January 28, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Factors affecting HBV vaccination in HD revised.docx).

Title: Factors affecting effectiveness of vaccination against hepatitis B virus in hemodialysis patients.

Author: Theodoros Eleftheriadis, Georgios Pissas, Georgia Antoniadi, Vassilios Liakopoulos, Ioannis Stefanidis

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6717

The manuscript has been improved according to the suggestions of the reviewers:

1. Format has been updated

2. Revision has been made according to the suggestions of the reviewer

(1) Response to the reviewer 00034635

Major Comments:

1) The necessity for HBV vaccination in any patient with decreased GFR has been noted in the manuscript.

In the patient-associated factors that affect HBV efficacy section: *"On the other hand, it has been confirmed that the earlier the vaccination against HBV in the course of chronic kidney diseases, the greater the response rate. Consequently, vaccination should be performed as soon as possible in the course of chronic renal failure, before patient reaches HD, since response is associated with the degree of renal function[66]."*

In the Conclusions section: *"A modifiable patient-associated factor is the stage of chronic kidney disease. It is recommended to vaccinate patients as early as possible in the course of chronic kidney disease in order to achieve higher response rates."*

2) The necessity for isolation of HBV carriers in HD units has been noted in the revised manuscript.

"Patients should be isolated and hemodialyzed in separate machines[6], since HBV is found in high titers and is potentially infectious for more than 7 days in the environment…"

3) In our opinion early antiviral prophylaxis with nucleoside analogues is not an appropriate measure due to the probability of resistance development to these drugs. The following statement has been added to the revised manuscript.

"*Although in HBV high prevalence areas, prophylaxis with nucleoside analogues in seronegative patients undergoing HD seems reasonable; in our opinion such a strategy could lead to the development of resistance to such medicines. For instance 15% of patients develop resistance to lamivudine after one year of treatment and 30% after two years due to mutation of RNA-dependent HBV DNA polymerase* [14-16]."

Minor Comments:

1) References have been modified according to the journal style.

2) In the revised manuscript two tables are included. Most of the other five reviewers demanded further information, although we tried to keep the text as short as possible.

(2) Response to the reviewer 00503536

1) The review is focused on factors affecting HBV vaccination in HD patients, directing us to a brief documentation of the immunological mechanisms that affect response to vaccination in this population. This passage was brief in order to maintain the body text of the whole document in a rational length.

"*Acquired immunity disturbances in HD patients are many and diverse. They are caused by uremia per se, co-morbidities, the HD procedure, chronic renal failure complications and therapeutic interventions for their treatment*[17]*. As a consequence these patients are susceptible to both bacterial and viral infections*[48,49]*. Current data support that acquired immunity disturbances in HD patients concern mainly the T-lymphocyte and the antigen presenting cells. The required for an effective immune response interaction between antigen presenting cells and T-lymphocytes is impaired in HD patients*[43]*, while defects have been detected in T-lymphocytes as well*[51]*. Disturbances of antigen presenting cells and T-cells have been related to the decreased efficacy of HBV vaccination in HD patients*[52-56]*. Also various other factors have been incriminated for the decreased response rate to HBV vaccination in HD patients, such as the increased levels of the immunosuppressive enzyme indoleamine 2,3-dioxygenase*[57] *, or the deficiency of the immunomodulatory vitamin D*[51]*. However, many of the patient-associated factors that are known to affect the immune response have not been evaluated in the context of the response to HBV vaccination and remain to be elucidated."*

2) Two tables have been included in the revised manuscript. The first one refers to "Factors that decrease HBV vaccination efficacy in HD patients" and the second refers to "Protocols that increase HBV vaccination efficacy".

3) The annotated sentence has been rewritten on a clearer form.

"*Nevertheless, in individuals with initial anti-HBs levels higher than 10 IU/L due to successful response to vaccination or natural infection, but with present anti-HBs levels lower than 10 IU/L, only one dose is recommended* [41,42]."

(3) Response to the reviewer 00070833

General Comment: The review is focused on factors affecting HBV vaccination in HD patients, directing us to a brief documentation of the immunological mechanisms that affect response to vaccination in this population. This passage was brief in order to maintain the body text of the whole document in a rational length.

"*Acquired immunity disturbances in HD patients are many and diverse. They are caused by uremia per se, co-morbidities, the HD procedure, chronic renal failure complications and therapeutic interventions for their treatment*[17]*. As a consequence these patients are susceptible to both bacterial and viral infections*[48,49]*. Current data support that acquired immunity disturbances in HD patients concern mainly the T-lymphocyte and the antigen presenting cells. The required for an effective immune response interaction between antigen presenting cells and T-lymphocytes is impaired in HD patients*[43]*, while defects have been detected in T-lymphocytes as well*[51]*. Disturbances of antigen presenting cells and T-cells have been related to the decreased efficacy of HBV vaccination in HD patients*[52-56]*. Also various other factors have been incriminated for the decreased response rate to HBV vaccination in HD patients, such as the increased levels of the immunosuppressive enzyme indoleamine 2,3-dioxygenase*[57] *, or the deficiency of the immunomodulatory vitamin D*[51]*. However, many of the patient-associated factors that are known to affect the immune response have not been evaluated in the context of the response to HBV vaccination and remain to be elucidated."*

Major Comments:

1)Regarding the fact that HD patients become chronic HBV carrier more frequently than the general population the following statement has been added in the revised manuscript

"*Both innate and adaptive immunity contribute to HBV clearance after an acute infection* [21,22]*. Since both arms of the immune system are disturbed in HD patients* [17,23]*, it is not surprising that there is a higher possibility to become chronic carriers after an initial HBV infection. For instance, the role of CD8+ T-cells in HBV clearance has been confirmed* [24]*. It is known that this T-cell subset is decreased both in number and in function in HD population*[17,23].

