Dear Editor,

Thank you for allowing us to submit a revised version of our manuscript. We would also like to thank the reviewers for their pertinent and helpful comments. We have considered each of these in depth and have incorporated the appropriate changes in the revised version of the manuscript. We are confident that this revised manuscript has addressed the reviewers' concerns. All changes we have made to address these comments are described below. In the revised manuscript, all changes in the Abstract, Text, Tables and Figure legends are highlighted. Changes were not highlighted in the References and Figures, which were substantially modified according to the reviewers' suggestions.

Referees' comment

Immune tolerant phase of HBV infection is thought to be the first`phase of this chronic infection in areas where vertical transmission of HBV is dominant. This phase and is characterized by HBeAg positive, normal ALT, high DNA-HBV levels and no or minimal histological damage. Most guidelines do not recommend treatment at this phase due to lack of effectiveness and the risk of antiviral resistance development. However, the long-term consequences of this infection is a high risk of developing HCC, cirrhosis and liver failure. The authors underwent a meta-analysis to evaluate the role of vitamin E administration to children with immune tolerant HBV infection. Results showed a nearly four-fold likelihood of achieving HBeAg seroconversion in those children receiving vitamin E. Sensitivity analysis showed that exclusion of any of the three studies did not change results. The main short-cut of this study is the small sample size with only three eligible studies, which preclude definitive recommendations on the role of this intervention.

Major comments:

1. A recent study conducted by Chan et al (Gastro 2014) using TDF or TDF+FTC showed a seroconversion rate of 5% and 0%, respectively over a 192 weeks. How can you explain such a high seroconversion rate in the Italian study?

This point has been considered in the section "Discussion" and the following considerations have been included in the text (page 9, lines 23-30, page 10, lines 1-28):

Therefore, according to preliminary observations on the beneficial role of vitamin E as therapy in patients with chronic hepatitis B in a randomized controlled pilot trial several years ago, we had the aim to understand whether this compound could exert useful and safety therapeutic effects against HBV in childhood. We performed a systematic review and meta-analysis to search studies available in literature, assessing Vitamin E ability to induce HBeAb seroconversion in children with HBeAg positive persistent hepatitis. We found only three trials meeting inclusion criteria for our meta-analysis, but, surprisingly, two of them reported high rates of HBeAg loss and HBeAb development.

In particular, in Fiorino's trial a very high percentage of children (7/23, 30.4%), receiving vitamin E supplementation for 12 months with a follow-up period of further 12 months, obtained HBeAg clearance versus 1/23 (4.3%), in the control arm at the end of the follow-up period (24 months). However, it has to be considered that a substantial percentage of HBeAg loss (23.2%) was also observed in the group of vitamin E-treated children for 6 months with an additional follow-up period of 12 months in comparison to placebo arm (8.7%) in Gerner's research. Hepatitis B e antigen seroconversion rates obtained in the above mentioned studies are higher in comparison to those observed in trials enrolling patients, who were treated with nucleotide/nucleoside analogs. A recent research performed by Chan et al (Gastro 2014), using TDF (tenofovir) or TDF+FTC in adult immune-tolerant patients, showed low percentage of HBeAg loss and HBeAb development (5% and 0% respectively) over a 192 weeks. The reasons explaining these high seroconversion rates in children supplemented with vitamin E in comparison to subjects treated with TDF analogs, mainly in Fiorino's study, are not completely understood and deserve further investigations. Nevertheless, some very interesting issues have to be considered:

1) different mechanisms of vitamin E and nucleotidic/nucleosidic analogs action;
2) duration of treatment period;
3) vitamin E dosage used.

Nucleos(t)ide analogs exert antiviral activities, by means of well-known a) direct as well as b) indirect mechanisms:

a) suppression of HBV replication mainly through the inhibition of reverse transcription process in the viral lifecycle; b) partial restoration of the impaired immune response, as shown by significant reductions in the percentages of $CD4^+CD25^{high}$ T regulatory cells, PD-L1 (programmed-death-L1 receptor) expression on $CD4^+$ T cells and pro-inflammatory cytokines production. Therefore, antigen-viral burden reduction is associated with the improvement of anti-HBV activity of immune cells. According to our current knowledge, Vitamin E exerts its beneficial effects by means of similar mechanisms . .

