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REVIEW ARTICLE

Antiviral therapy with nucleotide/nucleoside analogues in chronic hepatitis B: A meta-analysis of prospective randomized trials

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Abstract Nucleotide/nucleoside analogues (antiviral therapy) are used in the therapy of HBeAg positive and HBeAg negative chronic hepatitis B. We analyzed ten selected randomized controlled with 2557 patients to estimate the effect of antiviral drugs in chronic hepatitis B with compared to placebo. Virological response, biochemical response, histological response, seroconversion of HBeAg, and loss of HBeAg were estimated as primary efficacy measures. The included studies were subjected for heterogeneity and publication bias. The heterogeneity was assessed with $\chi 2$ and I^2 statistics. Publication bias was assessed by funnel plot. Greater rates of improvement obtained in antiviral group for virological response [43.96 % vs. 3.15 %, RR = 0.57, 95 % CI = 0.54–0.61, *p*-value <0.00001], biochemical response [58.37 % vs. 21.87 %, RR = 0.52, 95 % CI = 0.48–0.56, *p*-value <0.00001], histological response [58.99 % vs. 27.13 %, RR = 0.56, 95 % CI = 0.50–0.63, *p*-value <0.0001], seroconversion of HBeAg [10.66 % vs. 5.56 %, RR = 0.94, 95 % CI = 0.91–0.97, *p*-value = 0.0005], and HBeAg loss [14.59 % vs. 9.64 %, RR = 0.92, 95 % CI = 0.88–0.96, *p*-value = 0.0002]. The safety analysis were carried out for adverse events such as headache [17.22 % vs. 17.34 %, OR = 1.09, 95 % CI = 0.81–1.46, *p*-value = 0.58], abdominal pain [16.46 % vs. 14.34 %, OR = 1.24, 95 % CI = 0.90–1.72, *p*-value = 0.19], and pharyngitis [22.22 % vs. 18.23 %, OR = 1.12, 95 % CI = 0.86–1.45, *p*-value = 0.40]. Excluding adverse events, all primary efficacy measures shown statistical significant result for chronic hepatitis treatment (*p*-value <0.05). Antiviral therapy provided significant benefit for the treatment of chronic hepatitis B with no measurable adverse effects.

Keywords Antiviral drugs · Chronic hepatitis B · Meta-analysis · Nucleotide analogue · Randomized trials

Background

Chronic hepatitis B (CHB) is a severe disease affecting more than two billion of people with 350 million of chronic carriers of it all over the world creating major public health problem [1, 2]. CHB infection is associated with development of cirrhosis in liver and hepatocellular carcinoma. CHB is characterized by the presence of HBeAg or HBsAg in serum, elevated levels of HBV DNA, and alanine aminotransferase level [3]. The available treatments for treating for CHB are interferon (IFN) and antiviral therapy [3, 4]. Over the past three decades, first with interferon alpha, and more recently with the advent of nucleoside analogues, progressively more patients have received treatment.

The antiviral therapy includes different nucleoside/nucleotide analogues (Lamivudine, Adefovir, Entecavir, Emtricitabine, Tenofovir, and Telbivudine) for treating CHB and associated with certain adverse effect. Of these Tenofovir has the highest probability of reducing HBV DNA, normalizing alanine aminotransferase levels, and inducing HBeAg seroconversion after 1 year of treatment [5, 6].

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Pegylated interferon and nucleos(t)ides analogues (NUC) have advantages and limitations, as short-term interferon treatment induces a sustained virological response in a third of patients, whereas long-term suppressive therapy by NUC rapidly inhibits HBV replication in most patients but drug resistance and safety in the long-term will remain the most important unresolved questions [7, 8].

Here, we present a review and meta-analysis of studies that compared nucleoside/nucleotide analogue with placebo for the treatment of CHB.

Methods

We searched the literature published in English from 1990 to 2013. The databases searched included PubMed, Medline, Ovid, Embase, and Cochrane central library. The key words used for the search strategy were "Hepatitis B," "Lamivudine, " "Adefovir," "Entecavir," "Emtricitabine," "Tenofovir," "Telbivudine."

