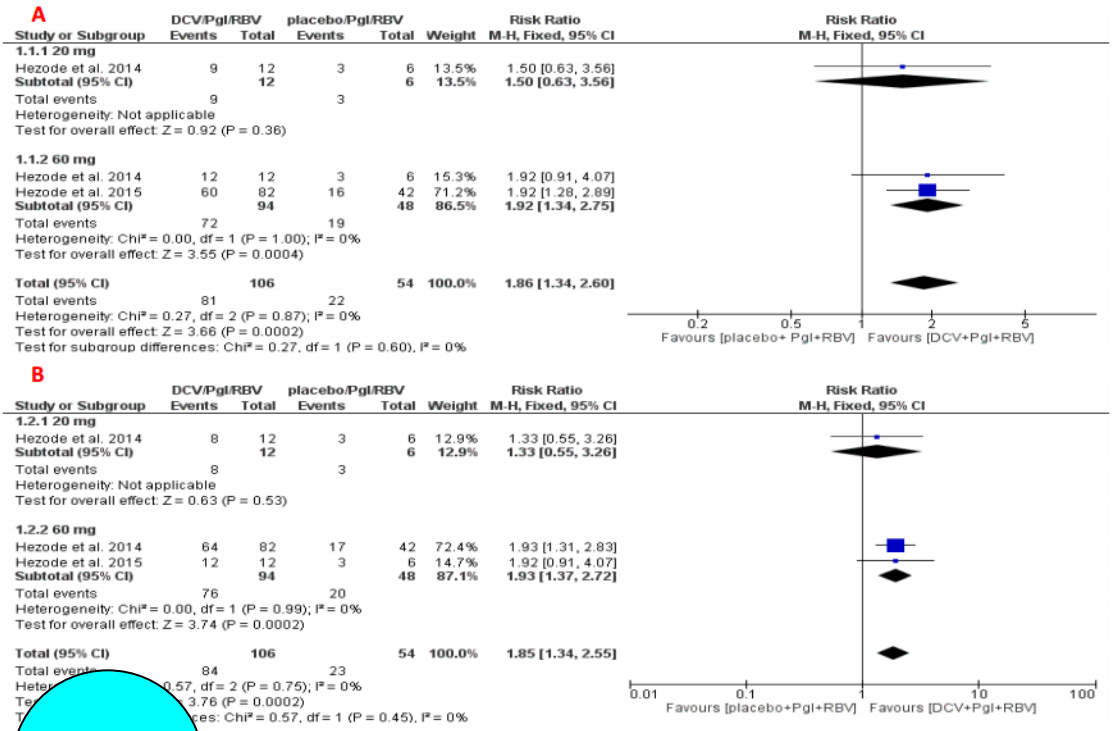

BJP

Bangladesh Journal of Pharmacology

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A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2017; 12: 12-22
ISSN: 1991-0088

Meta-analysis

Efficacy of daclatasvir plus peginterferon alfa and ribavirin for patients with chronic hepatitis C genotype 4 infection

Hussien Ahmed[1], Abdelrahman Ibrahim Abushouk[2], Mohamed Gadelkarim[3], Arwa Mohamed[1], Mohamed Gabr[1] and Ahmed Negida[1]

[1]Faculty of Medicine, Zagazig University, El-Sharkia, Egypt; [2]Faculty of Medicine, Ain Shams University, Cairo, Egypt; [3]Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Article Info

Received: 10 October 2016
Accepted: 13 February 2017
Available Online: 1 March 2017

DOI: 10.3329/bjp.v12i1.29940

Cite this article:
Ahmed H, Abushouk AI, Gadelkarim M, Mohamed A, Gabr M, Negida A. Efficacy of daclatasvir plus peginterferon alfa and ribavirin for patients with chronic hepatitis C genotype 4 infection. Bangladesh J Pharmacol. 2017; 12: 12-22.

Abstract

Clinical trials evaluating the safety and efficacy of daclatasvir for chronic hepatitis C virus (HCV) genotype 4 infection are scarce and yet with small sample sizes. Therefore, we conducted this systematic review to investigate the efficacy of daclatasvir in HCV genotype 4 treatment. A computer literature search of PubMed, Scopus, Embase, Ovid, Web of knowledge, and Cochrane central was conducted. We selected studies comparing daclatasvir plus peginterferon-alfa/ribavirin versus placebo plus peginterferon-alfa/ribavirin in patients with HCV genotype 4 infection. Pooling data from two randomized controlled trials (n = 154 patients) showed that daclatasvir/peginterferon/ribavirin treatment achieved a moderate sustained virologic response rate of 76% after 12 weeks and of 79% after 24 weeks. The daclatasvir containing regimen was superior to the placebo containing regimen in terms of virologic response rates after 12 weeks (RR=1.9% CI 1.3 to 2.6) and 24 weeks (RR=1.8% CI 1.3 to 2.5). More effective regimens are needed for HCV genotype 4.

