in decompensated cirrhosis, it cannot be placed in all patients. Contraindications of intra-gastric balloon include severe coagulopathy, upper gastro-intestinal tract conditions with potential bleeding risks (large or high risk esophageal varices, gastric varices, ulceration), presence of eating disorders, history of prior gastroesophageal surgery, presence of autoimmune connective tissue disorder affecting GI tract, significant hiatal hernia, esophageal stenosis, GI motility disorders, unwillingness for supervised diet and behaviour modification program and allergy to Silicon (product information). In conclusion, there is plenty of data about use of intra-gastric balloon for weight loss in morbidly obese patients and it has proven to be a safe modality. However, its use in morbidly obese patients with cirrhosis who are awaiting liver transplantation has not been studied.

The endoscopic administration of botulinum toxin type A in gastric wall is thought to aid in weight reduction by inhibiting antral motility and slowing gastric emptying by inhibiting acetylcholine release at the neuromuscular junction causing local paralysis of muscle. In published randomized placebo controlled trials no statistically significant weight loss has been shown[84,85]. When fundal injections were also applied, significantly greater short-term weight loss, reduction in BMI and prolongation of gastric emptying was achieved compared with controls[86]. A recent meta-analysis by Bang *et al*[87] analysed a total of 115 patients in 8 studies. Wide area injection including the fundus or body rather than the antrum only and multiple injections (> 10) were associated with weight loss. The safety and efficacy of this approach needs to be studied in cirrhosis.

There has been a lot of enthusiasm in gastric elec-

trical stimulation (GES) and devices innervating the stomach for bariatric applications. The exact mechanisms of GES are largely unknown, but causes delayed gastric emptying and increased satiety[88]. Recently in January 2015 the Maestro Rechargeable System, was approved[89]. These devices are generally implanted through open or laparoscopic means, but electrical stimulation systems deployed endoluminally has shown to be feasible and safe[90,91].

CONCLUSION

There has been a worldwide rise in patients having obesity associated with cirrhosis. Obesity with cirrhosis is a double trouble leading to early decompensation and also making liver transplantation difficult. Weight reduction is generally more difficult in this group of patients. Lifestyle changes should include a diet of around 30 kcal/kg and walk of greater than 5000 steps/d but optimal safe exercise regimen is unknown.

Among the current FDA approved anti-obesity drugs (orlistat, phenteramine/topiramate-ER, lorcaserin, naltrexone-bupropion ER and liraglutide) none are well studied in patients with cirrhosis but lorcaserin and liraglutide have similar pharmacokinetics in patients with mild hepatic impairment and are not contraindicated.

Bariatric surgery can be relatively safely performed in compensated cirrhosis patients with a slightly higher but acceptable complication rate.

Role of endoscopic intervention for management of obesity in cirrhosis especially intragastric balloon placement is evolving but promising and seems feasible even in those with decompensated cirrhosis.

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MINIREVIEWS

Update on hepatitis C: Direct-acting antivirals

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Abstract

Hepatitis C virus (HCV) was discovered 26 years ago. For decades, interferon-based therapy has been the mainstay of treatment for HCV. Recently, several direct-acting antivirals (DAAs) have been approved for treatment of HCV-infected patients and to help combat the virus. These drugs have revolutionized the management of HCV as all-oral regimens with favorable side effect profiles and superior rates of sustained virological response. Emerging real-world data are demonstrating results comparable to registration trials for DAA agents. Suddenly, the potential for eradicating HCV is on the horizon.

Key words: Hepatitis C virus; Direct-acting antivirals; Sustained virologic response; Management; Treatment

Core tip: Recently, several direct-acting antivirals (DAAs) have been approved for treatment of hepatitis C virus (HCV)-infected patients and to help combat the virus. These drugs have revolutionized the management of HCV as all-oral regimens with favorable side effect profiles and superior rates of sustained virological response. Emerging real-world data are demonstrating results comparable to registration trials for DAA agents. Suddenly, the potential for eradicating HCV is on the horizon.

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INTRODUCTION

Hepatitis C virus (HCV) was discovered 26 years ago in 1989, previously the HCV-related clinical entity was referred to as non-A, non-B hepatitis[1]. Currently, HCV has created a major health burden, with approximately 180 million infected people worldwide, representing about 2%-3% of the world's population[2]. This single-stranded, positive-sense 9.6 kb RNA-virus is globally

prevalent, showing geographic variation in its genotypic distribution and represents a major cause of end-stage liver disease[3,4]. About 4 out of 5 patients acutely infected with HCV develop a chronic hepatitis while only 20% of patients demonstrate spontaneous recovery with eradication of HCV[5]. Chronic hepatitis C (CHC) is a leading cause of cirrhosis and is complicated by development of hepatocellular carcinoma in 1%-4% of cirrhotic patients[6,7].

Until recently, interferon (IFN)-based therapies represented the mainstay of treatment for HCV infection. Modifications of the treatment-regimens including pegylation of IFN and the addition of ribavirin (RBV) resulted in suboptimal improvement sustained virologic response (SVR) and an unfavourable adverse effects profile. Based on the HCV genotype (GT) and the treatment-experience, only 40% to 70% of patients achieved SVR, with poorer outcomes among people infected with the more prevalent GT1[8]. The approval of the first-generation direct acting antiviral (DAA) agents, telaprevir (TLV) and boceprevir (BCV), in 2011 provided improvement in SVR for the targeted HCV GT1[9]. Unfortunately, TLV and BCV therapy was complicated by cumbersome schema of drug intake and the broad range of adverse events.

With the release of sofosbuvir in 2013 and 2014 in most Western countries, a new era in the treatment of CHC began. An all-oral, IFN-free antiviral treatment for CHC with DAA agents became available for the first time. In addition to sofosbuvir, approvals of other second-generation DAA agents, which target different proteins of HCV have improved the efficacy of antiviral therapy with better tolerance. The superior SVR rates from several phase Ⅲ trials have recently been confirmed by a number of real-life experience reports. We review various DAA-based antiviral regimens for HCV-infected patients.

MOLECULAR STRUCTURE OF HCV - TARGET SITES FOR DAA AGENTS

HCV is a member of the Flaviviridae virus family[10-12]. Its RNA is single-stranded and positive-sensed with a size of approximately 9.6 kb. The precursor-polyprotein is post-translationally processed and modified by a cooperation of cellular and viral proteases[13,14]. Bench molecular biology research on HCV has led to a better understanding of its replication cycle and has been instrumental in the discovery and development of molecules blocking viral proteins, specifically the DAAs[15-17]. The HCV-genome encodes for 9 proteins - 2 are structural (E1 and E2) and 7 non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B)[10,11,13,14]. These proteins provide targets for the DAAs, being mostly essential in the virus cycle of replication[10-14]. NS5B is a polymerase and a prime target for antiviral agents[18,19]. Antiviral agents are classified as inhibitors of nucleoside-type and non-nucleoside type. The active site of NS5B is highly conserved compared to other parts of the HCV-genome[18,19]. Currently, sofosbuvir is the only clinically available NS5B-inhibitor (nucleoside-type) with pan-genotypic antiviral activity and higher barrier to resistance compared to other DAAs[18,19]. It is a pro-drug and currently represents the backbone of most treatment-regimens[20,21]. Inhibition of the NS3/4A protease-complex is another potential target for DAAs. The first-generation DAAs, TLV and BCV were inhibitors of NS3/4A, and referred to as protease-inhibitors. Currently, two NS3/4A-inhibitors are approved in the United States and the European Union - paritaprevir, which is approved for the treatment of HCV GT1 in combination with ombitasvir and dasabuvir; and simeprevir, which is approved in combination with sofosbuvir for GT1 patients. DAAs targeting NS5A have also been approved[22-25]. Currently, three different NS5A-Inhibitors are approved in the United States and/or the European Union - daclatasvir, which is given in combination with sofosbuvir ± RBV for the treatment of the GTs 1-4; ombitasvir (ABT-267), which is approved for the treatment of GT1 in combination with paritaprevir and dasabuvir; and ledipasvir, which is approved for GT1, 3 and 4 in combination with sofosbuvir ± RBV.

DAA AGENTS - REGIMEN BASED ON HCV GT

With the approval of sofosbuvir in December of 2013 in North America (United States and Canada) and in January 2014 in Europe, an all-oral antiviral treatment for CHC with DAAs was available for the first time. In 2014, several studies analyzing the efficacy and the impact of the DAA-based therapies have been published. The response rates have been reproduced in real-life experiences (TRIO and TARGET 2.0) as well[26-29].

HCV GT1 is the most common GT with an overall prevalence of 46.2%. In particular, GT1 is more prevalent in the Western countries of North America and Western Europe (75.8% and 59.0% respectively). Accordingly, most studies have focused on the treatment of GT1. Patients with GT2 and GT3 are less prevalent worldwide (GT2 9.1% and GT3 30.1%) with a noticeable variation in distribution within Western countries - North America (GT2 12.0% and GT3 10.4%) and Western Europe (GT2 10.8% and GT3 24.8%). Patient with GT 4, 5 and 6 demonstrate the lowest prevalence (GT4 8.3%, GT5 0.8%, and GT6 5.4%) worldwide, with highest prevalence in low-income countries, and limited data on experience with second-generation DAA agents[4].

In phase-3 SAPPHIRE-Ⅰ clinical trial the combination of ritonavir-boosted ABT-450/r (protease inhibitor)-ombitasvir (NS5A inhibitor), and dasabuvir (non-nucleoside NS5B) with RBV were studied in treatment-naïve, non-cirrhotic HCV-infected non-cirrhotic patients with GT1. RBV was added according to body weight (≥ 75 kg 1200 mg/d or < 75 kg 1000 mg/d). Overall, 96.2% of patients achieved SVR (GT1b 98.0% and GT1a 95.3%). A higher stage of fibrosis and obesity were the negative predictive factors with SVR-12 rates still > 90% and thus satisfactory[30]. Treatment-experienced patients were studied in the SAPPHIRE-Ⅱ clinical trial.

Table 1 Direct-acting antiviral-based regimens for treatment-naïve hepatitis C virus-infected patients

Genotype	Recommended regimens options
GT1a	SOF/LDV × 12 wk
	PrOD + RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis)
	SOF + SMV ± RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis without Q80K variant)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis ± RBV)
GT1b	SOF/LDV × 12 wk
	PrOD + RBV × 12 wk
	SOF + SMV ± RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis ± RBV)
GT2	SOF + RBV × 12 wk (no cirrhosis) - 16 wk (cirrhosis)
	SOF + DCV × 12 wk (RBV intolerant)
GT3	SOF + PegIFN + RBV × 12 wk (PegIFN eligible)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis ± RBV)
	SOF + RBV × 24 wk (PegIFN ineligible)
GT4	SOF/LDV × 12 wk
	PrO + RBV × 12 wk
	SOF + RBV × 24 wk
	SOF + PegIFN + RBV × 12 wk
	SOF + SMV ± RBV × 12 wk
GT5	SOF/LDV × 12 wk
	SOF + PegIFN + RBV × 12 wk
GT6	SOF/LDV × 12 wk
	SOF + PegIFN + RBV × 12 wk

GT: Genotype; SOF: Sofosbuvir; LDV: Ledipasvir; PrOD: Paritaprevir + ritonavir + ombitasvir + dasabuvir; RBV: Ribavirin; SMV: Simeprevir; DCV: Daclatasvir; PegIFN: Pegylated interferon.

Table 2 Direct-acting antiviral-based regimens for treatment-experienced hepatitis C virus-infected patients

Genotype	Recommended regimens options
GT1a	SOF/LDV[1] × 12 wk (no cirrhosis) - 24 wk (cirrhosis[2])
	PrOD + RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis)
	SOF + SMV ± RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis without Q80K variant)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis ± RBV)
GT1b	SOF/LDV[1] × 12 wk (no cirrhosis) - 24 wk (cirrhosis)
	PrOD + RBV × 12 wk
	SOF + SMV ± RBV × 12 wk (no cirrhosis) - 24 wk (cirrhosis)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis and ± RBV)
GT2	SOF + RBV × 16-24 wk
	SOF + PegIFN + RBV × 12 wk
GT3	SOF + PegIFN + RBV × 12 wk (PegIFN eligible)
	SOF + DCV × 12 wk (no cirrhosis) - 24 wk (cirrhosis ± RBV)
GT4	SOF/LDV × 12 wk
	PrO + RBV × 12 wk
	SOF + RBV × 24 wk
	SOF + PegIFN + RBV × 12 wk
	SOF + SMV ± RBV × 12 wk
GT5	SOF/LDV × 12 wk
	SOF + PegIFN + RBV × 12 wk
GT6	SOF/LDV × 12 wk
	SOF + PegIFN + RBV × 12 wk

[1]Add RBV if previously treated with SOF + RBV or SOF + PegIFN + RBV; [2]Alternative option SOF/LDV + RBV × 12 wk. GT: Genotype; SOF: Sofosbuvir; LDV: Ledipasvir; PrOD: Paritaprevir + ritonavir + ombitasvir + dasabuvir; RBV: Ribavirin; SMV: Simeprevir; DCV: Daclatasvir; PegIFN: Pegylated interferon.

