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Improvement in Hepatitis "C" viral infection with advanced antiviral therapy treatment – a meta-analysis

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Abbreviations

EOT	End of treatment
eRVR	Extended Rapid viral response
ETR	End of treatment response
FDA	Food and Drug Administration
HCC	Hepatic Cellular Carcinoma
HCV	Hepatitis C Virus
INF	Interferon
LDV	Ledipasvir
NANB	non A non B
RBV	Ribavirin
RR	Relative Risk
RVR	Rapid viral response
SOF	Sofosbuvir
SVR	Sustained Virological Response

Abstract

Background

Hepatitis is the inflammation of liver and there are many reasons of hepatitis. Among these reasons the viral hepatitis due to hepatitis B virus (HBV) and hepatitis C Virus (HCV) are the common hepatic infections and the major causes of liver cancer and liver cirrhosis in the world especially in Asia and Medal east. HBV and HCV infections are having very high mortality ratio in Europe and every year millions of people become infected and die due to HBV or HCV in Europe and Worldwide. That's why viral hepatitis needs a serious consideration and perfect treatment.

Objective

Objective of the study was to analyze the role of ribavirin in HCV treatment and to observe the virological response and relapse against it by using statistical meta-analytical techniques.

Selection criteria and data collection

For this meta-analysis I used the publication databases PubMed, ScienceDirect and Google Scholar to collect all research articles describing treatment and response of HCV and using the antiviral drugs Ledipasvir and Sofosbuvir (control group) in combination with the active agent Ribavirin.

Method and Methodology

Meta-analysis was performed using the statistical package R and the package metafore.

Result and conclusion

The result shows that the use of ribavirin with control group (ledipasvir and sofosbuvir) did not show a significant impact on the sustained virological response (SVR) during and after treatment. But addition of ribavirin in control group effectively reduced the rate of relapse after treatment.

1. Introduction

1.1 Hepatitis C virus (HCV)

Virus that causes Hepatitis C was discovered as non-A non-B hepatitis virus (Choo *et al.,* 1989). The Hepatitis C virus (HCV) with a total genome of 9.5 kilo base (Kb) (Safi, *et al.,* 2010) belongs to genus Hepacivirus (Qadri *et al.,* 2004) in the *Flaviviridae* family (Schmaljohn and McClain *et al.,* 1996). It is a positive single stranded RNA virus (Lindenbach *et al.,* 2001). HCV is enveloped by two types of glycoproteins E1 and E2 (Op-De., et al 2003). HCV has seven genotypes (Nakano *et al.,* 2011) and can be differentiated on the basis of genetic makeup (Jose *et al.,* 2002). Among all seven genotypes, the genotype 1 is the major and very common genotype worldwide especially in the USA and Europe and it covers 46.2% of all HCV infected population of the world (Franciscus., 2015). HCV genotype 3 is the main genotype found in HCV patients of Pakistan (Safi, *et al.,* 2010) and is covering 30% of world wide HCV infected population (Franciscus., 2015). Genotype 5 is the prevalent genotype in South Africa while 6 is dominant in Hong Kong (Idrees & Riazuddin., 2008).

1.2 Hepatitis C Infection

Hepatitis C virus (HCV) mainly attacks the hepatocytes (liver cells) (Jacobson *et al.,* 2010) where it multiplies (replicates) and kill liver cells (Franciscus., 2015) and thus HCV positive patients may develop chronic liver disease and hepatocellular carcinoma (HCC) (Jacobson *et al.,* 2010). In 60 to 80% of HCV positive patients the acute state of hepatitis infection changes to chronic infection and then to hepatic steatosis (Qadri *et al.,* 2006; Waris *et al.,* 2007). Changes in lipid metabolism associated with HCV leads to steatosis (Marzouk *et al.,* 2007) and among all genotypes of HCV, steatosis is frequently found in patients who are infected by HCV genotype 3 (Guo *et al.,* 2007; Gupte *et al.,* 2006). Hepatic steatosis is a severe condition of fluid accumulation in the hepatocytes, and this fluid accumulation is the major indication of cirrhosis development (Yoon & Hu., 2006). Cirrhosis is a condition in which liver does not work properly and fibrosis starts in liver cells which leads to hepatocellular carcinoma. Hepatocellular carcinoma associated with Hepatitis B or Hepatitis C is one of the common malignancy with estimated annual cases from 500000 to 1000000 annually (Bosch *et al.,* 2004; Parkin *et al.,* 2002). Over the last 20 years the ratio of HCV mortality has been increasing continuously (Davis *et al.,* 2010). According to the Center for

Disease Control and Prevention, nowadays more than 3.2 million of the total world population is victims of the Hepatitis C virus (CDC., 2014). About 60% of these patients have the genotype 1 strain of Hepatitis C virus (Sharma & Sherker., 2009).