2) The necessity for HBV carriers isolation in HD units has been noted in the manuscript.

"Patients should be isolated and hemodialyzed in separate machine[6]*, since HBV is found in high titers and is potentially infectious for more than 7 days in the environment…"*

In our opinion early antiviral prophylaxis with nucleoside analogues is not an appropriate measure due to the probability of resistance development to these drugs. The following statement has been added to the revised manuscript.

"*Although in HBV high prevalence areas, prophylaxis with nucleoside analogues in seronegative patients undergoing HD seems reasonable; in our opinion such a strategy could lead to the development of resistance to such medicines. For instance 15% of patients develop resistance to lamivudine after one year of treatment and 30% after two years due to mutation of RNA-dependent HBV DNA polymerase* [14-16]."

Minor Comments:

1) Linguistic and type error have been corrected in the revised manuscript.

2) References have been modified according to the journal style.

(4) Response to the reviewer 00069294

1) Linguistic and type errors have been corrected in the revised manuscript.

2) The factors affecting HBV vaccination efficacy in HD patients have been included in a table in the revised manuscript.

3) A table regarding the several protocols for improving the HBV vaccination efficacy has been implemented in the revised manuscript.

4) As already noted from the 30% of HBV carriers that develop histologically confirmed chronic hepatitis only the 5% die from liver disease. *"Obviously, the decreased survival in these patients mainly due to cardiovascular diseases and bacterial infections contribute to the above relative low percentage"*.

5) We agree with the comment about the role of immunosuppression in life threatening exacerbations and increased rates of liver disease in renal transplant recipients. This is noted in the revised manuscript.

6) The results about HIV infected HD patients are included in the revised manuscript.

"Surprisingly in a study performed in HIV infected HD patients, the response rate to HBV vaccination was the same with a group of randomly selected non-HIV infected HD patients. Seventy percent n of the HIV infected responders maintained protective titers 6 months after vaccination[65]*."*

7) The possible causes for the faster decay of the anti-HBV titers in HD patients are noted in the revised manuscript.

"Malnutrition and increased protein loss due to PD could be responsible for the last observation since the anti-HBV titer is inversely to serum albumin[44]."

8) To our knowledge there are no available data about the long term efficacy of the third generation HBV vaccine. The HB-AS02 vaccine has been studied for a period of 36 months confirming its long term efficacy. This is already noted within the manuscript.

The side effects of adjuvanted HB-AS02 vaccine have been added in the revised manuscript.

"It should be noted that the overall reactogenecity of the HB-S04 vaccine was higher than in the non adjuvanted vaccine, although incidences of general symptoms were similar. The higher local reactogenecity is related to the higher incidence of pain. However, occurrence of grade 3 pain is very low and similar in both groups[87,88]. Regarding the adjuvanted HB-S02 vaccine, it was found to be more reactogenic than HB-S04 vaccine. On the other hand, local and systemic reactions are mild to moderate in intensity and transient in nature[89,90]."

The side effects of the third generation HBV vaccine are also included.

"Regarding its side effects, 15% of the subjects experience a local predominantly mild or moderate pain. General symptoms, including chills, dizziness, headaches and diarrhea are mild and transient[34]."

(5) Response to the reviewer 00503560

1) A table with all the factors that affect response to HBV vaccination is provided in the revised manuscript.

2) A second table regarding the several protocols for improving the HBV vaccination efficacy has been implemented in the revised manuscript.

3) The reported adverse events of the adjuvanted HBV vaccines are noted in the revised manuscript.

"It should be noted that the overall reactogenecity of the HB-S04 vaccine was higher than in the non adjuvanted vaccine, although incidences of general symptoms were similar. The higher local reactogenecity is related to the higher incidence of pain. However, occurrence of grade 3 pain is very low and similar in both group[87,88]. Regarding the adjuvanted HB-S02 vaccine, it was found to be more reactogenic than HB-S04 vaccine. On the other hand, local and systemic reactions are mild to moderate in intensity and transient in nature[89,90]."

(6) Response to the reviewer 00013176

1) According to your recommendation, in the conclusion of the revised manuscript we have provided our opinion for the best strategy in the case of unresponsiveness to conventional HBV vaccination.

" *According to the available literature in case of no response after two series of the above vaccine schedule, ID administration of the classical vaccine, of a third generation vaccine or of the adjuvanted HB-AS04 vaccine could be considered as a solution. In our opinion the most convenient strategy is the administration of the more modern vaccines, and especially of the adjuvanted HB-AS04, already available in many countries. However, it is imperative that all HD patients should be vaccinated against HBV…*"

2) The comment of the reviewer regarding the long lasting immune memory after HBV vaccination, even when anti-HBs titer becomes low is very interesting; and it is discussed in the revised manuscript.

"*At this point it should be noted that although the cut-off point of the 10 IU/L for anti-HBs titer is recommended as a target, it has been confirmed that long lasting immune memory persists after vaccination even when anti-HBs titer drops at even lower levels. This has been demonstrated experimentally regarding the persistence of memory T and B cells* [36]*. In additional observational clinical studies showed that HBV vaccination may offer protection even in those vaccinated subjects with undetectable anti-HBs antibodies* [37-38]*. However, there are no available data for HD population.*"

3. References and typesetting were corrected

We are grateful for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

Dr Theodoros Eleftheriadis