Introduction

Hepatitis C virus (HCV) infection is a globally prevalent disease that affects about 170 million individuals (Negro and Alberti, 2011). It is a major cause of liver cell failure and hepatocellular carcinoma, accounting for about 350,000 deaths annually (Armstrong et al., 2006; Hanafiah et al., 2013). Seven genotypes of HCV have been identified. Of them, genotype 1 is the most prevalent infection worldwide (Smith et al., 2014), while genotype 4 accounts for 20% of the global burden of HCV infection, with a high prevalence in the Middle East and Central and North Africa (Cornberg et al., 2011; Kamal, 2011). Multiple factors affect patients' response to antiviral therapy including viral genotype, viral load, host genetics, and patients' demographics (Ghany et al., 2009; Thompson et al., 2010).

Current treatment options for HCV genotype 4 include the NS5B polymerase inhibitor (sofosbuvir) or NS3 protease inhibitor (simeprevir) plus peginterferon-alfa and ribavirin (Peg-IFN/RBV) (Pawlotsky et al., 2015). However, the increased incidence of adverse events associated with interferon-based regimens triggered the development of interferon-free therapeutic combinations (Hézode et al., 2014). *In vitro* studies show that daclatasvir, an NS5A inhibitor, has a pan-genotypic activity with picomolar potency and pharmacokinetic characteristics allowing for single daily dosage regimens (Fridell et al., 2011; Wang et al., 2012). It acts via inhibiting the NS3 protease enzyme and therefore, preventing NS5A hyperphosphorylation, leading to inhibition of viral replication complex formation (Lee et al., 2011; Qiu et al., 2011).

Phase II clinical trials showed that the combination of daclatasvir (60 mg) with Peg-IFN/RBV exhibited more

efficacy than Peg-IFN/RBV alone with a similar safety profile and allowed for a shorter treatment duration (Dore et al., 2015; Hezode et al., 2015). Moreover, addition of asunaprevir to the former regimen of daclatasvir plus Peg-IFN/RBV achieved a sustained virologic response rate (SVR) of 100% after 12 weeks of treatment (Jensen et al., 2015).

Although more than 90% of HCV infections in the Middle East and Africa are of genotype 4 (Karoney and Siika, 2013), studies assessing the efficacy of daclatasvir in patients with HCV genotype 4 are scarce and yet with small sample sizes. Therefore, we conducted this systematic review and meta-analysis to investigate the efficacy of daclatasvir in treating patients with HCV genotype 4.

Materials and Methods

We followed the PRISMA statement guidelines during the preparation of this systematic review and meta-analysis (Moher, 2009).

Criteria for selecting studies to this review

We used the following inclusion criteria: a) Population: Cirrhotic or non-cirrhotic adult patients with chronic HCV genotype-4 infection, b) Intervention: 20 mg and/or 60 mg of daclatasvir plus Peg-IFN/RBV (triple regimen), c) Comparator: Placebo plus Peg-IFN/RBV (dual regimen), d) Efficacy outcomes: Measured in virologic response rates, and e) Study design: Randomized controlled trials. We excluded: a) Non-randomized trials, b) Studies comparing the efficacy of daclatasvir with other direct antiviral agents, c) *In vitro* and animal studies, d) Studies including patients, co-infected with Hepatitis B Virusor immunodeficiency virus, and e) Studies whose data were unreliable for analysis.

Literature search strategy

We searched PubMed, Scopus, Embase, Ovid, Web of knowledge, and Cochrane central through March, 2016 using relevant keywords (Daclatasvir OR BMS-790052 OR NS5A inhibitor). No language restrictions were imposed. We also manually searched the reference list of included studies for any missing citations.

Screening of records

Duplications between databases were removed and finally, retrieved references were screened for randomized controlled trials comparing daclatasvir plus Peg-IFN/RBV versus placebo plus Peg-IFN/RBV. References were screened in two steps: The first step was to screen titles/abstracts for eligibility and the second step was to screen full text articles of eligible abstracts.

Data extraction

Two independent authors (HA and AM) extracted data using an online data extraction form. Disagreements were resolved through discussion and consensus among the reviewers. The extracted data included the following domains: a) Characteristics of study design, b) Baseline criteria of included population and c) Study outcomes.

Primary efficacy measure

The efficacy of antiviral treatment was assessed by SVR and relapse rate. SVR is defined as patients with undetectable HCV RNA level at 12 or 24 weeks after cessation of treatment, while relapse rate is defined as detectable HCV RNA level during follow-up after achieving undetectable levels at any point of treatment.

Secondary efficacy measures

The secondary efficacy measures included: Rapid virologic response rate (RVR) [defined as undetectable HCV RNA at week 4 of treatment], extended rapid virological response (eRVR)[defined as HCV RNA <10-15 IU/mL at weeks 4 and 12 of treatment], complete early virological response (cEVR)[defined as ≥2 $\log_{10}$ reduction from baseline HCV RNA and the virus is undetectable], and end of treatment response (EOTR) [defined as undetectable HCV RNA at the end of treatment] (Lindsay, 1997; Yu et al., 2007).

Risk of bias assessment

The risk of bias of the retrieved clinical trials was assessed according to the Cochrane handbook of systematic reviews of interventions 5.1.0 (updated March, 2011) by two independent reviewers. Any discrepancies between the two assessors were resolved through discussion with a third assessor.