1.3 HCV treatments at a glance

In 1960's when only hepatitis A and hepatitis B were known, the treatment of these hepatitis were directed on strict bed rest, nutritious, diets and symptomatic medications (Seeff *et al.,* 1982). Corticosteroid were then used to reduce level of serum bilirubin but in many cases the use of corticosteroid possibly boost up the acute state of hepatitis B to chronic state of hepatitis B (Perrillo *et al.,* 2001). In 1970's when non-A non-B (NANB) hepatitis had been recognized a new era for treatment of different viral hepatitis was opened (Feinstone *et al.,* 1975) and various drugs were then checked out but almost all had slight positive outcome or literally generate very harmful side effects (Blumberg *et al.,* 1967; Ferenci., 1993). Acyclovir which is amongst the first antiviral drugs were also failed to treat viral hepatitis (Pappas *et al.,* 1985).

Among different medicines interferon (IFN) were seems to be effective for the treatment of viral hepatitis (Greenberg *et al.,* 1976) and use of recombinant interferon *alfa* was started for treatment of HCV infection (Hoofnagle *et al.,* 1986). But there were several disadvantages of interferon *alfa*, as it was available only in injectable form and the minimum treatment duration was 1 year of weekly injection with many side effects like myalgia, asthenia, cytopenia, influenza-like symptoms and depression (Sulkowski *et al.,* 2011; Heim *et al.,* 2013). However as compared with other antiviral drugs the encouraging results of interferon, open the way to use interferon for several dose administration, treatment length and drugs combination trials to treat NANB hepatitis (Davis *et al.,* 1989). An interferon trail dose of 3 times a week for 24 weeks, induced 6% sustained virological response (SVR) in almost one-third of patients but there were still high rate of rebooting of infection and relapse and when treatment length increased to 48 weeks the rate of SVR rose to 16% (Davis *et al.,* 1989; Hoofnagle *et al.,* 1986).

In 1990's a new drug ribavirin (RBV) which is a protease inhibitor were used to reduce the alanine aminotransferase, the side effects of ribavirin were lesser than any antiviral used for HCV treatment but using as a mono-therapy its efficacy was very low (Dusheiko *et al.,* 1996). However when RBV and interferon *alfa* were used in combination for 24 weeks the

rate of SVR raised up to 34% and when length of treatment exceeded to 48 weeks the rate of SVR raised up to 42% (McHutchison *et al.,* 1998; Poynard *et al.,* 1998) and patients showed long-term prognosis and this was the first milestone in HCV treatment (Maylin *et al.,* 2008; Pearlman *et al.,* 2011).

The next achievement was the increase of IFN's half-life through the process of pegylation, It result in the form of long-acting pegylated interferon (peginterferon) which lower down the frequency of injection and enhanced the SVR rate to 39% when used alone for 48 weeks (Reddy *et al.,* 2001). The SVR rate rose to 56% when peginterferon used in combination with RBV (Lindsay *et al.,* 2001). This combination accomplished a high level of sustained virological response (SVR) with reduction of treatment time (Jacobson *et al.,* 2011; Bacon et al; 2011), but on other hand combination of ribavirin with peginterferon increased the frequency of side effects and drug to drug interaction which results in premature discontinuation of treatment for many patients (Maasoumy *et al.,* 2013; Chen *et al.,* 2013).

In 2013 the Food and Drug Administration (FDA) approved the use of oral nucleotide inhibitor of the HCV NS5B polymerase for the treatment of HCV infection. This polymerase inhibiter is called Sofosbuvir (known as GS-7977; Gilead Sciences) which was initially used in combination with interferon and ribavirin (Lawitz and Lalezari *et al.,* 2013; Lawitz and Ghalib *et al.,* 2013). Another novel nucleotide analog inhibitor of HCV NS5A polymerase named Ledipasvir (known as GS-5885; Gilead Sciences) expressed potent antiviral activity against 1a and 1b genotypes of HCV (Lawitz *et al.,* 2012). Combinations of Sofosbuvir (SOF) and Ledipasvir (LDV) were found safe with no significant pharmacokinetic drug to drug interaction (German *et al.,* 2012).

1.4 Aims of study

The primary goal of this study is to systemically analyze the sustained virological response (SVR) and relapse of HCV infection, treated with a combination of Sofosbuvir and Ledipasvir with and without the addition of Ribavirin. The study is conducted as a meta-analysis using available randomized clinical trials for SVR during and after treatment with combinations of Sofosbuvir and Ledipasvir with or without ribavirin at 08, 12 and 24 week treatment regimes and to investigate the risk of relapse after treatment.

1.5 Research Question

What will be the effect of ribavirin on SVR and relapse of HCV infection when use with sofosbuvir and ledipasvir?