Again, a high grade of fibrosis and obesity were negative predictive factors, with an overall SVR-12 of 96.3% (GT1a 96% and GT1b 96.7%)[31].

The ION clinical trials (ION-Ⅰ, ION-Ⅱ and ION-Ⅲ) examined the efficacy of sofosbuvir and ledipasvir co-formulation with and without RBV for 12 to 24 wk in treatment-naïve (16% with cirrhosis) HCV-infected GT1 patients[31]. SVR-12 was 97%-99% in ION-Ⅰ clinical trial. There was no statistically significant difference between the duration of the treatment (12 wk *vs* 24 wk), HCV sub-GT (GT1a *vs* GT1b) or RBV use. Even the presence of cirrhosis did not impact the SVR[32,33]. Treatment-experienced HCV-infected GT1. Patients were treated with sofosbuvir and ledipasvir co-formulation ± RBV for 12 or 24 wk in ION-Ⅱ clinical trial. In these patients, addition of RBV did not impact the SVR. Previously treated patients with cirrhosis were the only sub-group that demonstrated a higher SVR with 24 wk of therapy. Therefore, 24 wk of treatment was recommended for previously treated patients with cirrhosis[34]. In The ION-Ⅲ clinical trial, the possibility of shortening the treatment to 8 wk in previously untreated patients without cirrhosis was evaluated. A high number of patients reached SVR in all groups (93% to 95%) without a significant impact of the duration of the treatment or the addition of RBV in the 8-wk treatment[35]. Based on secondary analysis, patients with baseline HCV RNA level greater than 6 million international units per milliliter demonstrated a higher risk of relapse with 8 wk of therapy. Therefore, 8 wk of therapy is recommended for treatment-naïve, non-cirrhotic HCV-infected patients with pre-treatment HCV RNA viral load of less than 6 million international units per milliliter[35].

In the COSMOS trial, the SVR to sofosbuvir and simeprevir combination in previous non-responders with METAVIR scores between F0 and F2 was compared to previous non-responders and treatment-naive patients with METAVIR scores between F3 and F4. The SVR-12 rates were similar in both groups, showing 90% SVR-12 in patients with METAVIR scores F0-F2 and 94% SVR-12 in patients with METAVIR score F3-F4. Neither the duration of the treatment (12 wk *vs* 24 wk) nor the addition of RBV seemed to influence the SVR[36].

The combination of sofosbuvir and daclatasvir DCV has been safe and effective, both, in previously treated and untreated HCV-patients with GT1[37,38]. In previously untreated HCV-infected GT1 patients, a SVR-12 of 98% was achieved with no significant impact of the duration of the treatment (12 wk *vs* 24 wk) or the addition of RBV[37]. In previously treated patients, 24 wk of treatment with sofosbuvir and daclatasvir demonstrated a SVR-12 of 97.5% with no influence from RBV addition[37].

Please refer to Tables 1 (treatment-naïve) and 2 (treatment-experienced) for treatment recommendation by HCV GT with DAA agents[38-44].

CONCLUSION

The current developments in the treatment of CHC are extraordinary. A significant improvement in efficacy

provided by the DAA agents has been long awaited. In addition to higher efficacy, DAA agents are tolerable with favorable adverse effects profile. Improved efficacy combined with easy tolerability is welcome news for a wide spectrum of patients who were not able to pursue interferon-based antiviral therapy for CHC. Impediments to DAA-based therapy include the high cost of therapy. Efforts are underway to make DAA agents affordable in Asia and Africa. Other issues include a cumbersome insurance authorization process in the United States. Importance of screening patients with risk factors for CHC and linkage to care remains a global issue. It is important to educate the patients that HCV treatment with DAA agents does not confer immunity and exposure to risk factors can lead to re-infection.

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MINIREVIEWS

Contributions of transgenic mouse studies on the research of hepatitis B virus and hepatitis C virus-induced hepatocarcinogenesis

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Abstract

Transgenic mouse technology has enabled the investigation of the pathogenic effects, including those on development, immunological reactions and carcinogenesis, of viral genes directly in living organism in a real-time manner. Although viral hepatocarcinogenesis comprises multiple sequences of pathological events, that is, chronic necroinflammation and the subsequent regeneration of hepatocytes that induces the accumulation of genetic alterations and hepatocellular carcinoma (HCC), the direct action of viral proteins also play significant roles. The pathogenesis of hepatitis B virus X and hepatitis C virus (HCV) core genes has been extensively studied by virtue of their functions as a transactivator and a steatosis inducer, respectively. In particular, the mechanism of steatosis in HCV infection and its possible association with HCC has been well studied using HCV core gene transgenic mouse models. Although transgenic mouse models have remarkable advantages, they are intrinsically accompanied by some drawbacks when used to study human diseases. Therefore, the results obtained from transgenic mouse studies should be carefully interpreted in the context of whether or not they are well associated with human pathogenesis.

Key words: Transgenic mouse; Hepatocarcinogenesis; Hepatitis C virus; Hepatitis B virus X; Hepatitis B virus; Hepatitis C virus core protein; Steatosis

Core tip: Transgenic technology offers researchers several advantages over *in vitro* experiments, including the ability to trace the pathogenic effects of viral genes in living organisms. Transgenic mouse studies have provided evidence that the direct action of viral genes, especially the genes encoding hepatitis B virus

X and hepatitis C virus core proteins, is involved in hepatocarcinogenesis. However, such results should be considered carefully as transgenic mouse experiments have intrinsic advantages and drawbacks. As such, the results including phenotypes and molecular mechanisms from transgenic mouse studies must always be verified by comparing them to those of human studies for evidence of an association.

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INTRODUCTION

Primary liver cancer has a high mortality rate worldwide and ranks 5th as the most common cancer among men and 7th among women; as such, therapeutic options to cure this disease are urgently needed[1,2]. Hepatocellular carcinoma (HCC) accounts for the majority of primary liver cancers. The most prevalent etiological agents of HCC are hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Once HBV- or HCV-related cirrhosis is established, HCC develops with an annual rate of approximately 4.3% or 7.1%, respectively in Japan, and 2.2% or 3.7%, respectively, in Western countries[3,4].

Individual viral proteins may play significant roles in and confer characteristics that are peculiar to the viral pathogenesis of HBV or HCV. Transgenic mouse strategies are applied to explore the functions of viral genes *in vivo* and can provide significant information on the mechanisms of viral pathogenesis by allowing the elucidation of the effects of individual viral proteins[5-7]. In this review, we summarize the past contributions of transgenic mouse studies to HBV- and HCV-induced viral pathogenesis with a particular focus on the evaluation of how well the results of these transgenic mouse studies correlate with human pathogenesis and how useful they are in the development of therapeutic strategies for viral disease, especially hepatocarcinogenesis.

MECHANISMS OF HEPATOCARCINOGENESIS DUE TO HBV AND HCV

When considering the contribution of transgenic mouse studies to the research on hepatocarcinogenesis caused by HBV or HCV, it is important to outline the mechanisms of the disease and consider for which mechanisms transgenic mouse studies can be applied to provide pathogenic and therapeutic contributions.

First, the common mechanism between HBV and HCV is as follows: Once these viruses infect liver, they skillfully evade host immune surveillance and induce chronic necroinflammation. These injuries cause fibrosis and result in liver cirrhosis. Hepatocytes, using their intrinsic regenerative capability, continue to proliferate in order to compensate for the necrotic tissues. Genetic alterations continuously accumulate during these processes, resulting in the formation of a pathogenic state such as cirrhosis from which HCC frequently arises.

Second, viral genes may be involved in hepatocarcinogenesis by directly affecting cellular machineries. The most representative genes of this type that have drawn clinical attention are the genes for HBV X (HBx) and HCV core protein. HBx is multifunctional and may induce the transactivation of many cellular genes[8]. On the other hand, the HCV core protein causes steatosis in the liver and subsequent HCC[9]. Transgenic mouse studies can shed light on the mechanisms of HBx and HCV core protein by enabling assays on the direct actions of these viral genes *in vivo*.

Third, a mechanism specific to HBV is its integration into the cellular DNA of the host; this may increase the genomic instability and cis-activation of the adjacent cellular genome that may possibly be involved in the regulation of the cell cycle[10]. Importantly, most integrated viral DNA retain the sequences encoding HBxAg, and the HBxAg expressed from the integrated HBV DNA further promotes genetic instability of the host by a variety of mechanisms[10].

ADVANTAGES AND LIMITATIONS OF RESEARCH USING TRANSGENIC MICE

Advantages

The mechanism of viral hepatocarcinogenesis comprises a number of complex factors[11]. Although the majority of research in this field has been performed using human samples, human resources have limitations in both ethical and quantitative terms, and it is sometimes difficult to extract significant conclusions from the final results that are dependent of a combination of vastly complex molecular events. The cell lines used for *in vitro* experiments are mainly derived from HCC cells in which carcinogenic events have already finished and little information on the real-time carcinogenic process can be obtained. Transgenic mouse research can compensate for these drawbacks.

Limitations

Transgenic technology offers the researchers several advantages over *in vitro* experiments[12,13]: It enables the investigation of the pathogenic effects, including those on development, immunological reactions, and carcinogenesis, of viral genes directly in living organisms in a real-time manner. Viral genes can be designed to be placed under their own or another appropriate promoter for specific expression in permissive cell types or tissues[14,15]. Thus, it is possible to identify particular cell types or organs that allow the expression of viral

proteins. In addition, it allows a sufficient number or amount of experimental material to be obtained at any specific condition, enabling the analysis of comprehensive and objective data.

Unfortunately, there are also several drawbacks to transgenic mouse research. First, because viral proteins of HBV or HCV, which normally only infect human or chimpanzee, are forced to be expressed in mouse tissue, the resulting protein-protein interactions may not be the same as those that would occur in the natural hosts and unexpected molecular reactions may result. Second, expressed viral proteins may become immune-tolerant in mice and cannot induce immunological response[16], and as eliciting an immune response is the main mechanism of viral liver pathogenesis, it becomes difficult to completely simulate this human disease; however, this may actually be an advantage as it may allow the evaluation of the direct role of viral proteins on liver pathogenesis without local inflammation[6]. Third, because transgenes are randomly integrated into the genome, the expression of transgene can be affected by the adjacent genomic structures. Phenotypes should be confirmed by the results obtained in mouse lines established using several founder mice independently. It is extremely difficult to establish viral replication in mice and cell culture systems are more suitable for analyzing viral replication, including the identification of viral receptors. Thus, because transgenic mouse models for viral diseases are not multipotent and can produce results that are easily hampered by experimental artifact, the obtained results should always be strictly scrutinized and evaluated in both scientific and clinical aspects. Namely, confirmation as to whether the phenotypes really correlate with the pathogenesis of human liver diseases is needed.

HBV AND TRANSGENIC MOUSE

HBV is a DNA virus with a length of 3.2 kb that replicates *via* an RNA intermediate for viral replication. Similar to retroviruses, which undergo reverse transcription for replication, it is integrated into the host genome.

Hepatitis B surface antigen transgenic mouse: Immunopathology of HBV

Chisari's group has made vast contributions to the clinical field by performing transgenic mouse studies to investigate immune-mediated hepatitis and hepatocarcinogenesis[17,18]. In the 1980s, they produced a transgenic mouse model that overexpressed the large protein of the hepatitis B surface antigen (HBsAg). It induced severe, prolonged hepatocellular injury that was characterized by inflammation and regenerative hyperplasia, resulting in the development of HCC[19]. This was the first transgenic mouse model in which the development of HCC was observed from the function of a single viral protein. However, these results were considered to be the outcome of the storage effect of the large HBsAg, since overexpression of the HBV core, precore, X, small or middle envelope protein was not associated with any evidence of liver disease in the transgenic mouse model due to immunological tolerance for the inherently expressed viral proteins[16]. In order to better mimic the HBV pathogenesis seen in human where immunological reactions to viral proteins are essential, HBsAg-specific cytotoxic T cells were transferred to the mice to induce viral antigen-mediated acute hepatitis; this provided direct evidence that hepatocellular injury in HBV infection may be immunologically mediated[20,21]. Moreover, further induction of cytokines such as interferon (IFN)-γ after acute liver injury may magnify the degree of inflammation, resulting in fulminant hepatitis[22]. These transgenic mouse studies clarified the immunological aspects of HBV infection, showing that the balance between viral load and the strength of the immunological reactivity towards HBV antigen determines the fate of the disease[18]. As for the immune pathogenesis of HCC, the adoptive transfer of CD8 lymphocytes to HBsAg transgenic mice generated a pathogenesis that closely resembled that of human chronic viral hepatitis and finally resulted in the development of HCC[23]. This model strengthened the notion that only immune-mediated hepatocellular injury, and not insertional mutagenesis from the integration of the HBV genome or the expression of the *HBx* gene, could cause hepatocarcinogenesis.