We restricted our search only for placebo-controlled double blind or single blind study. We examined the title and abstract of individual trial for identification. We contacted with authors and expert for any detail and further information about the study. For any unpublished data, we referred clinical trial organization website (clinicaltrial.org).

Inclusion and exclusion criteria

The following criteria are used for selection of trials

- The intervention treatment should be nucleoside/nucleotide analogue and compared with placebo
- It should be double blind randomized control trial
- The patient enrolled in study should be HBeAg positive, HBeAg negative, and HBsAg positive
- The HBV DNA and serum ALT level should be elevated than normal

The exclusion criteria also applied. In following cases, we excluded the study from our analysis

- Coinfection with HIV, HCV, or HDV
- Evidence of decompensated liver disease
- Previous treatment with nucleotide/nucleoside analogue, immunosuppressant, cytotoxic agents, corticosteroids, chemotherapeutic agents, or interferon
- Pregnant or breast feeding women
- Organ or bone marrow transplantation

After examining the inclusion and exclusion criteria, we have included finally 13 studies in analysis.

Extraction of data

The necessary data was extracted from the selected studies [9–18] for analysis. Baseline characteristics are represented in Table 1. The following data was extracted from each study.

- Name of study
- Number of patients
- Year of publication
- Study design
- Age of patients
- Randomization ratio of patients
- Male and female ratio in each study

Table 1 Baseline characteristic of patients involved in the analysis

	de Man et al. [9]	Zeng et al. [10]	Chan et al. [11]	Jonas et al. [12]	Marcellin et al. [13]	Hadziyannis et al. 2003 [14]	Hadziyannis et al. 2005 [15]	Lim et al. [16]	Lai et al. [17]	Tassopoulos et al. [18]
No. of patients	42	480	139	288	515	185	185	240	358	125
Year of publication	2001	2006	2007	2002	2003	2003	2005	2006	1998	1998
Age (years)	38.8/42.1	30/30	40/41	2–17	32/35	46/45	47/47	41/40	32/29	42/44
Male	85.29/87.5	84/82	84/83	–	75.29/71	83/82	82/82	73/69	73.5/72	83/77
Female	14.71/12.5	16/18	16/17	–	24.71/29	17/18	18/18	27/31	26.5/28	17/23
Study design	Double blind	Double blind	Double blind	Double blind	Double blind	Double blind	Double blind	Double blind	Double blind	Double blind
Therapy period	4 weeks	12 weeks	24 months	52 weeks	48 weeks	48 weeks	48 weeks	48 weeks	12 months	52 weeks
Dosage regimen	1 mg once daily	10 mg once daily	100 mg daily	3 mg per Kg of body weight	30 mg once daily	10 mg once daily	10 mg once daily	200 mg once daily	100 mg once daily	100 mg once daily
Intervention	Entecavir	Adefovir Dipivoxil	Lamivudine	Lamivudine	Adefovir Dipivoxil	Adefovir Dipivoxil	Adefovir Dipivoxil	Emtricitabine	Lamivudine	Lamivudine