2. Methods

2.1 Research articles search

A computerized search was done to retrieve research articles using PubMed, ScienceDirect and Google Scholar. For PubMed the filter was set to retrieve only those literatures which were full text, clinical trials and since 2014. Using this filter a search was done with four different search characters which were "HCV treatment, HCV treatment response, Ledipasvir & Sofosbuvir, HCV medication" and from this 204, 131, 21 and 9 articles were retrieved respectively. For ScienceDirect the filter option was set to retrieve Journals since 2015 and search character "Ledipasvir & Sofosbuvir" were used which come up with of 129 articles. In Google Scholar the filter sitting was set for articles since 2014 and word "HCV treatment" was searched which results in outcome of 117 articles. So total of 611 studies were retrieved.

The initial screening of total retrieved articles were done looking for ribavirin as a tested drug, sofosbuvir and ledipasvir as control group and same systematic plan for therapy with diet (Regimen) of 8, 12 and 24 weeks. To explain the initial screening criteria the articles with ribavirin as tested drug were selected because we were interested in ribavirin (a protease inhibiter) to check its efficacy and effect with control group of sofosbuvir and ledipasvir (nucleotide inhibitors) and this screening reduced the number of 611 articles to 44 articles.

To get more specific articles the final screening of 44 studies were done looking for same genotype (genotype 1), RNA measuring parameter of 25 IU/ml and reading time frame of sustained virological response (SVR) at 2, 4 and 12 week. Explaining the filter options genotype 1 were choose because the ratio of HCV cases with genotype 1 are higher than any other HCV genotype in the World and in Europe as well that's why we focused on genotype 1. IU/ml is the unit for viral RNA quantification and different researchers use different quantity of viral RNA to measure SVR (in the patient's blood sample) but to get same quantification measurement we choose 25 IU/ml because mostly researchers use this limit of quantification. Articles with reading time frame of sustained virological response (SVR) with 2, 4 and 12 weeks were choose because 2, 4 and 12 weeks of RNA detection provides you Early, Rapid and sustained virological responses respectively. However in this screening only 5 articles meet the required criteria (Figure 1). The final selected articles were Afdhal *et al.,* 2014; Kris *et al.*, 2014; Nezam *et al.,* 2014; Bourlière *et al.,* 2015 and Lawitz *et al.,* 2014.