These premises may help us to explain the positive preliminary results of our meta-analysis. It is conceivable to think that Vitamin e supplementation may contribute to improve the anti-HBV activity of immune system, by means of a direct anti-viral action both by decreasing its replication abilities and by boosting host's immune cell responses. The slow but progressive decline of HBV replication, associated with HBeAg loss/HBeAb seroconversion described in 2 of three studies, included in our meta-analysis, corroborates the hypothesis that Vitamin E acts as an immune-modulator resulting in a global antiviral activity. Interestingly, a delayed response has already been reported, with the use of immunomodulatory drugs for the treatment of adults with CHB. In Fiorino's study HBeAg seroconversion was also observed in additional 7, among 11 previously non-responders patients in the vitamin E supplemented group, who were followed-up for an additional period of 12 months.

2. The HBeAg seroconversion rate in immune tolerant phase is < 2% among children younger than 3 yrs and 4-5% among older children. I have been unable to find the age of the eligible population. Please, describe the age in Table-1 and report the seroconversion rate by age groups. May be the older children might respond better. Was there any relationship between dose and response?

This point has been considered in table 1 and in the section Discussion. The available data concerning the age and number of children, undergoing HBeAb seroconversion are reported (table 1), whereas in the section Discussion the following considerations have been included in the text (page 12, lines 14-22): fourth, it has to be taken into account that

HBeAg seroconversion rate in children in immune tolerant phase is < 2% among children younger than 3 yrs and 4-5% among older children and that data concerning HBV genotypes as well as children's seroconversion rates by age groups were available only for one of considered studies for the meta-analysis. Therefore, this limit precludes further proper assessment of the potential Vitamin E benefits in children lower than 14 years as well as of its potential efficacious dose. However, in the study by Fiorino, patients responders to vitamin E treatment were respectively 4 (1 child), 13 (2 children), 14 (2 children) and 17 (2 children) years old, at the enrollment (Table 1). This observation underlines the importance of the duration of the treatment period and might contribute to explain, at least in part, the absence of children responding to vitamin E therapy in Dickici's study. In this trial, the period of supplementation with this compound was only three months long, in addition the dosage used was equal to 100 mg/day, probably, not enough to induce an improvement of anti-viral immune response. Therefore, both dosage and duration of Vitamin E supplementation represent very important factors to assess, when its anti-viral efficacy is evaluated.

3. In population-based studies, long-term administration of vitamin E has been related with serious negative outcomes. Please, warn about generalization of vitamin E use in pediatric population before confirmation of effectiveness and safety of this compound.

This point has been considered in the secton "Discussion" and the following considerations have been included in the text (page 12, lines 24-32, page 13, lines 1-4):
In addition, it has to be considered that some meta-analyses have reported that long-term administration of vitamin E, at dosages exceeding 150 UI once a day, is associated with serious negative outcomes, such as an increase in all-cause mortality. However, although the conclusions of these studies are rather questionable, because of the meta-analytic approaches used, these results suggest caution in the generalization of vitamin E administration in pediatric population before confirmation of effectiveness and safety of this compound. Certainly, vitamin E, that has been administered to the children in the reported trials at high dosage, has to be considered as a drug, with possible benefits as

well as with potential risks. Therefore, further observational studies are required to definitively settle the issue.

4. HBV genotype A patients respond better to interferon-based therapies. Did you know genotypes in these studies?

HBV genotypes are available only for children in Fiorino's study, therefore no definitive conclusions can be obtained, concerning this issue. This limit has been underlined in the section "Discussion" (page .

Minor: There are several typo and grammar mistakes (Eg. die instead of day)

The typo and grammar form of article has been reviewed and underlined mistakes have been corrected