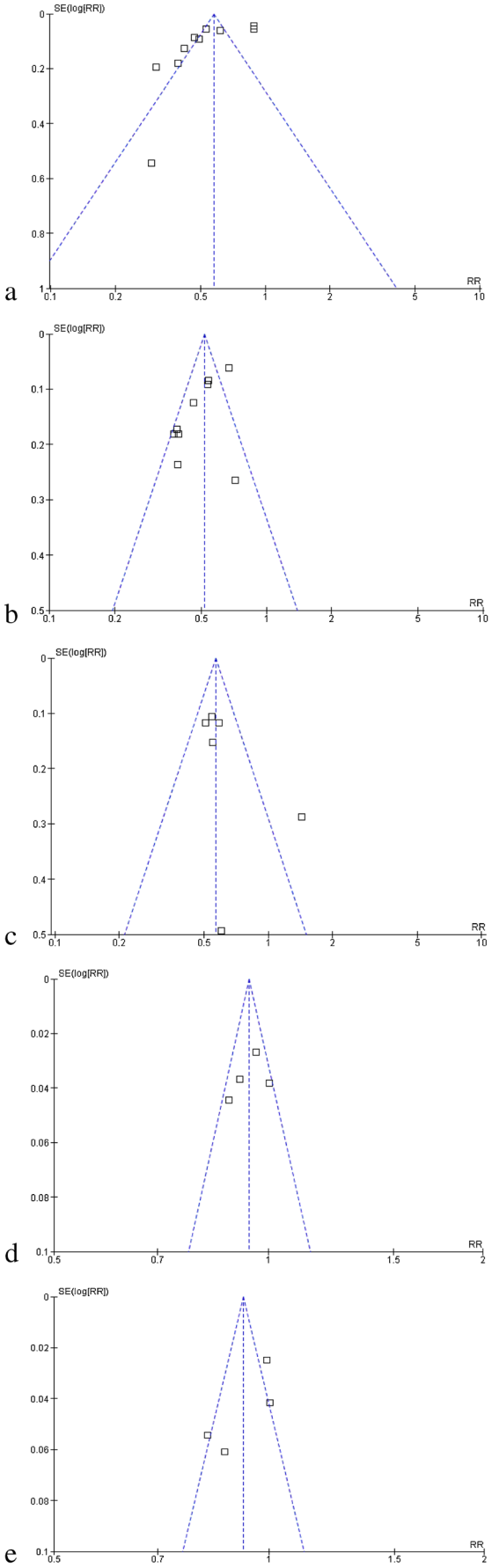


Fig. 1 Publication bias for included studies in meta-analysis. **a** Publication bias for virological response. No significant publication bias recorded for virological response. **b** Publication bias for biochemical response. No publication bias recorded for biochemical response. Studies are symmetrically distributed about estimated risk. **c** Publication bias for histological response. No publication bias recorded for histological response. Studies are systematically distributed about estimated risk. **d** Publication bias for seroconversion of HBeAg. No publication bias recorded for seroconversion of HBeAg as studies are symmetrically distributed about estimated risk. **e** Publication bias for loss of HBeAg. No publication bias has recorded for loss of HBeAg as studies are symmetrically distributed about estimated risk

- HBV DNA level
- Serum ALT level

Endpoint measure

The primary endpoint measures of treatment were sustained virological response (reduction in level HBV DNA and sustained biochemical response (normalization of serum ALT level). We also checked for adverse event resulting from the treatment. The other measures were seroconversion of HBeAg, HBeAg loss.

Statistical analysis

Forest plot was used for graphical representation of confidence interval and odds ratio. The funnel plot was used for detection of publication bias [19, 20]. Heterogeneity was calculated using $\chi 2$ and I^2 index statistics [21]. The effect size measurement was risk ratio and odds ratio. The confidence interval, odds ratio, and *p*-value were calculated for individual studies. We used Review Manager 5 for statistical analysis.

Results

Selection and characteristic of studies

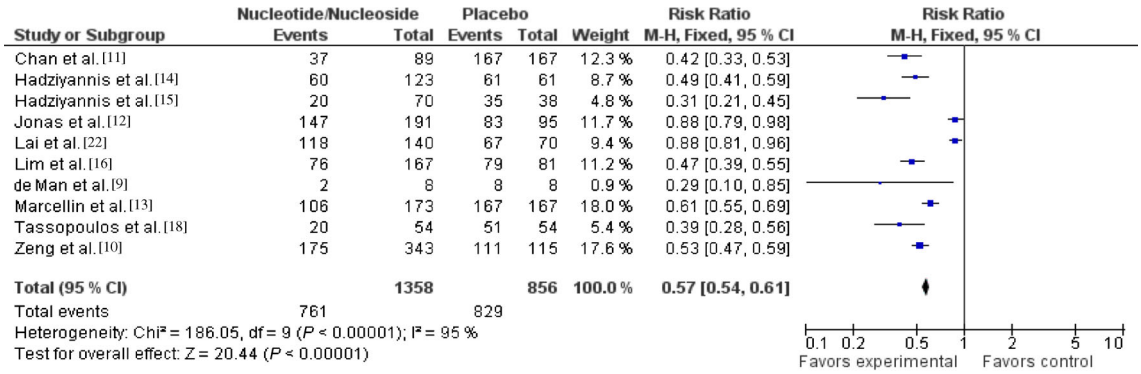
We searched the literature for selection of studies. Only studies published in English selected for meta-analysis. We got 13 studies [9–18, 22–24] and finally included ten studies for meta-analysis after applying inclusion and exclusion criteria [9–18]. Certain studies has given different doses of intervention, we evaluated patients only with high dose of intervention. We studied the baseline characteristic of patients (Table 1). We evaluated total 1987 ($n = 1987$) patients from ten studies. All trials contain nucleotide/nucleoside therapy as intervention treatment and placebo therapy as control treatments. Virological response and

