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Dear Editor,

Please find enclosed the edited manuscript in Word format (Gopalakrishnan200613final.docx).

Title: Hepatitis B Virus Subgenotype A1 Predominates in Liver Disease Patients from Kerala, India

Authors: Deepak Gopalakrishnan, Mark Keyter, Kotacherry Trivikrama Shenoy , Leena Kondarappassery Balakumaran, Thayumanavan Barathi, Varghese Thomas, KR Vinayakumar, Charles Panackel, Arun T Korah, Ramesh Nair, Anna Kramvis.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3715

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

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewers and our response is in **bold.**

Reviewer 00183339

This paper aimed to characterized HBV isolates molecularly and related them to the clinical manifestation of infection. The manuscript is well presented and of interest and although it is repetitive, it can contribute to increase the knowledge of this topic.

It was important to genotype HBV from liver disease patients from Kerala because this has not been previously done and the knowledge gap needed to be closed.

Reviewer 02458064

Some phrases and sentences need polishing. SPECIFIC COMMENTS The section "Introduction" should be simplified and the authors can only present the necessary backgrounds of this study. Figure legends should be simplified. In my opinion, Figure 1 can be crossed out. This scientific article should be written in formal English rather than oral English.

We have revised where we deemed it necessary. We are of the opinion that the Introduction is not too long and provides a good background. Figure 1 is retained because it highlights the differences between the distribution of the genotypes in Kerala and the other Indian states.

Reviewer 00053786

Deepak Gopalakrishnan et al's study shows that HBV genotype A1 is predominant regardless of the clinical status of chronic HBV-infected liver disease patients. The manuscript is well structured and well written. It contributes to the understanding of the relationship between the HBV variability and clinical outcomes in different populations worldwide. Minor comments: Revise carefully paragraph and word spacing before publishing.

Thank you for your comments and suggestions.

Reviewer 00054966

The points that need to be improved are related to the sample size that results to be to small to obtain reliable statistical conclusions: the total cases enrolled with HBV infection are 91 from a general population (Kerala state) reported by the authors to be of 33 million inhabitants.

Even though over a period of 4 years (2005 – 2009) 500 HBsAg-positive patients were recruited, HBV isolates from only 91 patients could be studied in depth

because of financial and capacity constraints. However, we are confident that the 91 samples are representative.

For the same reason, the statement reported in the results section (demographic, clinical and virological characteristics paragraph) where the authors affirm that the "HBeAg-negative HCC patients, infected with genotype A, were significantly younger than those infected with genotype D" is base on a sample size of 23 patients vs 5 (data deduced by the percentages reported by the authors) that seems to be insufficient.

Unfortunately this is a limitation of the study and we have added a statement in the Discussion.

The authors need also to better explain, in the "patients" section, how do they formulate the diagnosis of chronic hepatitis and in particular how many patients underwent liver biopsy (in our opinion the only reliable tool to obtain the diagnosis).

The authors report to have used "liver function tests to find necro-inflammatory activity" but this method is not validated and not universally accepted: we suggest to use histological parameters (HAI) only to asses the necro-inflammatory activity.

In our experience the criteria used are generally accepted.

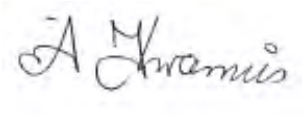
The conclusions need to be supported by a larger number of cases analyzed.

We agree and have thus added a *proviso* in the Discussion

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Yours sincerely



Professor Anna Kramvis
Leader: Hepatitis Virus Diversity Research Programme

L03 Prevalence of hepatitis B virus infection in general population of India

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Chronic HBV infection is a major global public health problem in spite of increasing awareness for hepatitis in general and hepatitis B in particular. In this study, an attempt has been made to update and analyze the available studies on the prevalence of chronic HBV infection in different parts of India and in high-risk groups. Of the 25 reports that have been taken for the meta-analysis of HBV infection in the general population of India, an HBsAg prevalence rate of 1.1%-12.2% was observed. When the states of India were classified based on the HBsAg positivity rate, Jammu & Kashmir and Kerala came under the below-2% zone. Karnataka, Maharastra, Delhi, Haryana, Himachal Pradesh and West Bengal showed 2%-4% HBsAg carrier prevalence. Tamil Nadu, Pondicherry, Andhra Pradesh, Madhya Pradesh, Uttar Pradesh and Arunachal Pradesh belonged to high HBsAg prevalence (above 4%) zones. However, the mean HBsAg carrier rate for India from these reports is 3.34%, which works out to about 34 million persons infected with HBV, with the baseline population being 1000 million in India.

The point to be remembered besides the formidable pool of HBV-infected population is the significant regional variations of chronic HBV infection within India. Analysis of HBV infection status in pregnant women has shown the range to be between 1%-12.3%, with a mean of 4.22%. Rajasthan came under the <2% zone. Maharastra and Tamil Nadu in the intermediate zone of 2%-4%, and Karnataka, Kerala, Delhi, Haryana and Uttar Pradesh as above-4% zones. Again, significant regional variation is observed. Community studies revealed a 5.7% HBsAg rate in Tamil Nadu, while a 5.3% positivity rate was observed in Bengal. The HBeAg positivity rate based on the seven reports revealed a range of 2.6% to 56.1%, with a mean rate of 24.22%. The HBeAg positivity rate in pregnant women showed a range of 7.8%-47.8%, with a mean of 24.01%. The HBeAg prevalence in the two community studies revealed 3.8% in Bengal and 23.6% in Tamil Nadu. In view of the available data on HBV in India, it has to be summarily decided to include hepatitis B vaccine in the Expanded Programme of Immunisation (EPI) in India without any further delay.

L04 Transfusion-associated hepatitis B (TAH-B) despite screening for HBsAg

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Over the last 25 years, routine screening for HBsAg has reduced but not eliminated the problem of TAH-B. Even in the 1990s, 1%-16% of TAH in different parts of the world continues to be due to HBV despite routine screening for HBsAg. Numerous reports have documented the occurrence of TAH-B after the transfusion of units testing negative for HBsAg. A variety of explanations have emerged for this phenomenon, e.g., human error, insensitive HBsAg EIA kits, the use of monoclonal versus polyclonal anti-HBs for sandwich EIA, low levels of viremia below the detection limit of routinely used EIA kits, the presence of surface mutants or antibody escape mutants (AEM), and the rare passibility of a unit being collected in the window period of an acute HBV infection. Except for the last situation, the presence of the virus is readily identified by the detection of high-titer anti-HBc and, in all cases, by the use of PCR amplification.

Fears that TAH-B occurring despite screening for HBsAg may be an increasing problem in intermediate- and high-prevalence regions of the world have been fueled by reports that 4%-24% of units collected from voluntary donors negative for HBsAg test positive for HBV-DNA in these areas. Until the dream of eliminating HBV from the world by universal immunization is achieved, the phenomenon of TAH-B occurring despite the use of HBsAg-screened blood may be held in check by taking steps to improve the safety of heterologous blood transfusion. Besides using rigorous criteria for accepting voluntary donors, various measures proposed to eliminate this problem have included improved donor unit screening with better tests for HBsAg detection, routine anti-HBc screening, universal vaccination of repeat voluntary donors, and routinely treating all donor units with virus inactivation procedures. The Japanese approach of rejecting all HBsAg-positive and isolated, high-titer anti-HBc positive donor units has succeeded in eliminating TAH-B from Japan and is an example that warrants wider emulation in high-prevalence areas of the world.