In the early era of transgenic mouse studies, several reports showed a high level of HBV replication *in vivo*, injecting a duplicated HBV plasmid[24-26]. These results demonstrated that the HBV genome integrated into the mouse chromosome acted as a template for viral gene expression, allowing viral replication. Although these mice did not show the HCC phenotype, they contributed to the detailed studies of the replication and expression of HBV and to pathological studies of hepatitis. In addition, HBs large envelope protein expression in transgenic mouse showed the inhibition of HBsAg secretion, suggesting an inhibitory effect of the pre-S-containing domain of the large envelope peptide[27].

HBxAg transgenic mouse: Direct hepatocarcinogenic role of HBV

The *HBx* gene is a known oncogene with pleiotropic functions that transactivate multiple cellular genes; it has attracted extensive clinical attention and has been regarded as a suitable target for transgenic mouse research[8,28,29]. Kim *et al*[30] first observed the occurrence of HCC in HBx transgenic mouse. Koike *et al*[31,32] further showed that HBx induced hepatocyte proliferation and contributed to hepatocarcinogenesis. Inspired by these studies, many researchers have attempted to produce the HCC phenotype in HBx transgenic mice, but most of them failed[33-36]. These inconsistent observations in terms of HCC development among transgenic mice might be partly due to differences in the sequences[37] or subtypes[38] of HBV or the genetic background of the mice used. However, strong and continuous expression of HBx might be a requirement for observing the HCC

phenotype[38,39]. Nonetheless, it remains unclear whether this high level of HBx expression is physiologically relevant to human pathogenesis[8]. Moreover, there is still a lack of clinical and molecular evaluation methods that can be used to measure how much the direct carcinogenetic function of HBx contributes to human HBV hepatocarcinogenesis where necrosis-regeneration sequence of hepatocytes by immunological reactions to HBV is generally considered to be an essential mechanism. How a single HBx gene is involved in this huge complex pathogenesis process remains to be clarified.

It is has been reported that fibrosis levels of liver complicated with HBV-HCC is milder than that of HCV-HCC[40-42]. In our cohort of 57 patients with HBV-HCC who were treated surgically, 25 (44%) did not have cirrhosis, and 22 (39%) had a mild level of fibrosis (F1 or 2) (Unpublished results). Therefore, the direct carcinogenic role of HBx or the dysregulation of the cell cycle due to insertional mutagenesis by the HBV genome might play a large role, especially in those who have a mild level of fibrosis, than cirrhosis, which is the final state of necrosis-regeneration sequence. In addition, our past studies have shown that the HBx transgenic mouse is a good model for testing the anti-hepatocarcinogenic function of IFN-β[29,43].

HCV AND TRANSGENIC MOUSE

HCV and steatosis: Close association with human pathogenesis

While HBx transgenic mouse research has been confronted by difficulties in correlating the research results with specific clinical or molecular landmarks, HCV transgenic mice have provided fruitful experimental observations in terms of steatosis[6,44] which is commonly observed in both HCV-infected humans and HCV-transgenic mice.

Steatosis has been reported to be a characteristic finding of chronic HCV infection[45-47]. Moriya *et al*[9] observed steatosis in HCV core gene transgenic mice at as early as 3 mo of age and found that about a quarter of the mice developed HCC in their late life, demonstrating that the HCV core protein itself has a direct role in hepatocarcinogenesis by virtue of steatosis[9]. Lerat *et al*[48] also observed hepatic steatosis and HCC in transgenic mouse models that express complete viral and structural proteins without immunological reactions.

It is well known that HCV genotype 3 directly induce steatosis in liver[44,49], supporting the observations obtained in transgenic mouse. Importantly, an association between steatosis and fibrosis has also been demonstrated in a meta-analysis of chronically infected patients with HCV[50], and hepatic steatosis is a risk factor for HCC in chronic hepatitis C patients[51,52]. These results indicate that the findings obtained from transgenic research are well associated with findings from human research.

HCV core protein and PRARα

In addition, HCV core gene transgenic mice became the base for further studies which explored the mechanisms of steatosis and its relationship with HCC development. Tanaka *et al*[53] generated peroxisome proliferator-activated receptor alpha (PRARα-homozygous, -heterozygous, and -null mice with HCV core protein expression and showed that severe steatosis developed in mice that had both PRARα alleles, revealing that the expression of PRARα, which is important in maintaining triglyceride homeostasis, was essential for the development of HCV core protein-induced steatosis and HCC[53]. Moriishi *et al*[54] showed that a knockout of the proteasome activator 28 gamma (*PA28γ*) gene induces the accumulation of HCV core protein in the nucleus of hepatocytes of HCV core gene transgenic mice and disrupts the development of both hepatic steatosis and HCC, thus revealing that PA28γ plays a crucial role in the development of HCV-induced liver pathogenesis[54].

HCV core protein and reactive oxygen species

Transgenic mouse studies have further provided significant findings on the mechanisms of the progression from steatosis to hepatocarcinogenesis. Okuda *et al*[55], reported that HCV core protein increases the production of reactive oxygen species (ROS) *via* a direct effect on the mitochondrial electron transport system. Korenaga *et al*[56] reported that reduced activity of electron transport complex 1 enhances the production of ROS in HCV core gene transgenic mouse. Consistent with these observations, both mitochondrial dysfunction[57] and high levels of oxidative stress have been demonstrated in HCV-infected patients[58,59]. 8-Hydroxy-2′-deoxy-guanosine which is generated by ROS and leads to an increased frequency of mutations, accumulates in HCV core gene transgenic mouse[60] and causes mutations in cellular genes[61]. In addition to its direct effect on mitochondria, HCV core protein has been shown to cause endoplasmic reticulum stress that results in an oxidized redox state in hepatocytes, interfering with immune responses and potentiating fibrosis and carcinogenesis[62]. Moreover, Klopstock *et al*[63] used HCV transgenic mice that were crossed with Mdr2-knockout mice to demonstrate that the HCV transgene accelerates inflammation-associated hepatocarcinogenesis, which has a pathogenesis similar to that of human HCV-induced carcinogenesis[63].

It was also reported in a transgenic mouse study that the production of ROS induces high levels of iron deposition in liver, resulting in an increased risk of HCC[64]. A strong correlation between hepatic DNA damage and iron overload has been confirmed in a human study[65]. Mitochondrial ROS may be linked to metabolic disorders such as insulin resistance, hepatic steatosis, and hepatic iron accumulation, all of which are characteristic features of chronic HCV infection[66].

Direct role of HCV core protein in hepatocarcinogenesis

The mainstream mechanism of HCV-induced hepato-

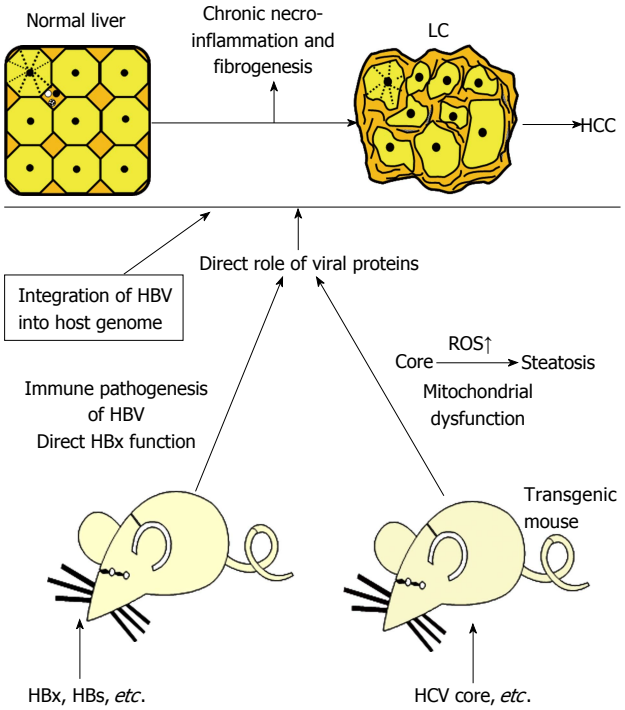

Figure 1 Outline of the overall aspects of this review. The mainstream mechanism of HBV- or HCV-induced hepatocarcinogenesis is necroinflammation and hepatocyte-regeneration sequences that eventually result in cirrhosis where HCC frequently develops. However, transgenic mouse studies have clarified the significant contribution of viral proteins to hepatocarcinogenesis by directly affecting cellular machinery. A close association of HCV core protein and hepatic steatosis has been established by transgenic mouse studies. In addition to the inherent insertional mutagenesis mechanism of HBV, results from transgenic mouse studies have also suggested the direct involvement of HBV proteins in hepatocarcinogenesis. HBV: Hepatitis B virus; HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; ROS: Reactive oxygen species; HBs: Hepatitis B surface; HBx: Hepatitis B virus X.

carcinogenesis is persistent necroinflammation that induces the irregular regeneration of hepatocytes, allowing the accumulation of genetic or epigenetic alterations. In addition to this, transgenic mouse studies have shown that the HCV core protein plays a significant role in hepatocarcinogenesis by inducing steatosis *via* the production of ROS from by the dysregulation of lipid metabolism or functional abnormalities of the mitochondria[5]. Thus, transgenic mouse studies have shown that viral proteins, especially the HCV core protein, directly interact with lipid-metabolizing pathways and contribute to HCC development; these are the major achievements of transgenic mouse studies on HCV-induced carcinogenesis.

CONCLUSION

The mechanisms of hepatocarcinogenesis common between HBV and HCV include persistent necroinflammation and the regeneration of hepatocytes that allows the accumulation of genetic changes. However, there are yet no reports on common genetic changes that can fully explain these complex pathways; rather, multiple dysfunctions resulting from abnormalities in a number of signal transduction pathways appear to converge to produce the common HCC phenotype. However, the use of transgenic mouse technology has clarified that even a single viral gene, such as the gene for HBx or HCV core protein can directly affect the cellular machinery and impact the mainstream mechanism of persistent necroinflammation-induced hepatocarcinogenesis (Figure 1). Especially for HCV, a good correlation has been found between the experimental findings from transgenic mouse studies and clinical observations. Thus, transgenic mouse models may provide an efficient method for evaluating the effectiveness of anti-hepatocarcinogenesis agents in the future.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Comparison of peg-interferon, ribavirin plus telaprevir *vs* simeprevir by propensity score matching

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Abstract

AIM: To compare efficacy of telaprevir (TVR) and simeprevir (SMV) combined with pegylated interferon (PEG-IFN) and ribavirin (RBV) while treating chronic hepatitis C (CHC).

METHODS: In all, 306 CHC patients were included in this study. There were 159 patients in the TVR combination therapy group and 147 patients in the SMV combination therapy group. To evaluate pretreatment factors contributing to sustained virological response at 12 wk (SVR12), univariate and multivariate analyses were performed in TVR and SMV groups. To adjust for patient background between TVR and SMV groups, propensity score matching was performed. Virological response during treatment and SVR12 were evaluated.

RESULTS: Overall rates of SVR12 [undetectable serum hepatitis C virus (HCV) RNA levels] were 79.2% and 69.4% in TVR and SMV groups, respectively. Patients in the SMV group were older, had higher serum HCV RNA levels, lower hemoglobin, higher prevalence of unfavorable interleukin-28B (*IL28B*) genotype (rs8099917), and poorer response to previous PEG-IFN and RBV treatment. Propensity score matching was performed to adjust for backgrounds (n = 104) and demonstrated SVR12 rates of 74.0% and 73.1% in the TVR and SMV groups, respectively. In the TVR group, discontinuation rates were higher because of adverse events; however, breakthrough and nonresponse was more frequent in the in SMV group. Multivariate analysis revealed *IL28B* genotype (rs8099917) as the only independent predictive factor of SVR12 in both groups.

CONCLUSION: SVR12 rates were almost identical following propensity score matching.