Data synthesis

Study outcomes were pooled as a risk ratio (RR) in a fixed effect model meta-analysis using Mantel-Haenszel method. A subgroup analysis, according to daclatasvir dose, was conducted whenever possible. For all outcomes, effect estimates of the two doses (20 vs 60 mg) were compared by chi-square test. All analyses were conducted by Revman software version 5.3 for Windows.

Assessment of heterogeneity

Heterogeneity was assessed using the chi-square test and extent was measure using the I-square tests.

Publication bias

According to Egger and colleagues, the assessment of publication bias is not reliable for less than 10 pooled studies. Therefore, in the present study, we could not assess the existence of publication bias by Egger's test for funnel plot asymmetry (Egger et al., 1997; Terrin et al., 2003).

Results

Search results

Our search retrieved 1,856 unique citations. Of them, 36 records were eligible for full text screening. Finally, 34 articles were excluded and two randomized controlled trials (with a total of 154 HCV genotype 4 patients) were included in the final analysis (Figure 1).

Risk of bias in included studies

The risk of bias in included studies was low according to the Cochrane risk of bias assessment tool. The summary of risk of bias assessment domains and authors' judgments with justifications are shown in the supplementary data in page 22.

Our analysis included 154 patients with HCV genotype 4 (daclatasvir: 106 patients and placebo: 48 patients). Baseline characteristics of each study population are shown in Table I and the summary of included studies and their main results are shown in Table II.

Sustained virologic response rate (SVR)

The daclatasvir plus Peg-IFN/RBV treatment achieved a SVR of 76% (81/106) after 12 weeks and 79% (84/106) after 24 weeks. Compared with the SVR rate in the placebo plus Peg-IFN/RBV group, the daclatasvir containing (triple) regimen was superior after 12 weeks (RR= 1.9% CI [1.3 to 2.6], p=0.0002; Figure 2A). Pooled studies were homogenous (p = 0.87; I^2 = 0%). The SVR after 24 weeks was also higher in the triple regimen group (RR= 1.8% CI [1.3 to 2.5], p=0.0002; Figure 2B). Pooled studies were homogenous (p = 0.45; I^2 = 0%).

Relapse rate

The relapse rate was lower in the triple regimen group, compared to the dual regimen group (RR= 0.2% CI [0.1 to 0.5], p=0.002; Figure 3A). Pooled studies were homogenous (p=0.032; I^2= 13%).

Rapid virologic response rate (RVR)

Daclatasvir plus Peg-IFN/RBV group was superior to placebo plus Peg-IFN/RBV group in terms of RVR (RR= 6.6% CI [3.1 to 14.2], p<0.0001; Figure 3B). Pooled studies were homogenous (p = 0.80; I^2 = 0%).

Extended rapid virologic response rate (eRVR)

Daclatasvir plus Peg-IFN/RBV group was superior to placebo plus Peg-IFN/RBV group in terms of eRVR (RR=5.3% CI [2.6 to 10.7], p<0.0001; Figure 4A). Pooled studies were homogenous (p = 0.89; I^2 = 0%).

Complete early virologic response rate (cEVR)

Daclatasvir plus Peg-IFN/RBV group was superior to placebo plus Peg-IFN/RBV group in terms of cRVR (RR= 1.7% CI [1.3 to 2.3], p =0.0002; Figure 4B). Pooled studies were homogenous (p = 0.81; I^2 = 0%).

End of treatment viral response (EOTR)

Daclatasvir plus Peg-IFN/RBV group was superior to placebo plus Peg-IFN/RBV group in terms of EOTR