biochemical response evaluated in ten studies with 1987 ($n=1987$). Histological response evaluated in six studies with 1021 patients ($n=1021$). Seroconversion of HBeAg and loss of HBeAg evaluated in four studies with 1247 ($n=1247$) and 1333 ($n=1333$) patients, respectively. We assessed the adverse event too for meta-analysis. We checked for heterogeneity of studies using statistics and I^2 statistics. The publication bias was detected for each efficacy measure using visual inspection of funnel plot.

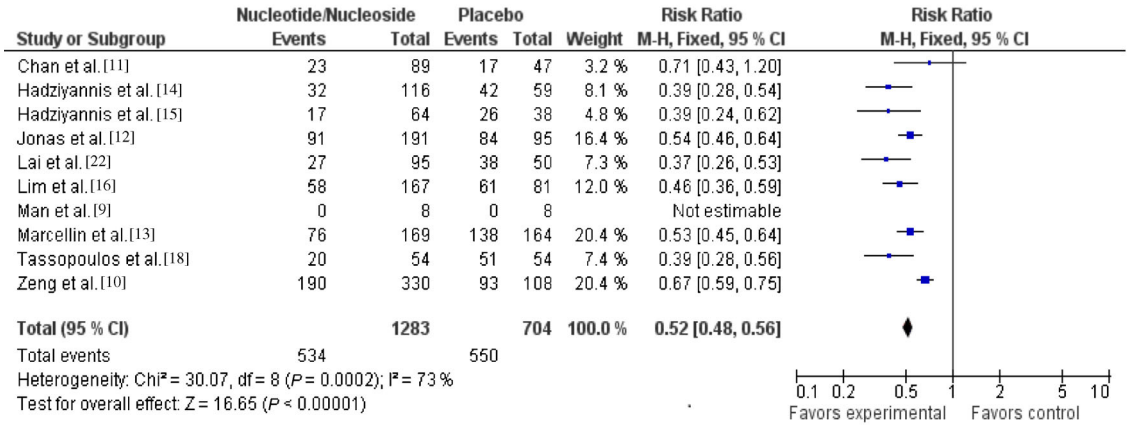
Virological response

Virological response was compared among nucleotide/nucleoside treatment and placebo treatment. Significant difference obtained as compared to placebo. Greater improvements were obtained in nucleotide/nucleoside therapy as compared to placebo [43.96 % vs. 3.15 %, RR = 0.57, 95 % CI = 0.54–0.61, p-value <0.00001] (Fig. 2a). We assessed the heterogeneity [$\chi 2 = 186.05$, $I^2 = 95$ %] (Fig. 2a). The