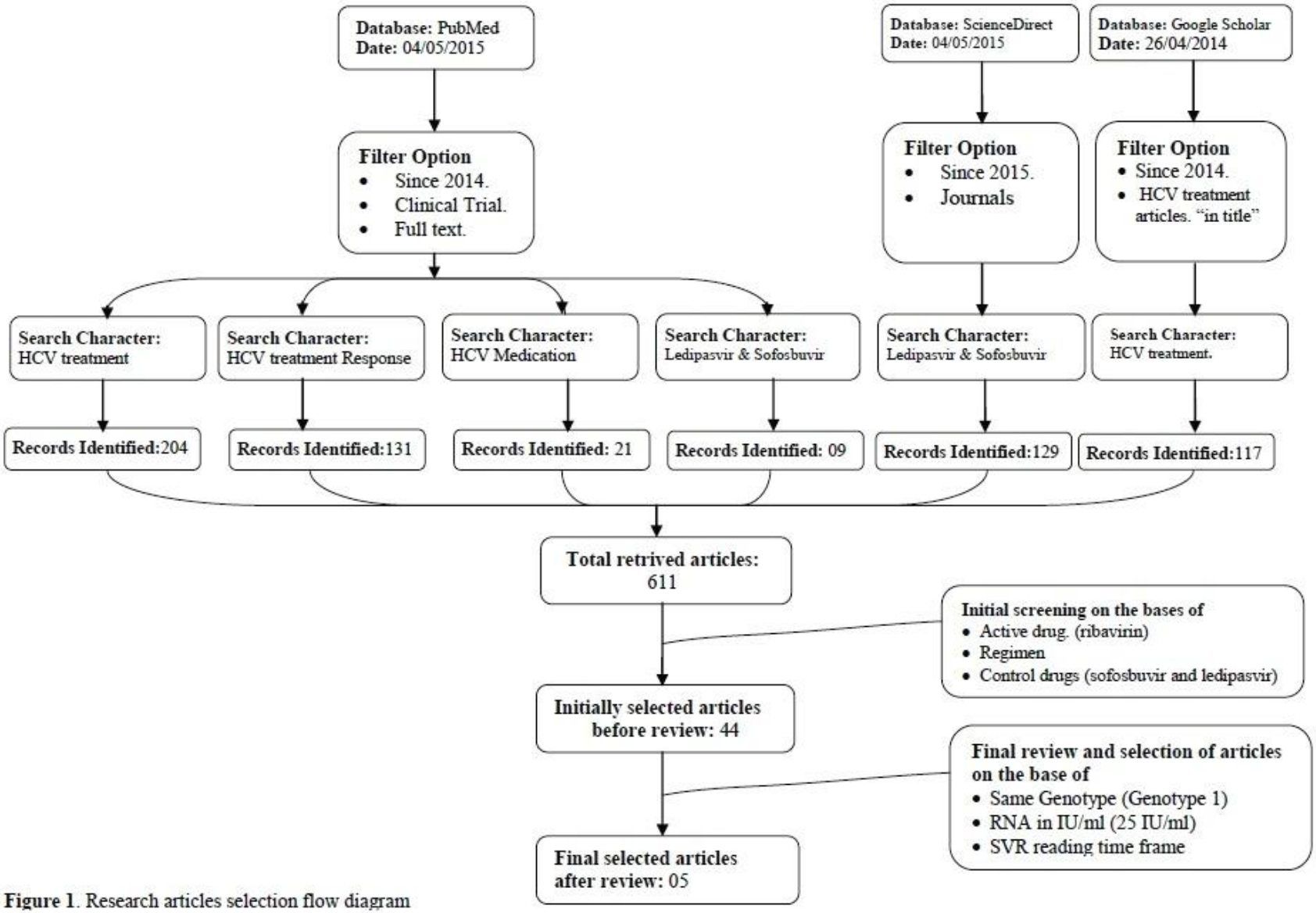

Figure 1. Research articles selection flow diagram

2.2 Meta-analysis

The effect of ribavirin in combination with sofosbuvir and ledipasvir was analyzed as the SVR during and after treatment and the risk for relapse after treatment calculated as the log risk ratio (logRR) and analyzed in random effects meta-analysis models in the statistical program R version 3.2.0 (R Core Team., 2015) and the package metafor (Viechtbauer., 2010).

3. Results

3.1 Data collection

From all selected studies the data of sustained virological response (SVR) during treatment (Table. 1) and after treatment (Table. 2) with observation time of 2 weeks 4 weeks and at the end of regimen was checked against Sofosbuvir and Ledipasvir (control group) with or without Ribavirin along with effect of Ribavirin on relapse after treatment (Table. 3). For all these tables the data were taken from 5 selected studies and the repetitions of same author's name in these tables are due to different regimen and SVR reading time frame. Different researchers followed different treatment time length and response observation period protocols.

Table 1. Response during treatment

Author and Year	**tresp**	**tnresp**	**cresp**	**cnresp**	**regim**	**time**
Afdhal *et al.,* 2014	92	19	89	20	12w	2w
Afdhal *et al.,* 2014	110	1	109	0	12w	4w
Afdhal *et al.,* 2014	111	0	108	1	12w	12w
Afdhal *et al.,* 2014	93	18	89	20	24w	2w
Afdhal *et al.,* 2014	110	1	108	1	24w	4w
Afdhal *et al.,* 2014	110	1	109	0	24w	24w
Kris *et al.,* 2014	195	21	190	25	8W	2w
Kris *et al.,* 2014	211	2	215	0	8w	8w
Nezam *et al.,* 2014	181	36	174	39	12w	2w
Nezam *et al.,* 2014	215	2	213	0	12w	4w
Nezam *et al.,* 2014	214	0	213	0	12w	12w
Nezam *et al.,* 2014	180	37	179	37	24w	2w
Nezam *et al.,* 2014	217	0	216	0	24w	4w
Nezam *et al.,* 2014	216	0	213	1	24w	24w
Bourlière *et al.,* 2015	75	2	75	2	12w	4w
Bourlière *et al.,* 2015	77	0	77	0	12w	12w
Lawitz *et al.,* 2014	21	0	20	0	8w	4w
Lawitz *et al.,* 2014	21	0	20	0	8w	8w

Treatment response=tresp. Treatment no-response=tnresp. Control response=cresp. Control no-response=cnresp. Regimen=regim. Observation time=time.

Table 1 is expressing the number of individual patients who develop or did not develop SVR against active and control drugs during treatment. In control Treatment combination of two drugs Ledipasvir and Sofosbuvir were used while in active treatment the testing drug Ribavirin with combination of control were used. Looking at the treatment and regimen time, different authors used different time line protocols of 8, 12 and 24 weeks and then the authors

observed first viral response during treatment either at 2nd week (rapid viral response (RVR)) or at 4th week (extended rapid viral response (eRVR)) from starting of medication and finally SVR at the end of individual regimens. At this position the SVR is called End of treatment response (ETR).