Key words: Chronic hepatitis C; Combination therapy; Pegylated interferon; Simeprevir; Telaprevir; Propensity score matching; Protease inhibitor

Core tip: We evaluated and compared the efficacy of telaprevir (TVR) and simeprevir (SMV) in combination with pegylated interferon and ribavirin in the treatment of chronic hepatitis C. patients in real-world clinical settings. In the TVR group, the proportion of patients achieving a virological response was higher than that in the SMV group according to the original data. After propensity score matching, the proportion of patients achieving a virological response during treatment and after 12 wk was almost identical between the two groups with no significant difference observed.

Fujii H, Nishimura T, Umemura A, Nishikawa T, Yamaguchi K, Moriguchi M, Sumida Y, Mitsuyoshi H, Yokomizo C, Tanaka S, Ishikawa H, Nishioji K, Kimura H, Takami S, Nagao Y, Takeuchi T, Shima T, Sawa Y, Minami M, Yasui K, Itoh Y. Comparison of peg-interferon, ribavirin plus telaprevir *vs* simeprevir by propensity score matching. *World J Hepatol* 2015; 7(28): 2841-2848 Available from: URL: http://www.wjgnet.com/1948-5182/full/v7/i28/2841.htm DOI: http://dx.doi.org/10.4254/wjh.v7.i28.2841

INTRODUCTION

Chronic hepatitis C (CHC) infection is associated with a greatly increased risk of liver cirrhosis and hepatocellular carcinoma. There are an estimated 130-170 million people infected with hepatitis C virus (HCV) worldwide[1] and approximately 1.5-2 million in Japan[2]. The combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) dual therapy was previously the standard care for CHC, and was administered for 48-72 wk in patients with genotype 1 and for 24 wk in genotype 2. Sustained virological response (SVR) rates are approximately 40%-50% in former group treated for 48 wk and approximately 80% in the latter treated for 24 wk[3-5].

Novel drug classes, including inhibitors of the NS3/NS4 protease of HCV polyprotein (protease inhibitors), have recently become available[6-8]. Of these, telaprevir (TVR) was the first to be approved in Japan for the treatment of CHC. In a clinical trial of TVR triple combination therapy (TVR, PEG-IFN, and RBV) for 24 wk in Japan, rapid reductions in serum HCV RNA levels were observed with a SVR rate of approximately 70%[9,10]. However, treatment discontinuation because of adverse events, including skin rash, anemia, and thrombocytopenia, occurred in up to 21% patients[11]. Thus, the TVR triple combination therapy is no longer recommended[12].

Simeprevir (SMV) is a second generation NS3/NS4 protease inhibitor[13]. The QUEST 1 and QUEST 2 phase 3 clinical trials demonstrated SVRs of 80% and 81% in patients treated with SMV triple combination therapy (SMV, PEG-IFN, and RBV), respectively. Similar results have been reported in phase 3 clinical trials conducted in Japan[14-16]. TVR and SMV were approved for use in clinical practice in Japan in December 2011 and December 2013, respectively. We previously treated patients with CHC using TVR or SMV as PEG-IFN and

RBV-based triple combination therapy with an NS3/NS4 protease inhibitor; however, "drug lag" between TVR and SMV, causing a difference in clinical backgrounds between the two regimens prior to treatment initiation, prevented fair comparison of the efficacy of TVR and SMV in real-world clinical practice. The aim of this study was to evaluate and compare the efficacy of TVR or SMV for the treatment of CHC patients in Japan.

MATERIALS AND METHODS

Patients

Patients were enrolled at Kyoto Prefectural University of Medicine and 8 affiliated hospitals in Kinki area of Japan (Kyoto, Osaka, Nara, Shiga Prefecture) from 2012 to 2014. Study protocols were approved by the ethics committee of each institution and conformed to the provisions of the Declaration of Helsinki. Patients enrolled in this study were diagnosed with CHC by board-certified hepatologists. Eligible patients were 20-80 years of age and had chronic HCV genotype 1 infection with HCV RNA levels of 5.0 log10 IU/mL or higher at screening.

Patients with decompensated liver disease, chronic hepatitis B, co-infection with human immunodeficiency virus, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, or Wilson's disease were excluded. Patients with uncontrollable hypertension or diabetes mellitus, and those with a history of alcohol abuse, were also excluded. Patients were followed-up monthly for the assessment of liver function and virological markers during treatment and until 12 wk after the completion of triple therapy. All patients gave informed consent to participate in this study.

In the TVR group, three patients were lost to follow-up and extreme protocol deviation (*e.g.,* extended PEG-IFN and RBV therapy for up to 48 wk) occurred in seven patients. In the SMV group, two patients were lost to follow-up and extreme protocol deviation (*e.g.,* extended PEG-IFN and RBV therapy for up to 48 wk) occurred in nine patients. Patients lost to follow-up or with extreme protocol deviation were excluded from the analysis.

The therapeutic outcomes of previous PEG-IFN and RBV therapy were classified into the following two groups: Undetectable serum HCV RNA levels at the end of the treatment period with quantifiable HCV RNA levels during follow-up (relapse group); and detectable HCV RNA levels at the end of the treatment period (other group).

Study design

Patients received per os telaprevir (Telavic; Mitsubishi Tanabe Pharma, Osaka, Japan) 2250 mg/d or simeprevir (Sovriad; Janssen Pharmaceutical K.K., Tokyo, Japan) 100 mg/d, combined with weekly subcutaneous injections of PEG-IFN alpha 2b (Peg-Intron; MSD, Tokyo, Japan) of 1.5 μg/kg and per os administration of RBV (Rebetol; MSD, Tokyo, Japan) of 600-1000 mg/d in accordance with prescribing information for 12 wk followed by PEG-IFN alpha 2b and RBV between weeks 12 and 24. In the TVR group, patients with lower serum hemoglobin levels began therapy at a reduced dose of TVR 1500 mg/d according to the judgment of treating physicians (2250 mg/d, 66 patients; 1500 mg/d, 93 patients). In the SMV group, patients began therapy at a dose of 100 mg/d. Dose reductions or discontinuation of TVR, SMV, PEG-IFN, and RBV were according to the judgment of treating physicians. Patients were followed-up for at least 12 wk after final treatment administration to assess SVR.

HCV RNA responses during therapy were classified into the following groups: Detectable HCV RNA levels at the end of the treatment period (nonresponse group); reappearance of HCV RNA during treatment (breakthrough group); and undetectable serum HCV RNA levels at the end of the treatment period with quantifiable HCV RNA levels during follow-up (relapse group). SVR12 was defined as undetectable serum HCV RNA levels at 12 wk after the end of treatment. Therapeutic effects were evaluated using intention-to-treat analysis.

Laboratory assessments

Blood samples were obtained for routine biochemical and hematological assessments at treatment initiation, on treatment weeks 2, 4, 8, 12, 16, 20, 24, at the end of treatment (EOT), and at 12 wk after EOT. The antiviral effects were assessed by measuring serum HCV RNA levels using the COBAS TaqMan HCV test (Roche Molecular Diagnostics, Tokyo, Japan) with a lower limit of quantitation of 15 IU/mL. Interleukin 28B (*IL28B*; rs8099917) genotyping was accordingly performed in the majority of patients. In brief, DNA was extracted from peripheral whole blood (100 μL) with DNeasy Blood and Tissue Kits (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Genotypes were determined using a Light Cycler (Roche, Osaka, Japan). Subsequent gene sequencing was performed to validate amplified polymerase chain reaction (PCR) products. Primers and probes used for PCR were as follows: Forward primer, 5'-CAACATGGAGAGTTAAAGTAAGTCTTG-3'; reverse primer, 5'-TGCTGGGCCCTAACTGAT-3'; probe 1, LC Red 640-TTGGGTGACATTGCTCACAGAAAGG-Phosphate; and probe 2, CCAGCTACCAAACTGTATACAGCATGGTTCCA-Fluorescein.

Statistical analysis

Baseline continuous data were expressed as median with interquartile ranges in parentheses, and categorical variables were expressed as numbers. Univariate analyses were performed using chi-squared or Mann-Whitney *U*-tests as appropriate. All *P*-values of < 0.05 of two-tailed tests were considered significant. Multivariate logistic regression was used to identify significant independent predictive factors of SVR12. Results were expressed as Odds ratios and 95%CI. All statistical analyses were performed using the SPSS 22.0 statistical package (SPSS Incorporated, Chicago, Illinois, United States).

To adjust for patient background between TVR and

Table 1 Baseline characteristics of patients who received triple therapy with pegylated interferon, ribavirin, and telaprevir or simeprevir

	Unmatched patients			Standardized	Propensity score matched patients			Standardized
	Telaprevir	Simeprevir	*P* value	difference	Telaprevir	Simeprevir	*P* value	difference
No. of patients	*n* = 159	*n* = 147			*n* = 104	*n* = 104		
Age (yr)	60 (51.0-65.0)	63 (54.5-70.0)	0.002	0.348	61.5 (53.0-65.8)	60.5 (52.0-67.0)	NS	0.0154
Gender (male/female)	77/82	67/80	NS	0.057	45/59	49/55	NS	0.0773
Body mass index (kg/m^2)	23.9 (21.7-25.7)	23.2 (21.1-25.0)	NS	0.202	23.6 (21.1-25.3)	23.4 (21.2-25.2)	NS	0.0747
Laboratory data								
Level of viremia (log IU/mL)	6.7 (6.3-7.0)	6.8 (6.3-7.2)	NS	0.210	6.7 (6.3-7.0)	6.6 (6.2-7.1)	NS	0.0158
Leukocyte count (/mm^3)	5060 (4200-5800)	4920 (4100-5800)	NS	0.094	5000 (4200-5700)	5020 (4150-5800)	NS	0.0272
Hemoglobin (g/dL)	14.1 (13.1-15.0)	13.8 (12.9-14.7)	NS	0.175	14 (13.0-14.8)	13.9 (12.9-15.0)	NS	0.0264
Platelet count (× 10^4/mm^3)	15 (12.5-19.8)	15.1 (11.7-20.1)	NS	0.053	15 (12.9-20.0)	15.1 (11.8-20.1)	NS	0.0032
SNP of IL28B (TT/non-TT/unknown)	99/34/26	89/43/15	NS	0.155	74/30	73/31	NS	0.0211
Other data								
Prior treatment response relapse/other	43/30	31/32	NS	0.196	31/22	23/20	NS	0.100

"Unmatched patients" refer to values prior to propensity score matching and "Propensity score matched patients" refer to values after adjustment by propensity score matching. Data are presented as numbers or medians with interquartile ranges in parentheses. *P*-values were calculated using the χ^2 or Mann-Whitney *U*-test for continuous variables. SNP: Single-nucleotide polymorphism; IL28: Interleukin 28B; NS: Not significant.

SMV groups, propensity score matching was performed. Propensity score models were estimated using a logistic regression model that adjusts for patient characteristics (age, gender, body mass index, HCV RNA level, leukocyte count, hemoglobin, platelet count, and *IL28* SNPs) listed in Table 1. Confounders were selected according to their potential association with the outcome on the basis of clinical knowledge and previous studies[17]. The propensity score matching model was validated by the Hosmer and Lemeshow goodness-of-fit test ($P = 0.638$) and by the value of the area under the curve (0.66, 95%CI: 0.594-0.724). One SMV patient was matched to one TVR patient using nearest neighbor matching without replacement. Propensity scores were matched using a caliper width 0.25 logit of the SD. The standardized difference was used to assess the covariate balance. McNemarr's tests were performed after matching.

RESULTS

Baseline characteristics

The baseline patient characteristics in the TVR group ($n = 159$) and SMV group ($n = 147$) are shown in Table 1 as "unmatched patients". Patients in the SMV group were significantly older than patients in the TVR group. High viral load, low hemoglobin levels, the non-TT *IL28B* genotype, and relapse following previous PEG-IFN and RBV treatment were more commonly observed in the SMV group compared with the TVR group.

Virological response to therapy and loss of HCV RNA during treatment

In the TVR group, the overall SVR12 was 79.2% (126 of 159 patients). Undetectable HCV RNA levels were achieved during treatment in 33.3% (41 of 123), 80.8% (118 of 146), 92.4% (146 of 158), and 91.2% (145 of 159) of patients at 2, 4, 8 wk, and EOT or 24 wk, respectively. In the SMV group, the overall SVR12 rate was 69.4% (102 of 147 patients). Undetectable HCV RNA levels were achieved during treatment in 23.8% (31 of 130), 69.4% (100 of 144), 89.3% (125 of 140), and 85.0% (125 of 147) of patients at 2, 4, 8 and EOT or 24 wk, respectively (Figure 1A).