a.Virological response. Nucleotide/nucleoside treatment vs. placebo



b. Biochemical response. Nucleotide/nucleoside therapy treatment vs. placebo



c. Histological response. Nucleotide/nucleoside therapy vs. placebo

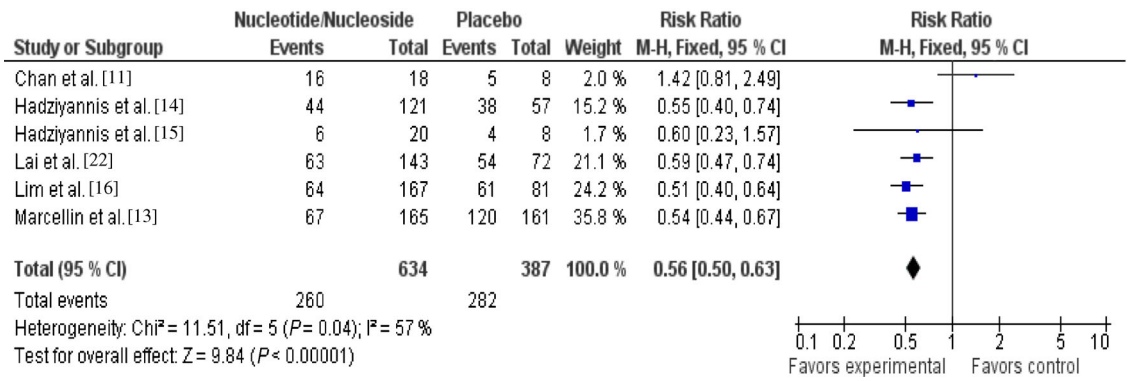


Fig. 2 Forest plot of meta-analysis. **a**. Virological response. Nucleotide/nucleoside treatment vs. placebo. **b**. Biochemical response. Nucleotide/nucleoside therapy treatment vs. placebo. **c**. Histological response. Nucleotide/nucleoside therapy vs. placebo

heterogeneity is present among studies so we use fixed effect model for meta-analysis. No significant publication bias was detected (Fig. 1a).

Biochemical response

Biochemical response was compared among nucleotide/nucleoside and placebo treatment. Greater improvements were obtained in nucleotide/nucleoside therapy as compared to placebo [58.37 % vs. 21.87 %, RR = 0.52, 95 % CI = 0.48–0.56, *p*-value <0.00001] (Fig. 2b). We assessed the heterogeneity [$\chi 2 = 30.07$, $I^2 = 73$ %] (Fig. 2b). No publication bias was recorded (Fig. 1b).

Histological response

Histological response was compared among nucleotide/nucleoside and placebo treatment. Greater improvements were obtained in nucleotide/nucleoside therapy as compared to placebo [58.99 % vs. 27.13 %, RR = 0.56, RR = 0.56, 95 % CI = 0.50–0.63, *p*-value <0.0001] (Fig. 2c). We assessed the heterogeneity [$\chi 2 = 11.51$, $I^2 = 57$ %] (Fig. 2c). Significant differences obtained between the two treatments. No publication bias was recorded (Fig. 1c).

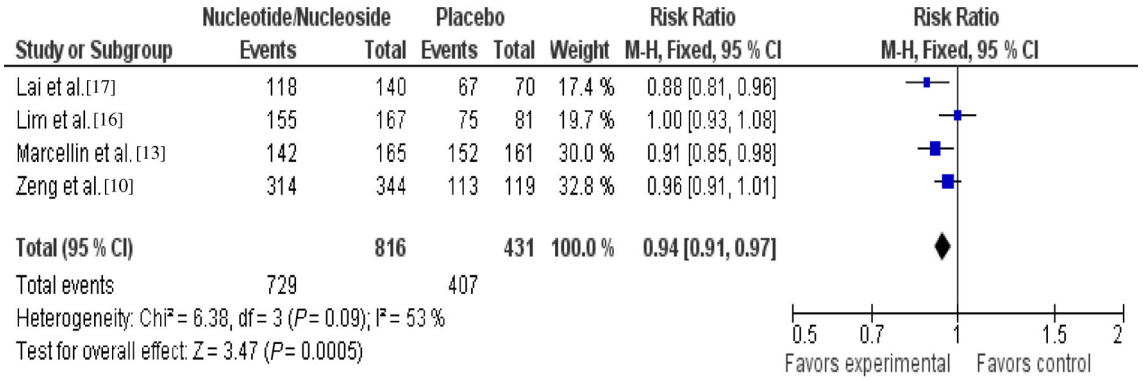
Seroconversion of HBeAg

Seroconversion of HBeAg was compared among nucleotide/nucleoside treatment vs. placebo treatment. Greater rate of formation of antibody (anti-HBeAg) were obtained in nucleotide/nucleoside therapy against placebo therapy [10.66 % vs. 5.56 %, RR = 0.94, 95 % CI = 0.91–0.97, *p*-value = 0.0005] (Fig. 3a). We assessed the heterogeneity [$\chi 2 = 6.38$, $I^2 = 53$ %] (Fig. 3a). No publication bias was detected (Fig. 1d).