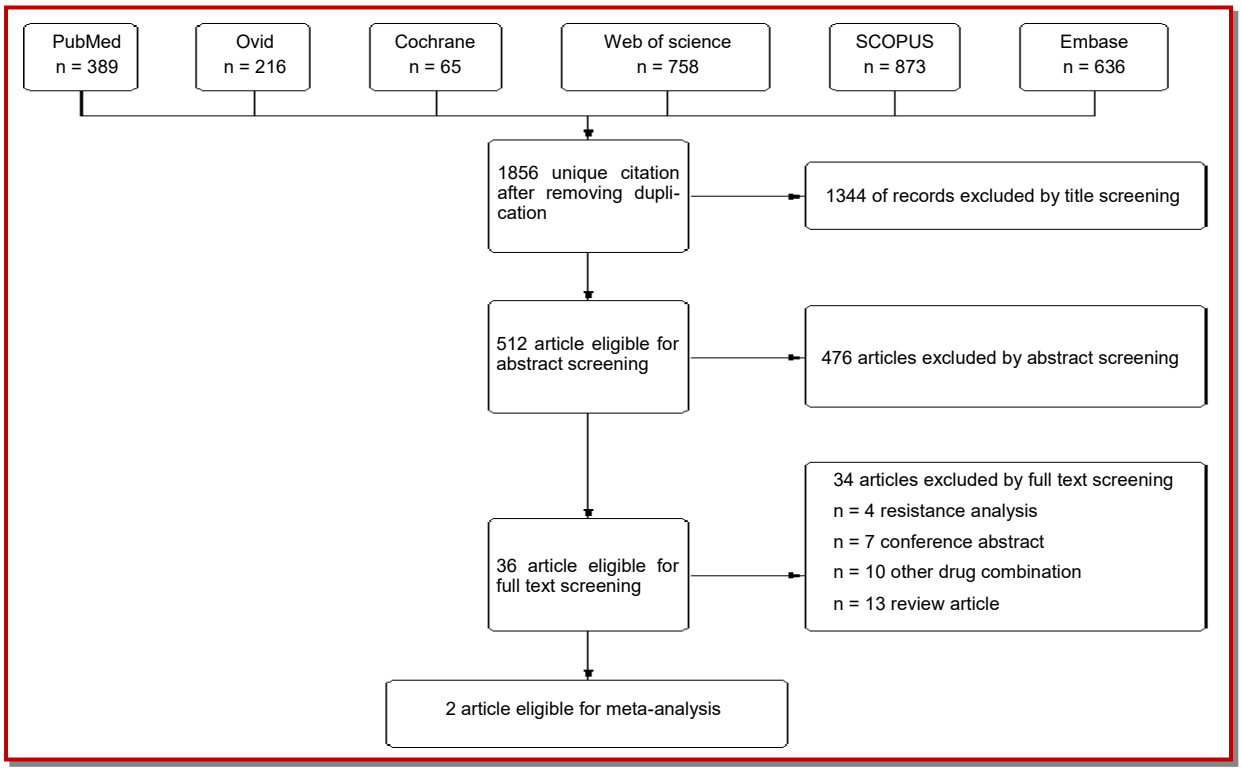

Figure 1: Flow diagram of search strategy and study selection

Table I

Baseline characteristics of the included population in the analysis

Study	Group	n	Age (Year)	Sex	Cirrhosis		HCV-RNA		IL28B GT (rs12979860), n (%)			HCV genotype, n (%) genotype 1		HCV genotype, n (%) genotype 4					BMI ≥30 kg/m2, n (%)
			Median (Range)	Male, n (%)	Re-ported	Not reported	Log 10 IU/mL, mean (SD)	≥800 000 IU/mL, n (%)	CC	CT	TT	1a	1b	4 un-specified	4a,c, or d	4e	4f	4h	
Hezode et al. 2015	Daclatasvir + peginterferon-alfa/ribavirin	82	48.5 (20-71)	61 (74.4)	9 (11.0)	4 (4.9)	5.8 (0.8)	39 (47.6)	22 (26.8)	40 (48.8)	20 (24.4)	26 (31.7)		1 (1.2)d	46 (56.1)	1 (1.2)	5 (6.1)	3 (3.7)	18 (22.0)
	Placebo + peginterferon-alfa/ribavirin	42	50.0 (32-61)	29 (69.0)	4 (9.5)	0	5.73 (0.6)	16 (38.1)	9 (21.4)	27 (64.3)	6 (14.3)	0		16 (38.1)	24 (57.1)	0	1 (2.4)	1 (2.4)	5 (11.9)
Hezode et al. 2014	Daclatasvir 20 mg + peginterferon-alfa/ribavirin	159	51 (22–70)	107 (67.3)	13 (8.2)		6.5*	133 (83.6)	53 (33.3)	82 (51.6)	17 (10.7)	106 (72.1)	41 (27.9)	12 (7.5)					31 (19.5)
	Daclatasvir 60 mg + peginterferon-alfa/ribavirin	158	50 (18–67)	103 (65.2)	8 (5.1)		6.5*	123 (77.8)	44 (27.8)	86 (54.4)	18 (11.4)	113 (77.9)	31 (21.4)	13 (8.2)					42 (26.6)
	Placebo + peginterferon-alfa/ribavirin	78	51 (25–66)	55 (70.2)	8 (10.3)		6.4*	61 (78.2)	23 (29.5)	38 (48.7)	11 (14.1)	56 (77.8)	16 (22.2)	6 (7.7)					23 (29.5)

Table II

A summary of included studies in the analysis

Study ID	Study design	Population	Sample size	Regimens		Findings
				Intervention	**Control**	
Hezode et al. 2014	Phase III, randomized, double blind, controlled trial	-Patients age 18-70 -Patients infected with HCV GT1 (90%) or GT4 (10%) with viral load ≥100 000 IU/mL -Treatment naive -No history or evidence of hepatic decompensation -No prior exposure to any agent with potential anti-HCV activity, -No coinfection with HBV or HIV, or evidence of chronic liver disease other than HCV.	N=359 GT1 = 365 GT4 = 30 -159 in intervention (20 mg) -158 in intervention (60 mg) - 78 in control	Daclatasvir (20 mg or 60 mg) + peginterferon-alfa/ ribavirin	Placebo + pegIFN-alfa -2a/RBV	The study shows that daclatasvir/ peginterferon-alfa/ ribavirin is superior to peginterferon-alfa/ ribavirin alone
Hezode et al. 2015	Phase III, randomized, double blind, controlled trial	-Patients aged ≥ 18 -infected with HCV Genotype 4 with viral load ≥ 10.000 IU/mL -Naive to interferon-based regimens or DAAs -With compensated cirrhosis -No co-infections with HBV or HIV, or any malignancy.	N=124 -82 in intervention group. -42 in placebo group.	Daclatasvir (60 mg) + peginterferon-alfa/ribavirin	Placebo + pegINF/ RBV	The study shows that daclatasvir/ peginterferon-alfa/ ribavirin is superior to peginterferon-alfa/ ribavirin alone