Table 2. Response after treatment

Author & Year	tresp	tnresp	cresp	cnresp	regim	time
Afdhal *et al.*, 2014	107	4	103	6	12w	4w
Afdhal *et al.*, 2014	107	4	102	7	12w	12w
Afdhal *et al.*, 2014	110	1	109	0	24w	4w
Afdhal *et al.*, 2014	110	1	108	1	24w	12w
Kris *et al.*, 2014	205	11	207	8	8w	4w
Kris *et al.*, 2014	201	15	202	13	8w	8w
Nezam *et al.*, 2014	213	1	211	3	12w	4w
Nezam *et al.*, 2014	211	3	211	3	12w	12w
Nezam *et al.*, 2014	215	2	215	2	24w	4w
Nezam *et al.*, 2014	215	2	212	5	24w	12w
Bourlière *et al.*, 2015	75	2	75	2	12w	4w
Bourlière *et al.*, 2015	74	3	75	2	12w	12w
Lawitz *et al.*, 2014	21	0	20	0	8w	4w
Lawitz *et al.*, 2014	21	0	19	1	8w	8w

Treatment response=tresp. Treatment no-response=tnresp. Control response=cresp. Control no-response=cnresp. Regimen=regim. SVR observation time=time.

Table 2 expresses the number of individual patients who develop or did not develop SVR against active drug and control group after treatment. Observing the treatment and regimen time, authors used different time period protocols of 8, 12 and 24 weeks for treatment. After the end of treatment (EOT) and before relapse, authors observed viral response after treatment first at 4th week and secondly on 8th week or either at 12th week.

Table 3. Relapse after treatment

Author and Year	trelap	tnrelap	crelap	cnrelap	time
Afdhal *et al.*, 2014	4	107	7	102	12w
Afdhal *et al.*, 2014	0	111	0	109	24w
Kris *et al.*, 2014	9	204	11	104	8w
Nezam *et al.*, 2014	0	217	1	213	12w
Nezam *et al.*, 2014	0	217	1	216	24w
Bourlière *et al.*, 2015	3	74	2	75	12w
Lawitz *et al.*, 2014	0	21	1	19	8w

Treatment relapse= trelap. Treatment no- relapse = tnrelap. Control relapse = crelap. Control no- relapse = cnrelap. Relapse reading time= time.

The treatment-free time period after completion of therapy during which the infection can rebound or reoccur is called relapse (Dienstag *et al.*, 2006). In case of HCV there are three

types of relapse, number one is “early relapse” in which HCV RNA rebound can be observed any time between end of treatment (EOT) and 12 weeks after EOT, second type is “late relapse” in which HCV RNA rebound observed between 12 and 24 weeks after EOT and third type is “very late relapse” in which HCV RNA rebound observed beyond week 24 of ETO (Medrano *et al.*, 2015). In table 3 the data is showing that the relapse was observed on 8^{th}, 12^{th} and 24^{th} week after EOT by different researchers.