Loss of HBeAg

Loss of HBeAg was compared among nucleotide/nucleoside treatment vs. placebo treatment. Greater rate of loss of HBeAg were obtained in nucleotide/nucleoside therapy compared to placebo therapy [14.59 % vs. 9.64 %, RR = 0.92, 95 % CI = 0.88–0.96, *p*-value = 0.0002] (Fig. 3b). We also assessed the heterogeneity among the

a. Seroconversion of HBeAg. Nucleotide/nucleoside therapy vs. placebo



b. Loss of HBeAg. Nucleotide/nucleoside therapy vs. placebo

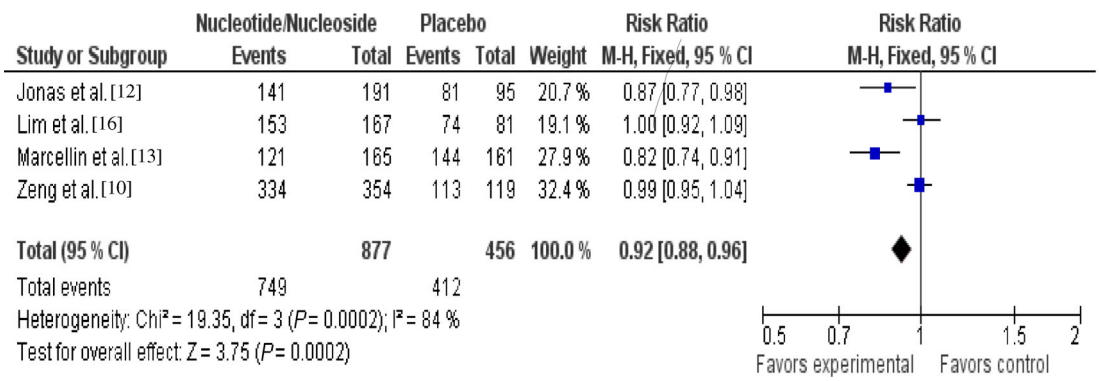


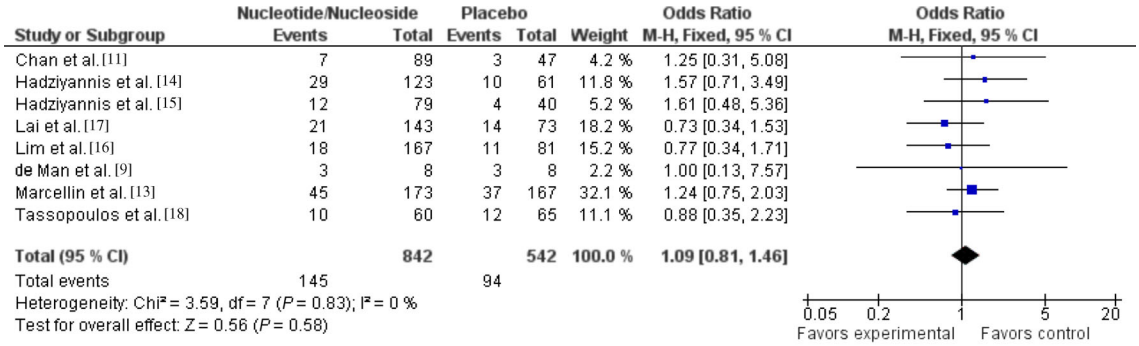
Fig. 3 Forest plot for meta-analysis. **a**. Seroconversion of HBeAg. Nucleotide/nucleoside therapy vs. placebo. **b**. Loss of HBeAg. Nucleotide/nucleoside therapy vs. placebo

studies [$\chi 2 = 19.35$, $I^2 = 84$ %] (Fig. 3b). No publication bias was recorded (Fig. 1e).