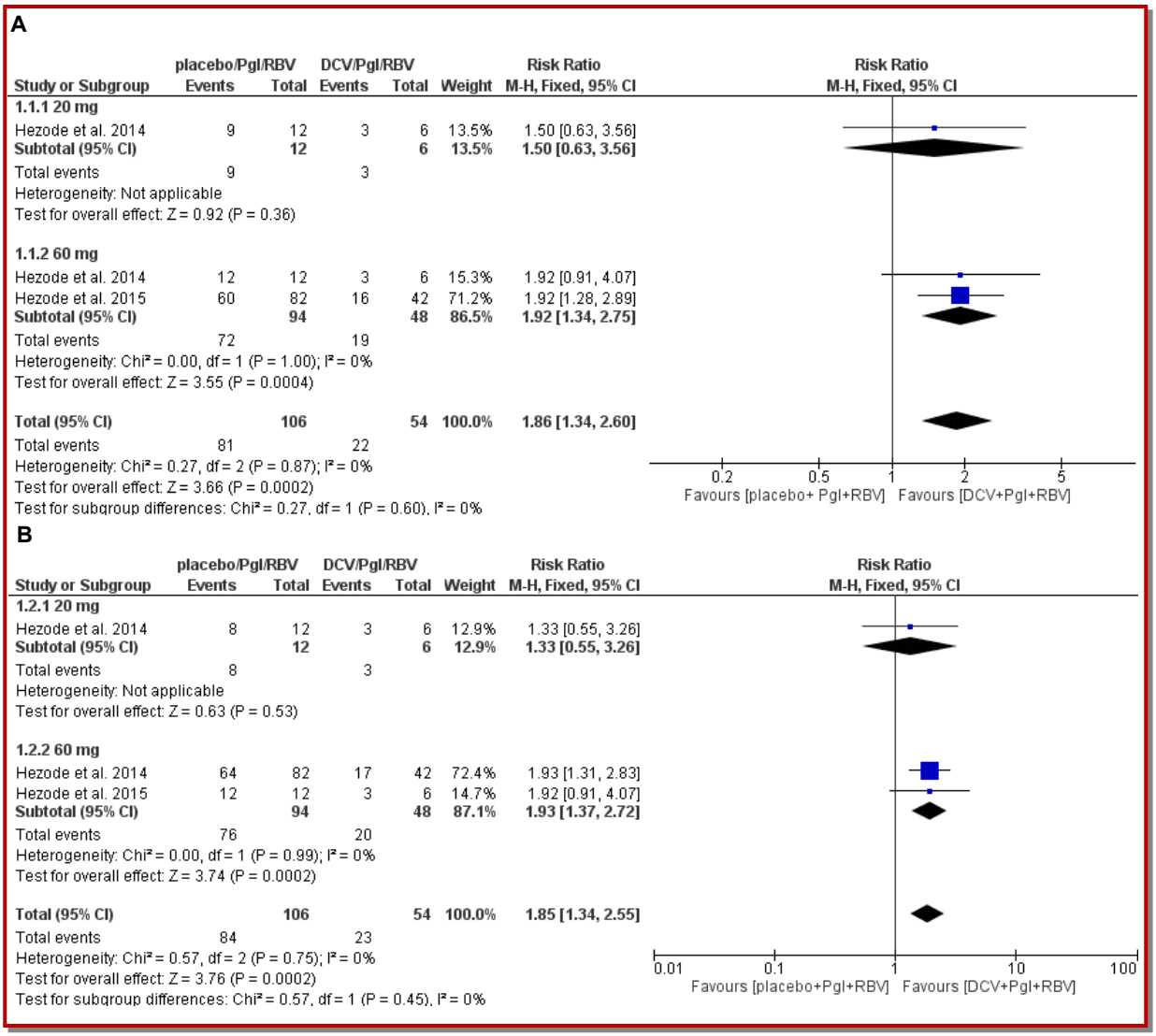

Figure 2: Forest plots comparing the dual and triple regimens in terms of SVR at 24 weeks (A) and 24 weeks (B)

(RR=1.7% CI [1.3 to 2.2], p=0.0001). Pooled studies were homogenous (p = 0.31; I² = 3.9%).

Daclatasvir dose 60 mg vs 20 mg

For all efficacy outcomes, data were presented in two subgroups according to the dose of daclatasvir (60 mg vs 20 mg). There was no statistically significant difference between the two doses in all efficacy outcomes (test for subgroup analysis: p>0.05).

Discussion

Summary of evidence

This study provides class one evidence that daclatasvir plus Peg-IFN/RBV regimen achieves moderate efficacy in treatment of chronic HCV infection genotype 4. The overall effect size of SVR, RVR, eRVR, cEVR, and EOTR rates was higher in the daclatasvir plus Peg-IFN/RBV compared to the placebo plus Peg-IFN/RBV group.

Daclatasvir dose 20 mg vs 60 mg

Two doses of daclatasvir (20 and 60 mg) were investigated in included clinical trials and were pooled in our analysis. Our results showed that both doses achieved comparable SVR rates at 12 and 24 weeks (Hézode et al., 2014). However, it is expected that the 60 mg dose might provide higher SVR rates in patients with insufficient response to other regimens such as patients with cirrhosis, those with an initially high viral load, and resistant polymorphism substitutions (Chan et al., 2012).