Safety and tolerability

In the TVR group, 10 patients demonstrated nonresponse, and breakthrough occurred in 4 patients. Relapse occurred in 19 patients. In patients with nonresponse, 8 patients discontinued TVR because of adverse events within the first 4 wk of treatment (four skin rash, one renal dysfunction, two appetite loss, one unknown). In the SMV group, 15 patients demonstrated nonresponse, and breakthrough occurred in eight patients. Relapse occurred in 22 patients. In patients with nonresponse, one patient discontinued within the first 4 wk of treatment (transient visual field defect). There was a trend toward greater rates of treatment discontinuation because of adverse events in the TVR group and nonresponse and breakthrough in the SMV group.

Pretreatment factors contributing to SVR12 in TVR and SMV groups

To evaluate pretreatment factors contributing to SVR12, univariate and multivariate analyses were performed in TVR and SMV groups including the following variables: Age, gender, body mass index, *IL28B* (rs8099917) genotype, viral load, leukocyte count, hemoglobin, and platelet counts (Table 2). In the TVR group, *IL28B* genotypes significantly correlated with SVR12 according to univariate analysis. In multivariable logistic regression analysis, *IL28B* genotype was found to be a significant independent predictor of SVR12 (OR = 4.316; 95%CI: 1.804-10.327, $P = 0.001$). In the SMV group, age and *IL28B* genotype significantly correlated with SVR12 according to univariate analysis. In multivariable logistic regression analysis, significant independent predictors of SVR were *IL28B* genotype (OR = 8.598; 95%CI: 3.388-21.817; $P < 0.001$), age (OR = 0.933; 95%CI:

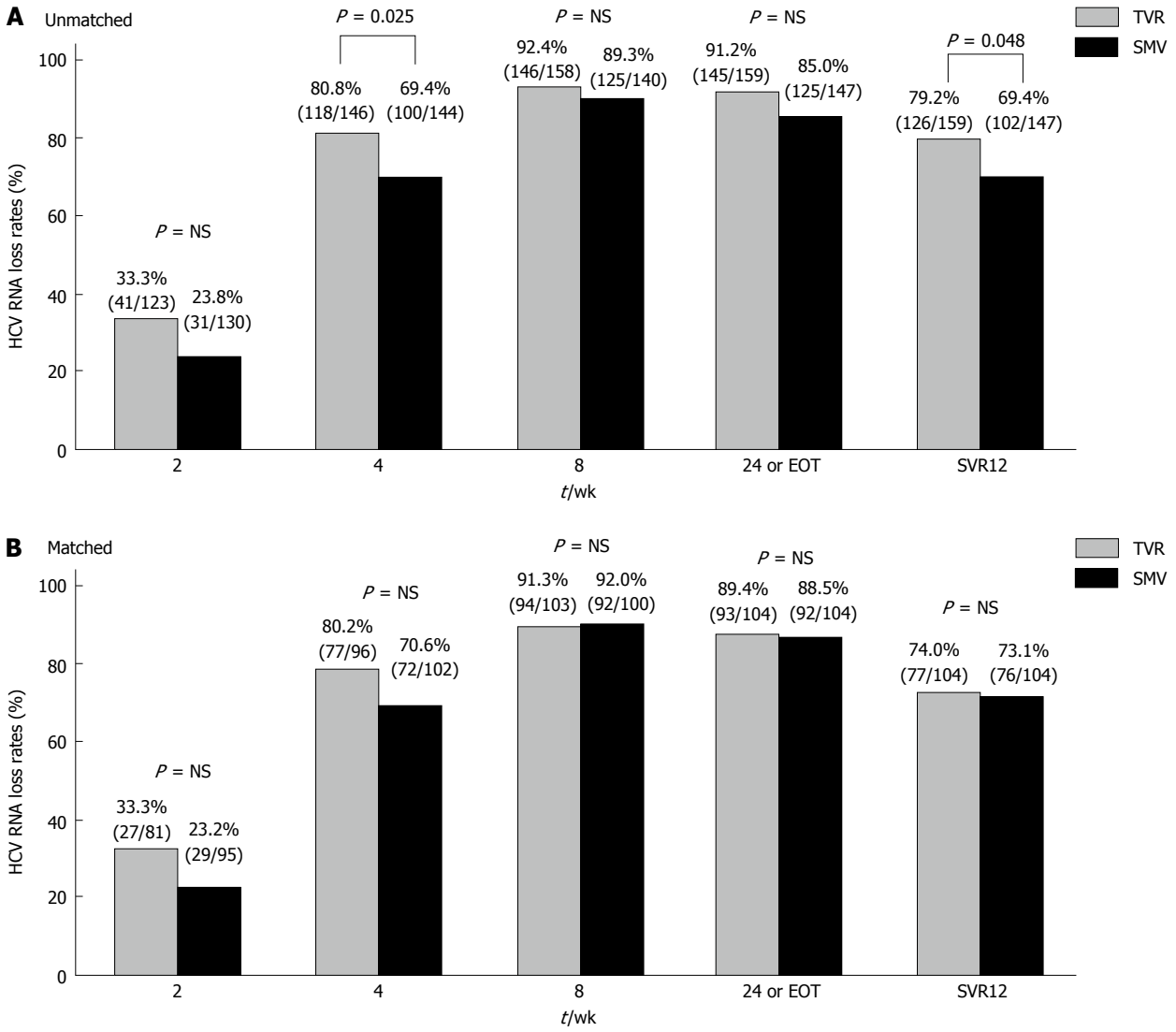

Figure 1 Rates of virological response to telaprevir and simeprevir according to serum hepatitis C virus RNA levels before and after adjustment by propensity score matching. Percentages represent the proportion of patients with undetectable serum hepatitis C virus (HCV) RNA levels. Patient numbers are shown in parentheses. *P*-values were calculated using the χ^2 test prior to matching and McNemarr's test after matching. A: Before adjustment. Rates of virological response at 4 and 12 wk after treatment were significantly different between the telaprevir (TVR) group and simeprevir (SMV) group; B: After adjustment. No significant difference in the virological response was observed between the two groups. NS: Not significant.

0.889-0.980; *P* = 0.006), and viral load (OR = 0.335; 95%CI: 0.157-0.715, *P* = 0.005). Propensity score matching analysis was subsequently performed to reduce bias caused by differing baseline patient characteristics between TVR and SMV groups (Tables 1 and 2, propensity score matched patients). Following one-to-one matching of the two groups according to propensity score, 104 patients from the TVR group and 104 patients from the SMV group were matched according to baseline characteristics (Tables 1 and 2). Majority of covariates were statistically similar between the two groups (Table 1, Propensity score matched patients). Multivariable logistic regression analysis demonstrated that *IL28B* genotype significantly associated with SVR12 in both groups (TVR: OR = 7.739; 95%CI: 2.111-28.375, *P* = 0.002; and SMV: OR = 8.594; 95%CI: 2.777-26.598, *P* < 0.001; Table 2).

Virological response during treatment and SVR12 after propensity score matching

Before adjustment, the proportion of patients achieving virological responses at 4 wk and after 12 wk treatment significantly differed between the TVR group and SMV group. In general, a greater proportion of patients in the TVR group had a virological response than that in the SMV group (Figure 1A). After one-to-one propensity score matching, the proportions of patients achieving a virological response during treatment and after 12 wk treatment were similar between the two groups (SVR12: TVR, 74.0%; SMV, 73.1%; Figure 1B).

DISCUSSION

In the present study, we evaluated and compared the efficacy of TVR and SMV in combination with PEG-IFN

Table 2 Univariate and multivariate analysis of factors associated with sustained virological response following pegylated interferon, ribavirin, and telaprevir or simeprevir triple therapy

Parameters	Telaprevir			Simeprevir		
	SVR	Non-SVR	*P* value	SVR	Non-SVR	*P* value
Unmatched patients						
Univariate analysis	*n* = 126	*n* = 33		*n* = 102	*n* = 45	
Age (yr)	58.0 (51.0-65.0)	62.0 (59.6-65.5)	NS	61.0 (52.8-67.3)	66.0 (56.5-71.0)	0.016
Gender (male/female)	62/64	15/18	NS	48/54	19/26	NS
Body mass index (kg/m^2)	23.8 (21.7-25.7)	24.6 (21.7-26.0)	NS	23.2 (21.0-25.1)	23.5 (21.3-26.0)	NS
Level of viremia (log IU/mL)	6.7 (6.3-7.0)	6.6 (6.3-7.0)	NS	6.8 (6.2-7.1)	6.9 (6.4-7.3)	NS
Leukocyte count (/mm^3)	5100 (4200-5700)	5100 (4400-6600)	NS	5000 (4300-5800)	4800 (3800-5800)	NS
Hemoglobin (g/dL)	14.1 (13.2-15.1)	14.1 (12.8-14.8)	NS	13.9 (13.1-14.7)	13.7 (12.7-14.8)	NS
Platelet count (× 10^4/mm^3)	15.0 (12.8-19.8)	15.0 (12.5-20.1)	NS	15.3 (11.9-20.5)	15.0 (11.1-18.3)	NS
SNP of IL28B (TT/non-TT)	84/19	15/15	< 0.001	72/19	17/24	< 0.001
Multivariate analysis	Odds ratio (95%CI)			Odds ratio (95%CI)		
SNP of IL28B (TT/non-TT)	4.316 (1.804-10.327)		0.001	8.598 (3.388-21.817)		< 0.001
Age (yr)				0.933 (0.889-0.980)		0.006
Level of viremia (log IU/mL)				0.335 (0.157-0.715)		0.005
Propensity score matched patients						
Univariate analysis	*n* = 77	*n* = 27		*n* = 76	*n* = 28	
Age (yr)	60.0 (52.5-66.5)	64.0 (60.0-65.0)	NS	59.0 (51.0-66.0)	65.0 (56.0-71.0)	0.021
Gender (male/female)	33/44	12/15	NS	37/39	12/16	NS
Body mass index (kg/m^2)	23.1 (20.6-25.0)	24.0 (21.4-26.6)	NS	23.2 (21.3-25.1)	23.6 (21.1-26.1)	NS
Level of viremia (log IU/mL)	6.7 (6.3-7.0)	6.7 (6.4-6.9)	NS	6.7 (6.1-7.0)	6.6 (6.3-7.2)	NS
Leukocyte count (/mm^3)	5000 (4100-5700)	5100 (4400-6700)	NS	5100 (4400-5900)	4600 (3500-5700)	NS
Hemoglobin (g/dL)	14.0 (13.1-14.8)	13.9 (13.0-14.9)	NS	14.0 (12.9-15.0)	13.8 (12.9-14.7)	NS
Platelet count (× 10^4/mm^3)	15.0 (12.3-19.7)	15.4 (12.9-20.3)	NS	15.1 (11.9-20.4)	15.0 (11.0-17.2)	NS
SNP of IL28B (TT/non-TT)	61/16	13/14	0.002	61/15	12/16	< 0.001
Multivariate analysis	Odds ratio (95%CI)			Odds ratio (95%CI)		
SNP of IL28B (TT/non-TT)	7.739 (2.111-28.375)		0.002	8.594 (2.777-26.598)		< 0.001

Values are presented as numbers or medians with interquartile ranges in parentheses. *P*-values were calculated using the χ^2 test or Mann-Whitney *U*-test for continuous variables. SNP: Single-nucleotide polymorphism; IL28: Interleukin 28B; SVR: Sustained virological response; NS: Not significant.

and RBV in the treatment of CHC patients in real-world clinical settings in Japan. Both regimens achieved higher SVR rates compared with that using the dual combination therapy with PEG-IFN and RBV[6-10,14-16]. In the TVR group, the proportion of patients achieving a virological response was higher than in the SMV group according to the original data. A number of patients discontinued TVR therapy because of adverse events at the beginning of treatment. After propensity score matching, the proportion of patients achieving a virological response during treatment and after 12 wk was almost identical between the two groups with no significantly difference observed (Figure 1B).

Patients in the SMV group appeared to have a greater prevalence of unfavorable baseline characteristics. Patients in SMV group were statistically older, had higher viral loads, lower hemoglobin levels, and a higher prevalence of unfavorable *IL28* genotypes (rs809997) compared with that in the TVR group. These pretreatment factors are known to influence the efficacy of IFN-based therapies[17]. As previously reported, Japanese patients infected with HCV genotype 1b are substantially older than Western patients[18]. A large proportion of patients able to tolerate IFN-based therapies were cured with previous therapies. Patients with unfavorable baseline characteristics remain untreated. In addition, according to academic guidelines[19], TVR therapy should be avoided in older patients with low hemoglobin levels in anticipation of future therapeutic options. As a result, a greater prevalence of unfavorable baseline characteristics were observed in patients in the SMV group.