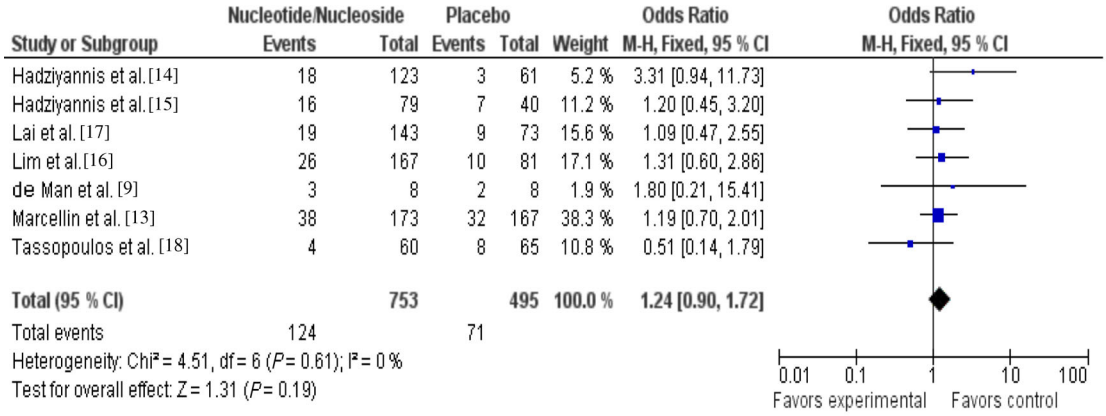
Safety analysis

Statistical analyses were performed for the common adverse events such as headache, abdominal pain, and pharyngitis during the therapy period. No statistical significant differences were obtained between the nucleotide/nucleoside treatments and placebo treatment. Headache [17.22 % vs. 17.34 %, OR = 1.09, 95 % CI = 0.81–1.46, *p*-value = 0.58] (Fig. 4a). Abdominal pain [16.46 % vs. 14.34 %, OR = 1.24, 95 % CI = 0.90–1.72, *p*-value = 0.19] (Fig. 4b). Pharyngitis [22.22 % vs. 18.23 %, OR = 1.12, 95 % CI = 0.86–1.45, *p*-value = 0.40] (Fig. 4c). We also assessed the heterogeneity for those adverse events. Headache [$\chi 2 = 3.59$, I2 = 0 %, *p*-value = 0.83] (Fig. 4a). Abdominal pain [$\chi 2 = 4.51$, $I^2 = 0$ %, *p*-value = 0.61] (Fig. 4b). Pharyngitis [$\chi 2 = 5.47$, $I^2 = 0$ %, *p*-value = 0.60]

a. Adverse event (headache). Nucleotide/nucleoside therapy vs. placebo



b. Adverse event (abdominal pain). Nucleotide/nucleoside therapy vs. placebo



c. Adverse event (pharyngitis). Nucleotide/nucleoside therapy vs. placebo

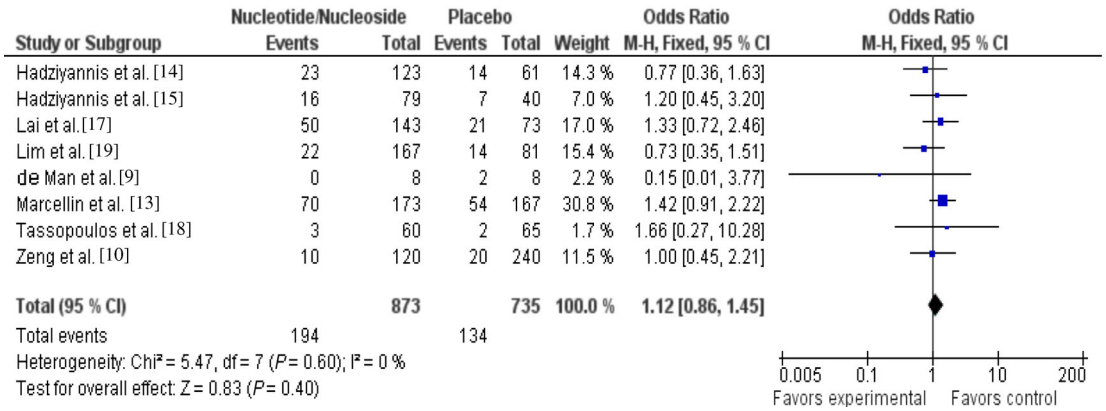


Fig. 4 Forest plot for meta-analysis. **a**. Adverse event (headache). Nucleotide/nucleoside therapy vs. placebo. **b**. Adverse event (abdominal pain). Nucleotide/nucleoside therapy vs. placebo. **c**. Adverse event (pharyngitis). Nucleotide/nucleoside therapy vs. placebo

(Fig. 4c). There was no statistical difference obtained between the two treatments for occurrence of adverse events viz. headache, abdominal pain, and pharyngitis. There was no heterogeneity among the studies.