Comparison to other regimens

Although daclatasvir plus Peg-IFN/RBV regimen achieved higher SVR rates than the dual regimen, it showed moderate SVR rates of 76% after 12 weeks and 79% after 24 weeks. In a previous study, genotype 4 patients treated with a combination of sofosbuvir and

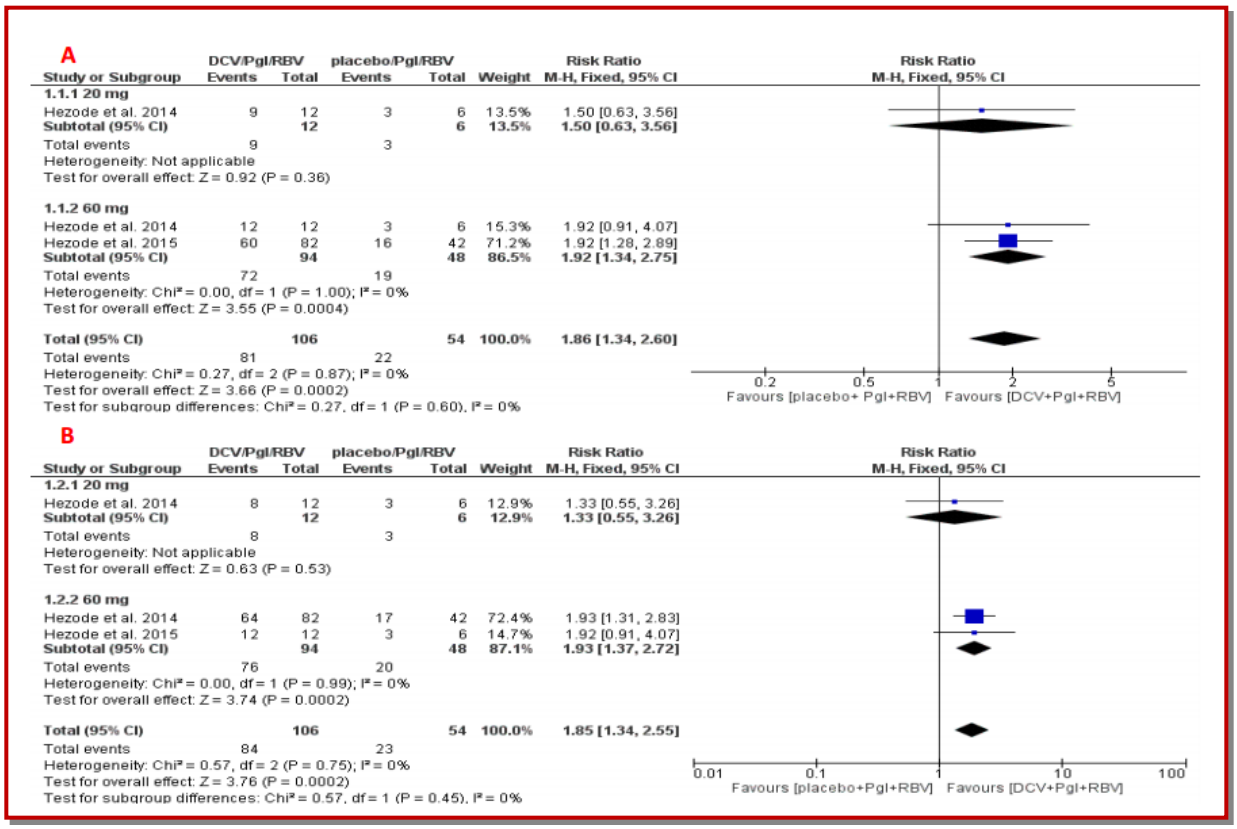

Figure 3: Forest plots comparing the dual and triple regimens in terms of relapse rate (A) and rapid virologic response rate (B)

Peg-IFN/RBV achieved a SVR rate of 96%. In another study, genotype 4 patients treated with simeprevir plus Peg-IFN/RBV achieved a SVR rate of 83% (Lawitz et al., 2013; Moreno et al., 2014). In comparison to these regimens, the daclatasvir plus Peg-IFN/RBV regimen is not strongly recommended for treatment of HCV genotype 4 patients. However, the evidence is insufficient and further trials are required to investigate the efficacy of this regimen in HCV genotype 4 patients.

Recent data in the literature suggests that most oral combinations of direct antiviral agents provide high SVR rates with shorter treatment duration, excellent tolerability, and low rates of virological relapse. Fortunately, most of these combinations allow for an interferon-free treatment regimen and therefore, less complications (Everson et al., 2014; Hassanein et al., 2014; Kumada et al., 2014; Suzuki et al., 2013).