3.2 Result of Response during treatment

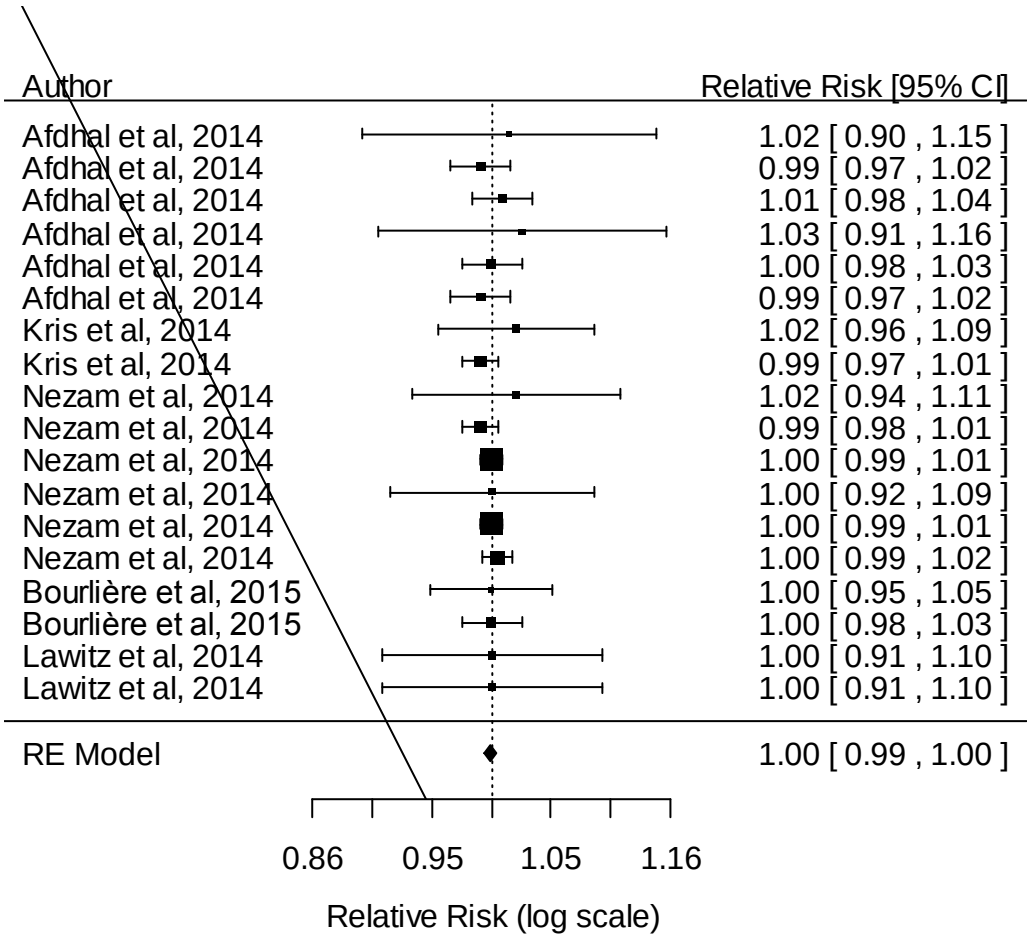

Figure 2. RR for sustained virological response during treatment with sofosbuvir, ledipasvir and ribavirin. The control treatment is sofosbuvir and ledipasvir. RR>1 indicate no treatment effect. The different entries for the same Author represent different regimens and different observation times.

There is no significant effect of ribavirin on the sustained virological response (SVR) during treatment as p-value is 0.6596. RR>1 is expressing the no or low efficacy of experimental drug ribavirin than the control group sofosbuvir and ledipasvir. Figure 2 is showing data from

the five selected studies and the different entries from the same study represent different treatment regimens and observation times.

3.3 Result of Response after treatment

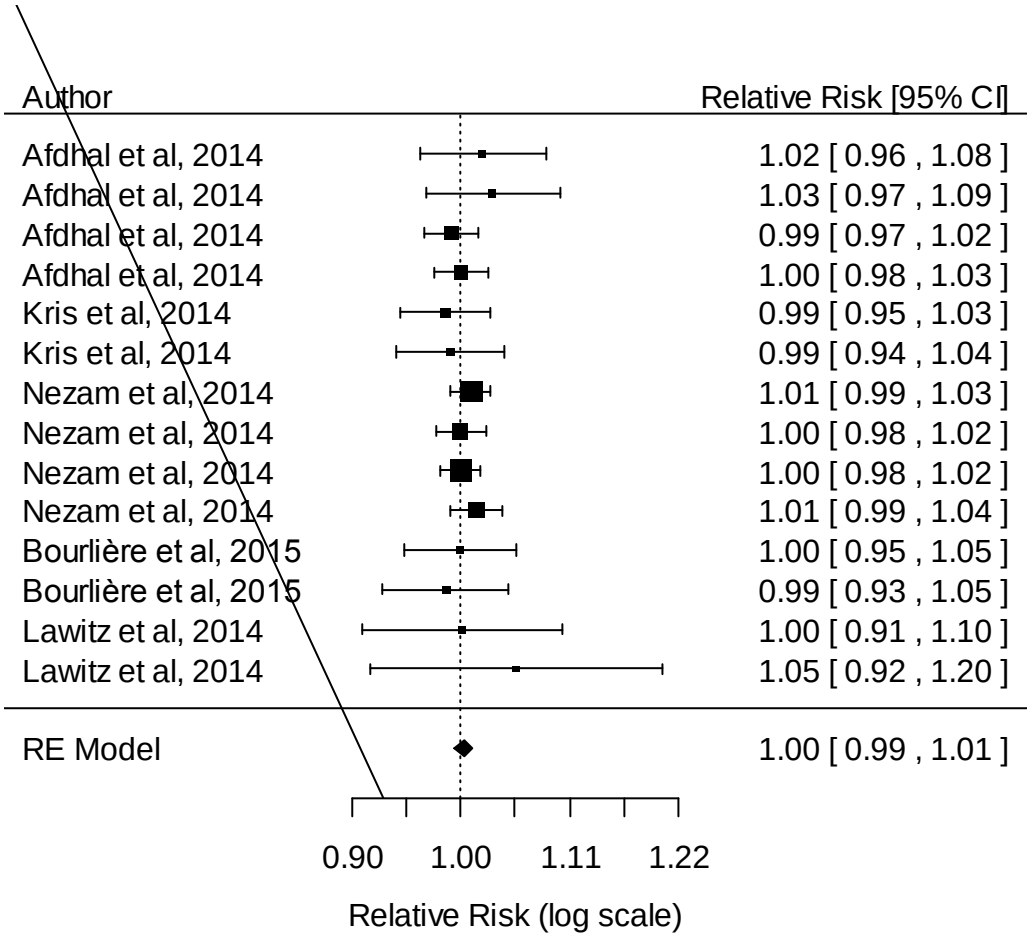

Figure 3. RR for sustained virological response after treatment with sofosbuvir, ledipasvir and ribavirin. The control treatment is sofosbuvir and ledipasvir. RR>1 indicate no treatment effect. The different entries for the same Author represent different regimens and different observation times.