Discussion

The meta-analysis of ten placebo controlled randomized trial has estimated that nucleotide/nucleoside therapy for treating the chronic hepatitis B is highly beneficial and shown greater statistical significant result compared to placebo. All of studies the treatment is given for the 1 year or less except one which has given 2 years of treatment. During the course of study, we had evaluated different endpoint of treatments. The dosage regimen given in each study was of different dose but we have considered only high dose population for our analysis. All of those of studies are carried out at different places. All included studies varied with respect baseline characteristic viz. age of patients, intervention treatment and therapy period, and we assessed the heterogeneity across the studies.

From the early study performed in 1998 to latest study published, all studies have shown the clinical improvement in the endpoint measures. We have estimated treatment in two groups that is nucleotide/nucleoside drug vs. placebo. A comparison between two treatments has shown the statistical significant result for the different efficacy measures. The *p*-value obtained for each endpoint measure virological response ($p < 0.00001$), biochemical response ($p < 0.00001$), histological response ($p < 0.00001$), seroconversion of HBeAg ($p = 0.0005$) and loss of HBeAg ($p = 0.0002$) are less than the 0.05. It means there was significant difference between the two treatments for treatment of chronic hepatitis B. Greater virological, biochemical, histological, seroconversion of HBeAg, and HBeAg loss rates were obtained as compared to that of placebo. We analyzed the heterogeneity across studies and studies have shown heterogeneity. Therefore, we have applied fixed effect model for meta-analysis. We recorded the publication bias for each efficacy measures. No significant publication bias was observed for virological response and it was absent in biochemical response, histological response, seroconversion of HBeAg, and loss of HBeAg.

Accordingly, we also evaluated secondary measure that is adverse events headache, abdominal pain, and pharyngitis of the two treatments. The adverse events are also compared between the two groups of treatments. There was no statistical significant result obtained for the adverse events during the therapy. We have calculated the odds ratio as an effect size of treatments. The *p*-value obtained after comparisons of adverse events headache ($p = 0.58$), abdominal pain ($p = 0.19$), and pharyngitis ($p = 0.40$) which is greater than 0.05. As the *p*-value obtained is greater than 0.05, we can say that there was no significant difference between two treatments. Conclusively, we believed that antiviral therapy is not solely responsible for any of adverse events. The heterogeneity was checked for the adverse events. There was absence of the heterogeneity across studies.

This meta-analysis may contain certain limitations that are due to the search strategy applied, inclusion and exclusion of studies, and most importantly publication bias. To minimize the publication bias, we searched for the unpublished data and also contacted to the authors where it seems data is insufficient. We applied standard protocol for extraction of data from various studies and analysis of data. The robust strategy for meta-analysis adopted in this work ensures a summarized result on all available clinical trials for chronic hepatitis B.

Our emphasis of study was on the treatment of chronic hepatitis where the antiviral therapy and interferon therapy are being used. We conducted meta-analysis of clinical trial for antiviral therapy, i.e. nucleotide/nucleoside treatments and explained the effectiveness of antiviral therapy with its details of any adverse effects due to this therapy. The advantages of nucleos(t)Ide analogs (NUC) analogues on patients with CHB-associated liver failure is notable for the betterment of patient survival, HBeAg serologic conversion, and brisk diminution of the levels of HBV DNA. In conclusion, the early initiation of adaptive nucleoside analogue drugs for antiviral therapy is the best available treatment in patients with HBeAg positive and HBeAg negative chronic hepatitis B without any significant adverse effects.

Compliance with ethical standards

Conflict of interest RHB, UR, SPM, and PKV declare that they have no conflict of interest.

Ethics statement Ethical approval does not apply as this is a review of earlier published studies and did not involve human or animal participants.

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