Combining daclatasvir with other direct antiviral agents such as asunaprevir, sofosbuvir or BMS-791325 have shown a high SVR-up to 100%-in treatment naïve genotype 1 and 2 patients (Everson et al., 2014; Sulkowski et al., 2014). A recent meta-analysis has shown that combining daclatasvir and sofosbuvir achieved a SVR rate of 88.8% at 12 weeks in patients infected with genotype 3 HCV (Swallow et al., 2015). Sofosbuvir plus daclatasvir combination has been recently approved in the US and EU for treatment of genotype 3 (Pawlotsky et al., 2015). Combining daclatasvir with asunaprevir plus Peg-IFN/RBV achieved a SVR of 100% ingenotype 1 and 4 non-responders (Jensen et al., 2015).

Because late relapse is extremely low, successful treatment is usually measured by achieving a SVR at any point of treatment (Smith-Palmer et al., 2015). Long-term follow-up studies have shown that achieving a SVR is associated with lower mortality rates and treatment costs with improvement of health related quality of life (Jafferbhoy et al., 2010; Larrey et al., 2014). Moreover, lowering the incidence of treatment emergent adverse events and shortening of the treatment course observed within the daclatasvir group in this study positively influenced the patients' adherence to treatment.

Although cirrhotic patients were not well represented in both included studies, Hezode et al. (2014) reported that cirrhotic patients treated with 20 and 60 mg of daclatasvir achieved SVR rates of 62 and 63% respectively, in contrast to 38% achieved by patients treated with Peg-IFN/RBV alone (Hézode et al., 2014). Further evaluation of the efficacy of this regimen in cirrhotic population is needed.

Different genotypic subtypes may have different response rates to daclatasvir treatment. Genotype 4a and 4d are the most prevalent phenotypes, especially in Egypt, Europe, and Saudi Arabia (Al Ashgar et al., 2013).

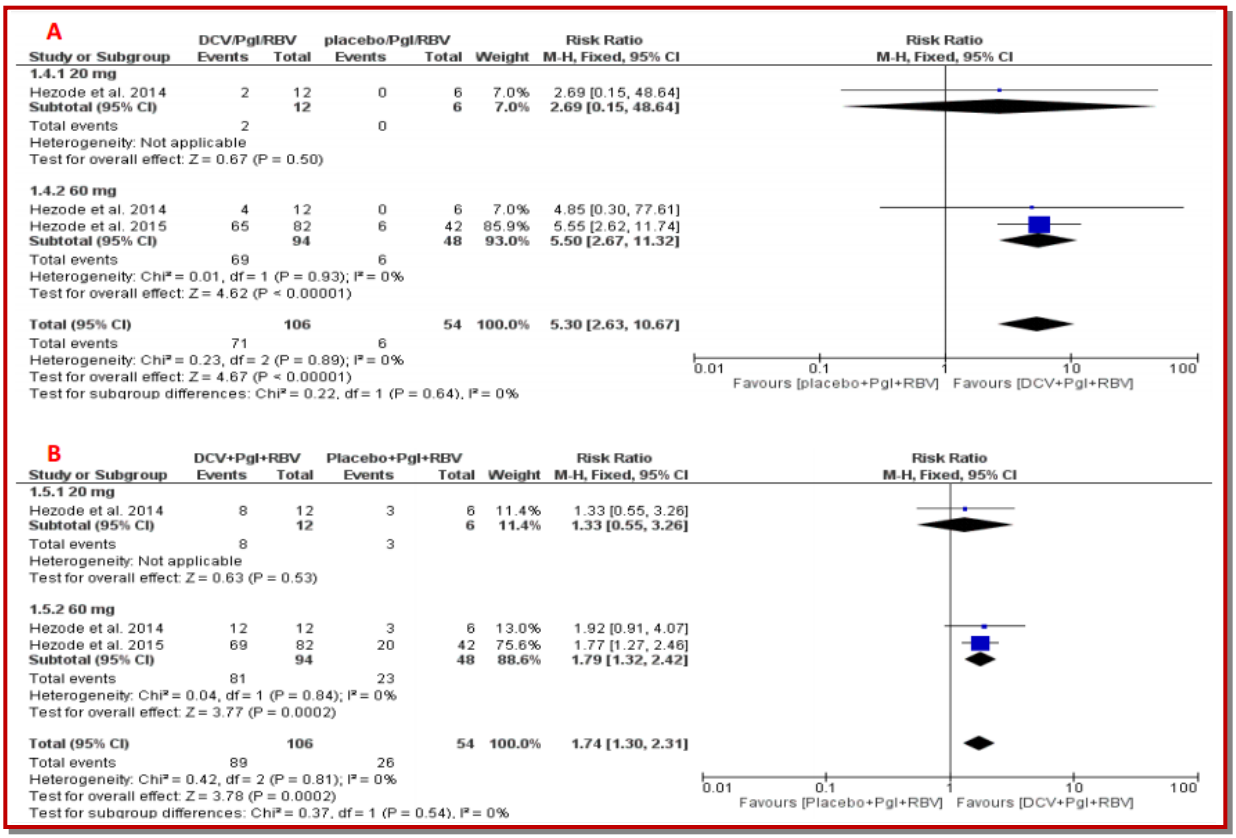

Figure 4: Forest plots comparing the dual and triple regimens in terms of extended rapid virologic response rate (A) and complete early virologic response rate (B)

Both subtypes were well represented in the included study by Hezode et al. (2015), which showed that the triple regimen achieved higher SVR rates in genotype 1b and 4, compared to genotype 1a (Hézode et al., 2015). Further evaluation of the impact of different genotypic subtypes on virological response rates to the daclatasvir-containing regimen is required in larger clinical trials.