There is no significant effect of ribavirin on the sustained virological response (SVR) after treatment as p-value is 0.5444. RR>1 is expressing the no or low efficacy of experimental drug ribavirin than the control group sofosbuvir and ledipasvir. Figure 3 is showing data from the five selected studies and the different entries from the same study represent different treatment regimens and observation times.

3.4 Result of Relapse after treatment

There is significant effect of ribavirin on relapse after treatment when used together with sofosbuvir and ledipasvir as p-value (0.0410) and RR<1 is expressing the significant effect of experimental drug ribavirin than the control group sofosbuvir and ledipasvir.

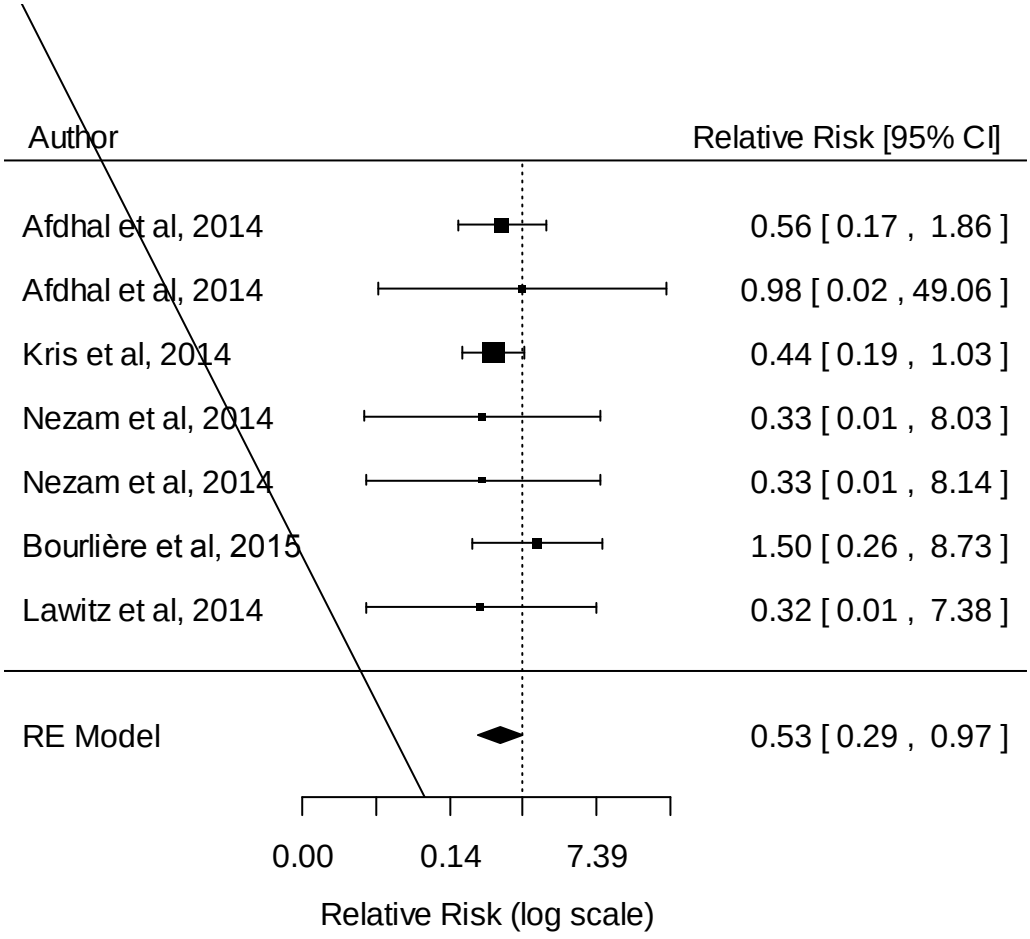

Figure 4. RR for relapse after treatment with sofosbuvir, ledipasvir and ribavirin. The control treatment is sofosbuvir and ledipasvir. RR<1 indicate treatment effect. The different entries for the same Author represent different regimens and different observation times.