In the present study, a greater proportion of patients in the TVR group discontinued treatment because of adverse events. Previously reported adverse events associated with TVR treatment include anemia, skin rash, and severe fatigue[11]. Cutaneous adverse effects caused by TVR have been frequently reported and are rare but are characterized by rapid development of lethal severe skin complications, such as Stevens-Johnson syndrome and drug-induced hypersensitivity syndrome[20,21]. Patients with these skin complications may have stopped the TVR treatment earlier. We administrated an initial dose of TVR 1500 mg/d in majority of patients to prevent treatment-induced anemia[22]. In contrast, the incidence of severe adverse events was low in the SMV group. Therefore, a smaller number of patients discontinued therapy in the SMV group.

Viral dynamics during treatment were similar to previous reports in both groups[16,23]. However, breakthrough and nonresponse was more frequent in the SMV group. Before matching, the TVR group had a higher SVR12 rate than that of the SMV group. After propensity score matching, this difference diminished and SVR12 rates were similar between the two groups. Reddy *et al*[24] reported a randomized control study between SMV and TVR for previous null or partial responders. Although the differences were observed in dosage, race, approved

combined interferon, and treatment duration in their report, viral breakthrough was more frequent with SMV therapy than with TVR therapy similar to the present report.

The SVR rate in the SMV group in the present study was lower than in the CONCERTO-4 study[16]. As our study was in a real-world clinical setting, patients were generally older (proportion of patients aged > 65 years, 42.3% *vs* CONCERTO-4, 22.8%) and had lower platelet counts (platelet counts < 15 × 10^4/mm^3: 47.7% *vs* CONCERTO-4, 31.6%) in our study. Baseline patient characteristics in our study may have resulted in a lower SVR12 rate.

The major limitation of the present study was the inability to evaluate several factors known to influence treatment efficacy. We did not examine amino acid substitutions of the HCV core region 70 and 91[23], NS5A interferon sensitivity determining region[25], interferon/ribavirin resistance determining region[26], or resistance-associated mutations of HCV NS3/NS4 proteases[27-29].

Treatment approaches to CHC are rapidly changing worldwide[30,31]. At present, direct-acting antiviral agent (DAA) combination therapy (daclatasvir and asunaprevir) is available for patients with HCV genotype 1 in Japan. Interferon-free DAA combination therapy has demonstrated an overall SVR12 rate of 85%[32]. Although the majority of patients with HCV infection may be treated with DAAs combination regimens, PEG-IFN and RBV-based treatment may still have utility in a small number of patients that do not respond to DAAs therapies.

In conclusion, both TVR and SMV regimens achieved high SVR12 rates. In the original analysis, TVR appeared to demonstrate an increased anti-viral efficacy compared with that of SMV. After propensity score matching, the proportion of patients achieving a virological response during treatment and after 12 wk treatment was almost identical between the two groups. Treatment discontinuation was more frequent in the TVR group because of adverse events at the beginning of treatment; however, nonresponse and breakthrough were more frequently observed in the SMV group.

COMMENTS

Background

The combination of pegylated interferon (PEG-IFN) and ribavirin (RBV) dual therapy was previously the standard care for chronic hepatitis C (CHC). Novel drug classes, including inhibitors of the NS3/NS4 protease of hepatitis C virus (HCV) polyprotein (protease inhibitors), have recently become available. Of these, telaprevir (TVR) triple combination therapy (TVR, PEG-IFN, and RBV) was the first to be approved in Japan in December 2011. Simeprevir (SMV), second generation protease inhibitor, was approved in Japan in December 2013. "Drug lag" between TVR and SMV, causing a difference in clinical backgrounds between the two regimens prior to treatment initiation, prevented fair comparison of the efficacy of TVR and SMV in real-world clinical practice.

Research frontiers

The authors' group evaluated and compared the efficacy of TVR or SMV for the treatment of CHC patients in Japan with propensity score matching to adjust for patient background between two groups.

Innovations and breakthroughs

Before adjustment, the proportion of patients achieving virological responses significantly differed between the TVR group and SMV group. In general, a greater proportion of patients in the TVR group had a virological response than that in the SMV group. After one-to-one propensity score matching, the proportions of patients achieving a virological response during treatment and after 12 wk treatment were similar between the two groups.

Applications

In the TVR group, the proportion of patients achieving a virological response was higher than in the SMV group according to the original data. A number of patients discontinued TVR therapy because of adverse events at the beginning of treatment. Breakthrough and nonresponse was more frequent in the SMV group. After propensity score matching, this difference diminished and sustained virological response 12 rates were similar between the two groups.

Terminology

TVR is the first inhibitor of the NS3/NS4 protease of HCV polyprotein (protease inhibitors) in Japan. SMV is a second generation NS3/NS4 protease inhibitor. Propensity score matching attempt is used to reduce the background difference between TVR and SMV groups.

Peer-review

The article is well written. It's clear and can help the authors' to understand the new drug treatment efficiency in a big cohort of HCV patience.

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SYSTEMATIC REVIEWS

Epidemiology of hepatitis C virus exposure in Egypt: Opportunities for prevention and evaluation

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Abstract

AIM: To critically evaluate the current epidemiology data on exposures, rather than infection, to hepatitis C virus (HCV) transmission and recommend epidemiologic strategies to fill gaps.

METHODS: Standard methods for identifying and evaluating relevant epidemiologic literature and available data were used.

RESULTS: There is a large body of literature on the epidemiology of HCV transmission in Egypt that collectively identifies ongoing iatrogenic exposures as the major driver for HCV transmission due to short comings in infection control and standard procedures. Additional epidemiologic studies on HCV transmission that requires the participation of human subject is unwarranted. Alternatively, very little literature was found on the epidemiology of exposure to HCV, infection control, and safe injection practices. The information that is available on patterns of HCV exposure shows high frequencies of inadequate infection control, problems in sterilization in health care facilities, low rates of hand washing, untrained personnel, lack of stated policies in facilities, HCV contamination of instruments and very large injection frequencies with low but very significant syringe and needle reuse. There is an important need to increase the number, size, and diversity of epidemiologic studies on HCV exposures, patterns of risk factors for infection, infection control, and safe injection practices. In addition to health care facilities evaluation, relevant knowledge attitude and practice studies are recommended.

CONCLUSION: Epidemiologic methods on HCV ex-

posure can be used to characterize the magnitude of exposures to HCV infection, target interventions to reduce exposures, and provide the best method for evaluating interventions by demonstrating the reduction of exposure to HCV infection.

Key words: Epidemiology; Hepatitis C virus; Egypt; Exposure; Prevention; Epidemic

Core tip: Much has been published on the epidemiology of hepatitis C virus epidemic in Egypt. The exposures that drive this epidemic are iatrogenic. This review focuses on what has been published on the epidemiology (patterns, distributions, and related factors) of the iatrogenic exposures. The review found that very little has been published on epidemiology of the exposures driving the epidemic. This is essential for developing effective interventions and evaluating prevention programs. Recommendations are given.

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INTRODUCTION

Kamel *et al*[1] reported in 1992 an unusually high prevalence of anti-hepatitis C virus (HCV) antibodies in over 2000 first time healthy Egyptian blood donors in Cairo, Egypt. The prevalence was 10.1%, five to ten times higher than what had been reported elsewhere in the world[2]. This was a population based study of first time blood donors in urban Egypt and likely underestimated anti-HCV antibodies prevalence in the general population.

A follow up to the blood donor study, Kamel *et al*[3] completed a rural population based community study of anti-HCV antibodies in 1994. The study included the entire population of a remote village in the northern Nile Delta. The overall anti-HCV antibodies prevalence in the village was 17.6%. In both of these population based epidemiologic studies, prevalence of anti-HCV antibodies increased strongly with age as shown in Figure 1. In the blood donors and the village study, the prevalence of anti-HCV antibodies was similar in both sexes.

These were the first two population based studies reported in Egypt providing the initial evidence that there was an extraordinary HCV epidemic unlike anywhere else in the world. These and many similar studies that followed[4-9], including two national studies[10,11], reinforced these observations. The 2008 national estimate of anti-HCV antibodies prevalence was 14.7% and the estimate for HCV RNA prevalence was 9.7%[10]. Given a national population of about 80 million persons, 7.8 million were estimated to be asymptomatically infected with HCV comprising a large reservoir of HCV in the population. It is now well established that Egypt has a HCV epidemic, which is the largest HCV epidemic in the world, and the epidemic is ongoing[2,9,12-14].

Epidemiologic tools are needed in Egypt to evaluate the magnitude and patterns of exposure to HCV transmission. Exposure to HCV is similar in concept to risk factors or independent variables associated with HCV transmission. The distribution and determinates of HCV exposures and related factors are fundamental for rationale allocations of resources for intervention by reducing exposure to HCV infection. Secondly, an extension of these epidemiologic tools is needed to evaluate intervention programs by demonstrating a reduction in the magnitude and patterns of HCV exposure.

The aim of our study was to first briefly summarize the epidemiology of HCV transmission (HCV T), identify all epidemiologic studies completed in Egypt to date that were designed to describe the magnitude, patterns and determinates of exposures (predominately iatrogenic exposures) to HCV transmission (HCV E), characterize gaps in HCV E and finally demonstrate and provide examples for the application of HCV E for the evaluation of public health interventions to reduce exposure to HCV.

MATERIALS AND METHODS

A literature review was conducted to identify publications on the epidemiology of HCV transmission and on the epidemiology of iatrogenic exposures in Egypt using methods previously published by us[14]. Briefly, a search of all published peer-reviewed literature (English language) from 1992 to 2015 on HCV and Egypt was made using the National Library of Medicine, PubMed, Google Scholar, Web of Science, Biological Abstracts, manual review of citations in search-identified publications and in Egypt for reports available only locally. Studies that: (1) reported HCV prevalence or incidence; (2) described the serologic methods; (3) were of cross-sectional or prospective epidemiologic design; and (4) could be abstracted for the purposes of the study were included. Studies on infection control were included as proxy for iatrogenic exposures.

One of our objectives was to build a complete bibliographic database on iatrogenic exposures and infection control practices in Egypt. From this, an assessment of epidemiologic methods for investigations on iatrogenic exposures or HCV E could be evaluated. Methodologically sound studies were sought as examples for investigation as well as methods for evaluation of intervention programs to reduce and prevent iatrogenic transmission of HCV in Egypt. Additional data were based on personal onsite visits in Egypt and anecdotal observation as some potentially iatrogenic practices have not been formally published and appear to be unique to Egypt. An example is the widely practiced re-use of latex gloves or not using

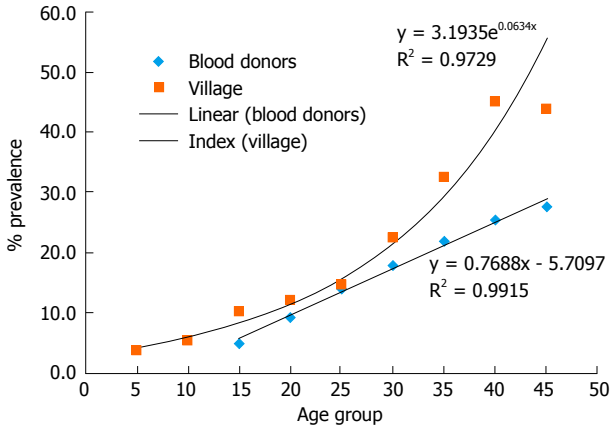

Figure 1 Prevalence of anti-hepatitis C virus antibody in urban blood donors and rural villagers.

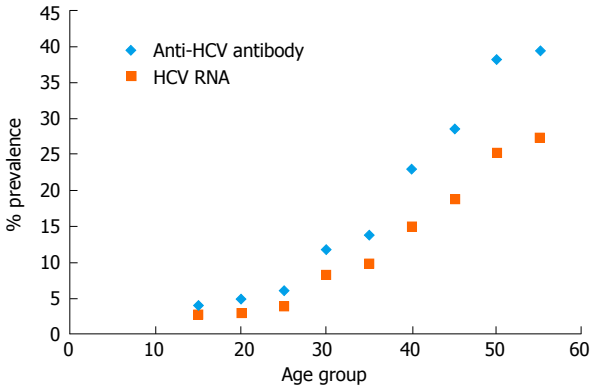

Figure 2 Prevalence by age of anit-hepatitis C virus antibody and hepatitis C virus RNA in 2008 Demographic Health Survey survey. HCV: Hepatitis C virus.

latex gloves when indicated.