Few reports described that NS5A genetic polymorphisms can influence virological response to daclatasvir in HCV genotype 4 patients (Fridell et al., 2011; Gao et al., 2010; Hézode et al., 2014; Zhou et al., 2016). One hundred thirty four out of 229 HCV genotype 4 patients had NS5A polymorphisms (Zhou et al., 2016). They found that the most common NS5A polymorphism was L30R substitution, which is estimated to decrease the response to daclatasvir by 10 folds, compared to patients without this polymorphism (Zhou et al., 2016). Hezode et al. (2014) reported that patients with CC IL28B genotype had a higher chance of achieving SVR, compared to those with a non-CC genotype, regardless of therapy (Hézode et al., 2014). Also, as shown by *in vivo* and *in vitro* studies, the combination of L30 polymorphism and IL28B non-CC genotype can significantly increase daclatasvir resistance (Wang et al., 2012; Wang et al., 2014).

Although safety outcomes of daclatasvir were not eligible for quantitative analysis, both included studies reported that adding daclatasvir to Peg-IFN/RBV did not increase the rate of adverse events, compared to the dual regimen. The most frequently reported adverse events in both regimens were headache, fatigue and nausea (Hézode et al., 2014; Hézode et al., 2015).

Overall completeness of evidence

Of the 154 patients included in this analysis, there were 39 discontinuations (25.3%) in the two included trials (daclatasvir 23/106 and placebo 16/48). However, we believe this is unlikely to affect the analysis outcomes because the investigators of both studies analyzed their data in an intention to treat approach by considering all patients allocated to study arms, regardless of any discontinuation following randomization

Strength points

Both studies included in this analysis were of low risk of bias as indicated by Cochrane risk of bias assessment tool. We followed the PRISMA statement guidelines during preparation and reporting of this meta-analysis and conducted all steps in accordance to Cochrane handbook of systematic reviews of interventions. We also conducted a subgroup analysis to evaluate the effect of daclatasvir dose on the patients' response.

Limitations

The relatively small number of available studies discussing our objective limits the generalizability of our findings. Both included studies enrolled only treatment naïve patients. Future studies should evaluate the efficacy of the same regimen in partial or null responders.

Conclusion

The present meta-analysis shows that the triple regimen of daclatasvir plus Peg-IFN/RBV achieved higher response and lower relapse rates than the dual regimen of Peg-IFN/RBV. Both daclatasvir doses (60 mg vs 20 mg) achieved similarly moderate virological response rates. However, the current evidence is not sufficient and further randomized controlled trials are needed.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflicts of interest

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Author Info
Abdelrahman Ibrahim Abushouk (Principal contact)
e-mail: Abdelrahman.Abushouk@med.asu.edu.eg

Hezode, 2014		
Domain	**Risk of Bias**	**Author judgment (quoting from the text)**
Random sequence generation (selection bias)	Low	Quote: "phase2b study Using a randomized block design stratified & randomization ratio is 2:2:1 as follow daclatasvir-20mg ,60mg ,placebo groups" Comment: Probably done "patients were randomly allocated"
Concealment of allocation (selection bias)	Low	Quote:" using interactive voice response system" Comment: Probably done "central allocation"
Blinding of participants &personnel (performance bias)	Low	Quote: "double blind" Patient & study site were blinded to HCV-RNA value &patient randomization"" Comment: Probably done
Blinding of outcome assessors (detection bias)	Low	Quote: "double blind" Sponsor was blinded to treatment assignment"" Comment: Probably done
Incomplete data reporting (attrition bias)	Low	Quote:" Modified intention to treat analysis done to all patient "
Selective outcome reporting (reporting bias)	Low	The protocol is available & all pre-specified outcomes have been reported in pre-specified way
others	Unclear	

Hezode, 2015		
Domain	**Risk of Bias**	**Author judgment (quoting from the text)**
Random sequence generation (selection bias)	low	Quote: " Multicenter phase 3 study ,randomization using block design 2:1 to DCV: placebo within each block ,stratified" Comment: Probably done "patients were randomly allocated
Concealment of allocation (selection bias)	low	Quote:" using validated centralized interactive voice response system for randomization" Comment: Probably done "central allocation
Blinding of participants &personnel (performance bias)	low	Quote :"Double blind(reported in protocol: blinded patient & investigator)" Comment: Probably done
Blinding of outcome assessors (detection bias)	low	Quote :"Double blind(regard protocol sponsor is blinded)" Comment: Probably done
Incomplete data reporting (attrition bias)	low	Quote:" Modified intention to treat analysis done with patient with missing HCV RNA at post treatment week 12 considered as failure"
Selective outcome reporting (reporting bias)	low	The protocol is available & all pre-specified outcomes have been reported in pre-specified way
others	Unclear	-Protocol deviation One patient with GT1a infection was incorrectly enrolled in DCV plus peg IFN/RBV group &was included in the analysis due to the pre-specified MITT analysis -The limitation was a limited sample size

Supplementary data shows the results of risk of bias assessment for included studies