4. Discussion

Hepatitis C infection is the infection caused by blood-borne hepatitis C virus (HCV) (Franciscus., 2015). HCV is a positive-sense, 9.5 kilo base (kb) single-stranded RNA(ssRNA) hepatitis C virus (HCV) is the member of genus *Hepacivirus* and family *Flaviviridae* (Schmaljohn and McClain *et al.,* 1996) Till date seven genotypes (Nakano *et al.,* 2011) and approximately 100 subtypes of HCV have been observed (Gower *et al.,* 2014). Among these genotypes the genotypes 1 is most common, and found all over the world (Franciscus., 2015). HCV attacks cells in the liver, causes liver inflammation and eventually kills hepatocytes (Jacobson *et al.,* 2010) and 60 to 80% of HCV infected patients become chronically infected (Qadri *et al.,* 2006; Waris *et al.,* 2007). Mostly people in the state of chronic hepatitis C infection do not have clear signs and symptoms and live a normal life (Franciscus., 2015). However in 10–25% of people with chronic HCV, the disease lead to serious liver damage, cirrhosis and finally hepatocellular carcinoma (HCC) (Jacobson *et al.,* 2010). Viral associated HCC is fifth common type of malignancy in the world and majority of HCC cases occurs in developing countries (Bosch *et al.,* 2004; Parkin *et al.,* 2002). There are mainly two types of virus which cause HCC, hepatitis B virus (HBV) and hepatitis C virus. HBV is a DNA while HCV is an RNA virus and thus at molecular level these two viruses are significantly different and the mechanisms by which these viruses cause cancer are sufficiently distinct mechanisms. HBV has inherent oncogenic property and is able to cause direct HCC without entering in the state of cirrhosis while HCV first cause cirrhosis and then HCC (Fung *et al.,* 2009).

Annually millions of people become infected and die because of HCV. Due to high morbidity and mortality rate of HCV its treatment and cure is one of the burning issue and need of time. In the past different drugs with different regimens, different treatment length and different dosage were used. Looking at the treatment of HCV, the cure of HCV is depending on many factors, like which drug is using to treat, which genotype is treating, what is the state of liver damage at start point of treatment, what was the previous treatment and many more factors. Keeping these factors in mind we will discuss and compare the results of efficacy with other studies at different aspects.

In a study by Lawitz *et al* (2013) the SVR of triple therapy sofosbuvir, peginterferon *alfa* plus ribavirin was compared with dual therapy of peginterferon and ribavirin in HCV genotype 1 infected patients at 12 week treatment regimen. As a result the SVR of triple therapy were

higher than peginterferon and ribavirin only. Another study by same author Lawitz *et al* (2012) showed that HCV infected patients treated with sofosbuvir and ribavirin for 12 weeks got higher SVR then those who were treated with peginterferon and ribavirin for 12 weeks. A study by Bourlière *et al* (2013) resulted that the patients who were treated with triple therapy of peginterferon, telaprevir and ribavirin for 12 weeks got higher SVR as compare to the SVR rate of those patients who were only treated with peginterferon and ribavirin.

Comparing the same treatment combination against different HCV genotypes, Osinusi *et al* (2015) trailed the use of sofosbuvir and ribavirin for 24 weeks regimen in patients infected with HCV genotype 1, 2 and 3. As a result of study the treatment efficacy and SVR of sofosbuvir and ribavirin against infected patients with genotype 2 and 3 were highly remarkable but against genotype 1 this trailed drug group of sofosbuvir and ribavirin become failed and patients infected with genotype 1 developed relapse. A study by Zeuzem *et al* (2011) patients with genotype 2 HCV infection were treated with combined therapy of sofosbuvir and ribavirin for 12 week treatment length while patients with genotype 3 HCV infection were treated with combined therapy of sofosbuvir and ribavirin for 24 week treatment length. In these 90% of patients infected by genotype 2 and 77% of patients infected by genotype 3 achieved SVR.

With aspect to the above studies if we look at our project in which the treatment of HCV genotype 1 with selected drugs sofosbuvir and ledipasvir with or without ribavirin were studied and interpreted for sustained virological response (SVR) and relapse. And as a result the tested drug failed to show significant efficacy on response but it expresses significant effect on relapse after treatment. It means that the significant effect of ribavirin on relapse after treatment is the novel finding of this project.

As we know that relapse is the reoccurrence of unhealthy state of infection at the end of therapy. In my opinion relapse is equally important to SVR because a relapse occurs after a long tension-full period of infection, treatment with side effects, unlike diet and uncomfortable routine and when relapse occurs the patients become emotional stressed and it affects his physical and sexual life. So our finding that ribavirin is helpful to stop relapse of HCV infection is very important.

Conclusion

In this study the drug ribavirin was observed in combination with sofosbuvir and ledipasvir. From the result we can conclude that there is no additive effect for the response during and after treatment. The possible reason of non significant efficacy of ribavirin on HCV response during and after treatment may be that the combined therapy of control drugs sofosbuvir and ledipasvir are showing such high level of sustained virological response which neglects the presence of ribavirin. The second possible reason may be drug to drug interaction of ribavirin with sofosbuvir or ledipasvir which keeps the ribavirin suppressed. So efficacy of ribavirin must be tested with other control to investigate the possible right reason of insignificant role of ribavirin in HCV response during and after treatment. But in relapse after treatment the addition of ribavirin in control group of sofosbuvir and ledipasvir showed significant effect. It means that the use of tested drug ribavirin is important to reduce the chance of HCV re-infection.

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