RESULTS

History of HCV epidemic in Egypt

Following the pioneering work of Kamel *et al*[1,3], many similar studies in rural communities and selected health clinics followed without adding any significant new findings. Beyond the national blood donor screening program, no national program for the prevention of HCV emerged in the first decade after discovery. In part, this may have been due to the natural history of HCV infection. In a primary HCV infection, there is rarely an acute phase. Manifestations of liver dysfunction and disease usually do not occur until one or more decades later. HCV became a silent epidemic.

The origins of the HCV epidemic in Egypt are not clear but thought to be due to past and ongoing iatrogenic exposures[15,16]. Iatrogenic exposures and failure in infection control could be frequently seen on visits to health care facilities throughout the large Egyptian health care system.

A report in 1997 showed an association between anti-HCV antibodies and a history of parenteral therapy for schistosomiasis and surgery[17]. In 2000, a report in the *Lancet* suggested that the epidemic was due in part to the previous wide spread rural campaigns of parenteral anti-schistosomiasis therapy (PAT)[15]. The Egyptian medical care system embraced this report as the cause of the epidemic. More importantly, Egypt's physicians concluded that since these campaigns had ended more than three decades ago, the cause of the epidemic, PAT, had ended and therefore transmission had ended as well. If this was true, then epidemiologically the prevalence of anti-HCV antibodies in Egyptians 30 years old and younger should be similar to other countries. That is from 1% to 3% or less. As shown in Figures 1 and 2, anti-HCV antibodies prevalence increase from the earliest age. There is now abundant evidence that there is an ongoing HCV epidemic in Egypt.

The first formal epidemiologic study on infection control and iatrogenic exposures (HCV E) to HCV transmission in Egypt was in 2006[18]. This was a survey of large and small health care facilities. The study showed a complete lack of infection control practices in all facilities. In this study the presence or absence of infection control programs was an index of iatrogenic exposure to HCV. The American Centers for Disease Control and Prevention reported in 2012 that Egypt continued to face an ongoing HCV epidemic and that a comprehensive prevention plan was needed[13].

HCV transmission in Egypt

HCV is a blood borne pathogen and the transmission and epidemiology of HCV is well established[16]. The virus is inherently liable with exponential die off in 24 h under laboratory conditions[19,20], is less infectious than hepatitis B virus (HBV) and slightly more infectious than human immunodeficiency virus (HIV)[21].

The probability of HCV transmission varies by different routes of exposure as shown in Table 1. Note that the significance relative to the ongoing epidemic in Egypt of a given exposure route of transmission has been included in this table. For example, the probability of HCV transmission by contaminated blood or organ donation results in all recipients becoming infected. In this case the probability of transmission and infection is one (100%). Contaminated blood and organ donation have the highest HCV transmission. Nationally mandated HCV blood donor screening program in 1994 reduced exposure frequency to the point that blood transfusion, once a significant route of transmission in Egypt and elsewhere, no longer plays a significant role in regard to the general population[22].

Injection drug users (IDU) were found to have very high prevalence of HCV infection presumably due to the reuse and sharing of drug injection equipment[23,24]. Contaminated drug injection equipment has a high probability of transmission although there is no exact probability estimate available. The exact population of IDU in Egypt is not well defined although considered small. Moreover, HCV transmission with IDU groups, unlike HIV, poses a very low probability of exposure to

Table 1 The probability of hepatitis C virus transmission by different routes of exposure

Exposure route	Exposure frequency	Transmission probability	Population exposed	Significance[1]	Ref.
Blood transfusion	Very low	1 (100%)	[2]300 k/yr	Zero	[2,16]
Organ donation	Very low	1	< 100/yr	Zero	[2]
Injection drug users	High	⩾ 0.8	Very small	Very low	[23,24]
Hemodialysis	High	⩾ 0.75	Small	Very low	[26-28]
Sexual	High	Unk[3]	Adults	Zero	[29]
Intrafamilial	Unknown	Unk	General	Very low	[15,16]
Needle stick	High	⩽ 0.02	Occupational	Low	[31]
Injections	[4]4.1/p per year	⩽ 0.02	General	High	[17,30,53,60]
Maternal	High	0.02	New born	Low	[33,43]
Dental	High	⩽ 0.02	General	High	[62-65]
Iatrogenic	High	⩽ 0.02	General	High	[14,42,43,45,49,50,54]

[1]Significance refers to the extent that the specific exposure route contributes to overall national HCV transmission in Egypt; [2]1000 persons per year; [3]Unknown; [4]Persons per year. HCV: Hepatitis C virus.

the general population[25].

It has been long recognized that HCV infection, like HIV, was a risk for hemodialysis patients[26-28]. In Egypt, this became a national scandal. From 46.1% to 100% of HCV negative dialysis patients would convert to HCV positive within a year in dialysis centers throughout the country[16,26,28]. Considerable efforts both in the public and private sector have reduced HCV transmission in local dialysis centers. However, the general population exposed to this risk is small and the significance of HCV positive dialysis patients to the overall epidemic is very low.

Sexual and intra-familial transmission of HCV remains to be unequivocally established in Egypt or elsewhere. Sexual transmission of HCV is controversial. HCV discordant monogamous couples showed almost no transmission for long periods and recovery of HCV from semen or other genital fluids has proved to be difficult[29]. Sexual transmission does not play a significant role in Egypt. The studies of intra-familial transmission of HCV in Egypt[30-32] have validity issues (small numbers, confounding, selection biases) and have not been replicated. No specific intra-familial exposure to HCV transmission has been identified. Familial sharing of any medical equipment such as syringe and needles or diabetic testing equipment could result in exposure to HCV transmission, but this remains to be established.

Confirmed occupationally related accidental needle sticks from HCV positive patients have a probability of infection slightly greater than HIV but much lower relative to the probability of HBV infection. The probability of transmission has been estimated to be approximately 0.01 to 0.02[21]. Accidental occupational needle sticks in Egypt is a significant exposure[33-36]. However, the extent that this exposure contributes to transmission in the general population is not known but not likely to be significant.

Transmission of HCV infection from mother to new born however is well established. In Egypt, we have estimated that there are 5000 newborns infected with HCV every year[37].

Iatrogenic transmission of HCV has been documented globally[2,24]. Iatrogenic transmission can be complex due to the many possible routes of exposure from contaminated medical and dental instruments, sharps, needles, invasive procedures, contaminated multi-dose vials, blood, or blood product transfusion, organ transplantation or any of many kinds of medical/dental percutaneous exposures.

A key element of iatrogenic transmission is patient to patient exposure where the first patient is knowingly or more likely unknowingly asymptomatically infected. This patient is a key to the exposure and contamination of medical or dental instruments, sharps, or needles. Failure in infection control or standard procedures to prevent a second patient to be percutaneouslly or parenterally exposed to a contaminated instrument or sharp has a relatively low probability of HCV transmission and infection[21].

Iatrogenic transmission of HCV in Egypt is well documented[7,17,38-54]. These reports identify iatrogenic transmission as the principal driver of the HCV epidemic in Egypt. Accordingly, in Egypt, if the probability of iatrogenic exposure is the same across a health care system, then the probability of transmission will be greater in patient populations who have a higher prevalence of chronic HCV RNA infection, symptomatic or not. This reservoir of chronic HCV infected patients is known to be high in Egypt. Overall, 10% of the Egyptian population is HCV RNA positive[10]. This varies with rural populations having a higher prevalence of HCV RNA relative to urban populations. In Egypt, like anti-HCV antibodies, HCV RNA positivity increases directly with age as shown in Figure 2. The reservoir of HCV infection is therefore higher in older patients relative to younger patients.

Epidemiology of HCV transmission and epidemiology of HCV exposure in Egypt

The epidemiology of HCV T in Egypt is defined as reports which estimate patterns of HCV infection in people and associations with a possible exposure to HCV infection. For example the report by el-Sayed *et al*[17] showed an odds ratio (OR = 7.9) with a history of

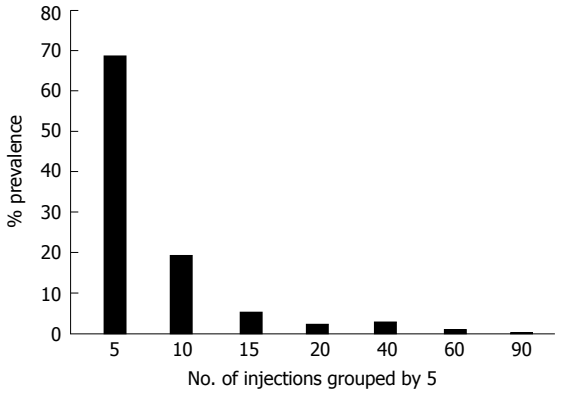

Figure 3 Percentage and number of injections reported in the past 6 mo.

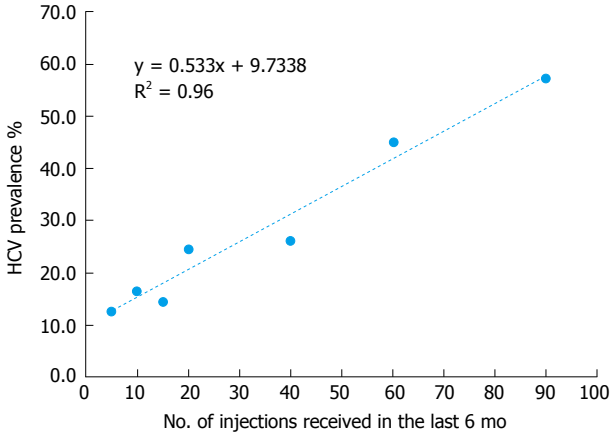

Figure 4 Injections received in last 6 mo and hepatitis C virus antibody percent prevalence. HCV: Hepatitis C virus.

PAT exposure. It is important to note that, as shown in this study, there is an abundance of similar HCV T literature on the prevalence, incidence, and risk factors for infection. Additional HCV T studies are unwarranted. There is no continued justification to test individuals for HCV antibodies or HCV RNA for the purposes of HCV T studies. Conversely, the epidemiology of exposure to HCV transmission (HCV E) would provide data on the patterns and frequency of exposures. Examples of HCV E are given below.

Injections: Reuse of inadequately sterilized needles and syringes during the PAT campaigns in rural Egypt over 30 years ago is cited as a major factor in the origin of the Egyptian HCV epidemic[15,21,33,34,55-60]. The PAT hypothesis has been challenged on the bases that there was a greater concurrent abundance of iatrogenic exposures throughout Egypt in addition to PAT exposures[49]. Accordingly, HCV E studies would characterize the patterns and frequency of injections and identify the magnitude and determinates of safe and unsafe injection practices.

Talaat *et al*[60] has estimated a high rate of injections in Egypt at 281 million per year. These injections were administrated by public and private sector physicians, pharmacists, barbers, doctor assistants, housekeepers, relatives and friends and 8.4% reported that the syringe was not taken from a closed packet[60]. Many in rural areas resort to informal nonprofessionals for injections[56].

Table 2 The number of persons who received one or more injections in the last 6 mo by gender

Gender	Yes	No	Total	Prevalence %	95%CI (lower-upper)
Female	1493	4661	6154	24.3	23.2-25.4
Male	625	4403	5028	12.4	11.5-13.3
Total	2118	9064	11182	18.9	18.2-19.6
OR = 2.3					2.0-2.5

Demographic Health Survey 2008[10]. OR: Odds ratio.

Safe injection practices were directly observed in a cross sectional study among 1100 healthcare workers located in 25 healthcare facilities in the Gharbiya governorate in the Nile Delta[33,34]. Noted was a lack of supplies needed for safe injections and safe practice was infrequent. Important policies were lacking including an infection control committee and dedicated infection control personal. Most importantly, there were an estimated 13.2% of syringes and needles reused. The important data on exposure in this study included lack of supplies, safe injection practices, essential policies, lack of infection control committee and personal and syringe and needle reuse.

Shown here for the first time is an analysis of injection data collected by the 2008 Egyptian Demographic Health Survey[10]. Shown in Table 2 is the frequency of injections reported by participants in the 2008 nationally representative study. Almost 19% (18.5%) received one or more injections in the last six months. Women reported receiving twice as many injections as men (OR = 2.3; 95%CI: 2.0-2.5). Figure 3 shows the pattern of participants reporting multiple injections in groups by 5. By far, the largest group received 1 to 5 injections in the last 6 mo followed by a sharp decline. Figure 4 shows the relationship between HCV antibodies prevalence and the pattern of multiple injections. A strong (R^2 = 0.96) and direct relationship of increasing HCV antibody prevalence and the number of injections is shown. These results suggest that increased frequency of injections has a direct relation with increased HCV antibodies positivity. An alternate interpretation is that individuals who are receiving multiple frequent injections have serious medical conditions and more likely have additional iatrogenic exposures.

The measure of exposure to HCV was the following question: "The last time you had an injection from a health worker, did the person who gave you that injection take the syringe and needle from a new, unopened package?". Of those that received injections, 14.8% answered "No". Data are shown in Table 3. There may be concerns about how this question was interpreted by the participant. The injection may have been prepared in a separate area from the patient. The patient may not know or be able to confirm that the syringe and needle came from a new unopened package. However, this estimate is similar in magnitude

Table 3 To answer the question: "The last time you had an injection from a health worker did the person who gave you that injection take the syringe and needle from a new, unopened package?" Demographic Health Survey 2008[10]

Question[1]	Yes	No	Total	Prevalence %	95%CI (lower-upper)
New Unopened Package	1273	221	1494	14.80	13.0-16.6

[1]Among those who received an injection and replied to the question.

to that from the study conducted in Gharbiya[33,34], in which Talaat *et al*[60] estimated about 8.4% in an answer to a similar question.

Dental health care: Dental health care has a significant potential for iatrogenic HCV transmission[61-63]. Only a single report of HCV iatrogenic exposure in Egyptian dental clinics was found published by Hashish *et al*[64] in 2012. The study measured the presence of HCV RNA by reverse transcription polymerase chain reaction (RT-PCR) on various dental instruments in selected dental clinics in Alexandria. The study found that 18% of the dental instruments in the dental clinics visited were positive for HCV RNA indicating the presence of the virus. This study demonstrates a method for measuring iatrogenic exposure to HCV infection from dental health care by showing the presence of HCV contaminated dental instruments.

The exact reason for the contamination of these instruments was not given. The authors did suggest that the lack of sterilization equipment for instruments was a short coming. What was not provided was the number of sets of instruments, patient load, hand washing practices, use of latex gloves, how instruments were cleaned and disinfected, the presence of operating sterilization equipment, and if there had been specific efforts and policies present for training and preventing HCV transmission.

The prevalence of HCV is lowest in Alexandria most likely due to better infection control than most other areas of Egypt, especially rural areas. The observed results are therefore most likely an under estimate.

The correct method to expand this epidemiologic approach to HCV exposure in dental clinics would be to include other measures of infection control mentioned above in recording data. Secondly, this study design could be used at the community level to describe the epidemiology of HCV exposure in dental clinics by obtaining a list of all dental care facilities in the community and decide on drawing a representative sample of dental care facilities or to include all facilities in the study. Results of a sample could be used to describe the magnitude of HCV contamination and provide justification for professional improvement or training programs. Results based on facility specific level would be used for compliance and licensing.

Detection of HCV RNA by RT-PCR could be resource challenging. A lower resource approach would have the same primary step of community and facility identification and sampling. Each facility would provide details on cleaning and sterilization methods and an inventory of dental equipment sets and daily patient loads. Large patient to equipment ratios would suggest a trigger for compliance or licensing issues.

Health care facilities: Egypt has a national health care system dating back to the 19th century. Health care facilities in Egypt are a complex organization of public and private sector facilities that include medical care, dental care, clinical laboratories, and pharmacies.

Pharmacies in Egypt give injections, intravenous fluids, and provide testing for glucose levels. Glucometers are an overlooked exposure to HCV transmission. Testing is carried out by pharmacy technicians who may have only secondary school education and do unsupervised home visits. No studies have been done on exposure to HCV infections related to Egyptian pharmacies.

El-Zanaty *et al*[65] carried out a national service provision assessment survey in 2004. A portion of this study was on "Systems for Infection Control". Medical care facilities rather than individuals were surveyed in a nationally representative sample using very specific standardized data forms. This report has considerable details representing all regions of Egypt and covering all types of medical care facilities. Private clinics, private pharmacies, and dental services were not included.

A statement in a summary of the findings reported a significant decrease from 2002 to 2004 in almost all indicators of infection control. It was concluded that infection control practices were extremely weak. Only 4% of all facilities were adherent to all infection control measures. New disposable syringes and needles were however universal. The study could uniquely compare changes over time to their previous publication in 2002[66].

Informal health care providers: Egypt has a large undocumented sector of informal health care provides. These providers do not have formal education or training and provide services for injections, dentistry, wound treatment, and male circumcision. Traditional birth attendants were reported to oversee > 50% of all births. "Injectionists" included barbers and staff at pharmacies. A study of Informal health providers in two Egyptian villages found that these providers, "knew little about HCV" and its transmission[56].

A number of studies in Egypt have included barbers as a possible exposure to HCV[41,43,44,56,67,68]. This is based on the assumption of percutaneous exposure by shaving. Most studies found that there is no evidence for transmission. This is consistent with viral fragility and the unlikelihood of the virus remaining viable in the soap used in shaving. In a study of barbers in the Gharbia governorate[68], anti-HCV antibodies were detected in 12.3% of barbers and 12.7% of clients. Knowledge of HCV prevention was reported to be high among the majority of participating barbers and good practices

during shaving and hair-cutting were observed for the majority of barbers.

HCV prevention in Egypt

The transmission of HCV is entirely preventable. Throughout the world, HCV transmission is prevented by good medical and dental care practices which reduce and eliminate iatrogenic exposure to infection. This includes following well established aseptic techniques, standard procedures and universal precautions[13]. Measures should and can be taken to reduce and eliminate all HCV transmission routes listed in Table 1.

HCV prevention in the Egyptian health care educational system: Aseptic techniques, standard procedures, and universal precautions are or should be introduced in all health care curricular in the Egyptian health care educational system and reinforced at every level of health care service. This educational intervention is not intellectually complex or challenging to teach. In fact, teaching and training could be done entirely using modern computer based social media. Moreover, the interventions to reduce HCV exposure do not require new or costly technology. There is no publicly available information on the evaluation of the Egyptian health care educational system in regard to HCV prevention curricular. Technically, evaluation of the Egyptian health care educational system is straight forward.

Recommendation: Conduct an examination on a sample of recent and past graduates from medicine, nursing, dentistry, and pharmacy on HCV prevention. This is an essential data for status on current knowledge and a benchmark to which future evaluation can be based.

Evaluation of knowledge, attitudes, and practices (KAP) of health care providers on standard procedures and infection control is necessary for the reduction of exposure to HCV. Well-designed repeated KAP studies based on representative samples stratified by health care provider categories can provide direct evidence of HCV exposures defined as incomplete knowledge and practice errors. The large Egyptian health care syndicates can provide needed sampling frames. These syndicates can also provide opportunities to spread important messages about HCV exposure prevention.

Recommendation: KAP study on health care providers on HCV prevention is needed.

In addition to curricular interventions, professional development or continued health care education should be developed for online certification or re-certification for preventing exposure to HCV infection.

Screening of blood and blood products: Globally, blood donations are screened for HCV. Egypt mandated a national blood donation HCV screening program by 1994[22]. Before this national program, approximately 9% of blood and blood products recipients would have become infected. Assuming the probability of HCV transmission at 100% among exposed recipients and 300000 recipients per year, this program has prevented to date at least half a million people from becoming infected. Maintaining and enhancing Egypt's national blood donation HCV screening program is essential.

DISCUSSION

The number and extent of studies which test for the presence of HCV in individuals (HCV T) have been considerable dating from the first reports in 1992. Given the abundance of literature on HCV T, additional HCV T studies are unwarranted. There is no continued justification to test individuals for HCV antibodies or RNA for the purposes of HCV T studies. Aside from cost, there are additional methodological limitations for both cross sectional and prospective HCV T studies in Egypt that further support discontinuation of these investigations. Due to the natural history of HCV, there are serious validity issues for cross sectional, prospective, and case control study designs undermining hypothesis testing related inferences. Population prevalence estimates are not useful for evaluating interventions and prevention measures[69]. Generating national incidence rates requires very large samples, is very costly, requires long term follow ups, and has limited validity determining past exposures. Prospective incidence studies are strongly discouraged especially for intervention assessment and project evaluation. The difficultly of using prospective incidence studies for intervention assessment is not unique to this public health problem but is widely recognized by public health practitioners.

Successful interventions will reduce exposure to HCV infection which will decrease the incidence. It is strongly recommended to use HCV E studies, rather than prospective incidence studies to evaluate the control of the ongoing epidemic. For that, benchmark HCV E data are needed which can be obtained from cross sectional studies.

Few studies on HCV E were found. However, these studies illustrate the basic low cost low technology methodology needed to document exposures to HCV infection. The only national level study using facility surveys completed by El-Zanaty *et al*[65,66] in 2002 and 2004 is a good example. These facility studies could be modified to focus more on infection control evaluation. This would provide direct data on the frequency and patterns of HCV exposures. These observational based studies can be complemented by interview based KAP studies adapted to the unique Egyptian epidemic directed at infection control documentation and evaluation.

It is essential to conceptualize the methodological approach epidemiologically for HCV E. The objective is to epidemiologically describe the prevalence of a specifically defined set of health care practices and procedures which can be evaluated as being correctly or incorrectly done with regard to standard procedures[64,65] in a given health care setting. As mentioned above this

should include both observational and interview based data collection. Specific modifications are needed for the different types of Egyptian health care facilities.

Methods for standardizing data collection on infection control in general and injection practices in specific for Egypt are needed. Standardized methods are also needed for direct and indirect observational data collection and data collected by interview. Standards for collecting photographic documentation would be useful. Pilot testing with independent verification is advisable. This capacity exist in Egypt as demonstrated by the studies carried out by El-Zanaty *et al*[10,65].

There are many unique infection control violations in Egypt which have the potential for exposure to HCV infection. These exposures unique to Egypt are poorly documented and should be thoroughly investigated and included in any comprehensive prevention program to reduce exposure to HCV transmission. There is a large unregulated informal health care system in Egypt that contributes significantly to injections and other poorly regulated procedures[18,60].

It is recommended that efforts be made to develop strong HCV E studies that generate a comprehensive inventory of all typical and unique iatrogenic exposures, where these exposures are occurring, the magnitude of these exposures (number of individuals potentially exposed), probability of transmission and create an index of iatrogenic transmission. The HCV index of transmission (HCV IT) would incorporate the magnitude of population exposed and probability of transmission. For example, HCV contaminated blood transfusion has 100% probability of transmission[2,16], a restricted population exposed (blood donor recipients) and with the current level of blood donor screening an overall low index. Given the magnitude of injections received in Egypt with much lower probability of transmission *via* contaminated drug vials or syringe and or needle reuse, the overall HCV IT is likely to be very significant[53].

Strong well designed HCV E studies have a dual function. The first objective is to better and more precisely document the distribution and determinates of specific iatrogenic exposures. This is essential to provide a baseline for evaluation. The second objective is evaluation of intervention programs. That is to quantitatively demonstrate a reduction in exposure to HCV transmission by an improvement in infection control measures and safe injection practices by direct and indirect measures[10,60,64,65] with follow up HCV E studies.

COMMENTS

Background

Egypt has the largest epidemic of hepatitis C virus (HCV) in the world. A review of the epidemiologic literature on HCV was completed.

Research frontiers

HCV is entirely preventable. The exposure factors to HCV infection driving the epidemic in Egypt have been thoroughly identified as iatrogenic. The authors' aim was to assess the magnitude of the epidemiologic literature on iatrogenic exposures in Egypt.

Innovations and breakthroughs

The authors found that the amount of epidemiologic information on iatrogenic exposures needed for designing the prevention of HCV transmission was very limited, especially in contrast to the epidemiology on HCV infection or transmission.

Applications

The epidemiology of exposure to HCV transmission, that is predominately iatrogenic exposures, is essential information and knowledge needed to design, guide, and evaluate interventions to reduce iatrogenic exposures and prevent HCV transmission. The application of epidemiologic investigation on exposures does not include human subjects which vastly reduces the cost and complexity of data collection. Recommendations and suggested epidemiologic approaches and designs were given.

Terminology

The authors refer to classical epidemiology of HCV infection, transmission, and prevalence as HCV transmission. Individuals participating is these types of studies have to provide a specimen for HCV testing, understand the consequences of being found HCV positive, and if viremic, referred for treatment. The authors refer to the epidemiologic investigation of exposures to HCV infection as HCV E. Knowledge, attitude, practice (KAP) studies are an example of methodology that could be adapted to exposure epidemiology. The KAP of injection preparation and administration is an example.

Peer-review

The review is very interesting and gives a fairly comprehensive overview of the situation in Egypt.

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