Dear Editor,

We really appreciate your constructive and valuable comments on our manuscript. We revised our manuscript entitled "Different pre-S deletion patterns and their association with hepatitis B virus genotypes" by Bing-Fang Chen. We have responded your comments and incorporated them into our revision (underlined) as shown in the next page. We hope this revision can meet the requirements of yours and the reviewers'. If you have any questions or need any further information, please feel free to contact us.

Sincerely yours,

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**Name of Journal:** World Journal of Gastroenterology
ESPS Manuscript NO: 27652

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

1. Format has been updated.

2. References were checked.

3. Revision has been made according to the suggestions of the reviewers and editor, and highlighted in the updated version of the manuscript (underlined).

**Reviewer 00506552's comments:**

(1) Author of this manuscript described the differential patterns of pre-S deletions in association with hepatitis B virus genotypes. It is very useful information to predict the prognosis and clinical outcomes. It would be better to compare/describe the pre-S/S mutations including pre-S deletions, too.

**Responses:** As suggested by the Reviewer, additional description was added as follows:
**Page 14, line 1-3:**
Moreover, pre-S/S mutations are associated with fulminant hepatitis, fibrosing cholestatic hepatitis, and the development of cirrhosis and HCC[6, 19-22].

(2) In Table 1, author calculated the p value as whole the progressive liver diseases between WT pre-S vs pre-S deletion. I suggest that the individual p values by CH, LC, NC-HCC, and LC-HCC between WT pre-S vs pre-S deletion. Same thing is applied to Table 2, too. The individual p values by CH, LC, NC-HCC, and LC-HCC between HBV genotype B vs HBV genotype C.

**Responses:** As suggested by the Reviewer, the individual p values were added in Table 1 and 2. (**Page 24 and 25**)

**Reviewer 02992674's comments:**

(1) It has been reported by several groups that pre S deletions and mutations are associated more with the progression of liver diseases to HCC than asymptomatic carrier (Chen CH, 2007, Dake Zhang, 2012; Lin CL, 2007;Chao Wang, 2015;) The rate of preS deletion is higher in HBV genotype C than genotype B. Here the authors have looked into the association of preS1, PreS2 and surface mutants with the progression of liver diseases and effect of HBV genotype B and C. This is a repeat study in HBV infected Taiwaneese people. Study has been conducted with enough number of patients with different disease stages and a group of new mutations has been reported. There is no explanation of using 43 patients in each of the genotype B and C group though they have more than 60 patients in respective groups.

**Responses:** As suggested by the Reviewer, the explanation of using 43 patients in each of the genotype B and C group was added as follows.

**Page 9, line 11-14:**

To examine the role of viral factors and to exclude the influence of HBV infection duration on the occurrence of pre-S deletion, 43 age-matched HBV/B and 43 age-matched HBV/C infected carriers were selected to examine the associations of different types of pre-S deletion with HBV genotypes.

(2) In Table 1: total number of patients in progressive disease stages is 92 while collectively four stages show 96 in wild type preS mutant. There are few typing mistakes.

**Responses:** Thank you very much for reviewing my manuscript, the mistake in Table 1 was corrected. (**Page 24**)

**Reviewer 00227403's Comments:**

(1) Why only 86 out of 126 HBV carriers who harbored the pre-S deletion mutants were examined according to genotype?

**Responses:** As suggested by the Reviewer, the explanation of using 43 patients in each of the genotype B and C group was added as follows.

**Page 9, line 11-14:**

To examine the role of viral factors and to exclude the influence of HBV infection duration on the occurrence of pre-S deletion, 43 age-matched HBV/B and 43 age-matched HBV/C infected carriers were selected to examine the associations of different types of pre-S deletion with HBV genotypes.

(2) In the section Patients and methods when you start with a sample change the number in letters (see, for example 55, 218)

**Responses:** As suggested by the Reviewer, the description was revised.

**Page 7, line 4-9:**

The study population comprised 55 asymptomatic HBV carriers with a normal serum alanine aminotransferase level for at least 3 years according to periodic biochemical examinations (every 3 or 6 mo) and 218 HBsAg-positive patients with histologically verified chronic liver disease. Among the HBsAg-positive patients, 55 had CH with active viral replication, 55 had LC without HCC, 53 had liver cirrhotic HCC (LC-HCC), and 55 had noncirrhotic HCC (NC-HCC).

(3) In the included cohort, has the author excluded the others standard causes of liver disease? This should be reported. -Has the author a pattern of HDV profile on this cohort?

**Responses:** As suggested by the Reviewer, additional information was added as follows:

**Page 7, line 10-12:**

None of them were coinfected with hepatitis C virus or hepatitis D virus. Other causes of hepatitis, including autoimmune hepatitis and alcoholic liver diseases were excluded clinically and serologically.

(4) In the section discussion "etc" should be avoided.

**Responses:** As suggested by the Reviewer, "etc" was deleted.

(5) In the section discussion when the author discussed the association between HBV genotype prevalence and the patterns of pre-S deletion associated with progressive liver diseases (as HCC), it should be highlighted that this is another piece in a field of investigations that involves other aspects as mutations in X region, mutations in BCP

region and in pre-core regaion (Lee et al. World J Gastroenterol 2016;22:5393-9), MicroRNA (MiRNA) (Petrini et al. Panminerva Medica 2015; 57:201-9).

**Responses:** As suggested by the Reviewer, additional discussion and references were added as follows:

**Page 14, line 25-31 and page 15, line 1-5:**

It is suggested that multiple risk factors may contribute to the pathogenesis of HBV infection. Chronic inflammation, the effect of cytokines, and the integration of HBV DNA into the host cellular genome are crucial factors in the development of HCC. In addition, HBV mutations in X, BCP, PC, and the pre-S/S region are associated with the severity of liver disease and the development of HCC[6, 19-22, 51, 52]. The dinucleotide substitution (A1762T, G1764A) is the most common mutation in BCP. This BCP mutation is associated with the higher occurrence of HCC and LC[6, 19, 20, 51]. Mutations in PC (G1896A) and X (C1653T and T1753V) are also associated with the development of HCC[6, 51, 52]. Moreover, pre-S/S mutations are associated with fulminant hepatitis, fibrosing cholestatic hepatitis, and the development of cirrhosis and HCC[6, 19-22]. Recent researches suggested that microRNA is involved in HBV-related HCC[53], All of these studies indicated that both viral and host factors affect HBV pathogenesis. Additional studies should be conducted to define their role in the progression of liver disease.

**Page 23, line 3-12:**

**Zhang ZH,** Wu CC, Chen XW, Li X, Li J, Lu MJ. Genetic variation of hepatitis B virus and its significance forpathogenesis. *World J Gastroenterol* 2016; **22**: 126-144. [PMID: 26755865 doi: 10.3748/wjg.v22.i1.126]

**Lee D,** Lyu H, Chung YH, Kim JA, Mathews P, Jaffee E, Zheng L, Yu E, Lee YJ, Ryu SH. Genomic change in hepatitis B virus associated withdevelopment of hepatocellular carcinoma. *World J Gastroenterol* 2016; **22**: 5393-5399 [PMID: 27340355 doi: 10.3748/wjg.v22.i23.5393]

**Petrini E,** Caviglia GP, Abate ML, Fagoonee S, Smedile A, Pellicano R. MicroRNAs in HBV-related hepatocellular carcinoma: functions and potential clinical applications. *Panminerva Med* 2015; **57**: 201-209 [PMID: 25897630]

**Reviewer 01800523's comments:**

**Comments to authors**
None

**Confidential Comments to Editor**
Chen et al investigated the associations of different types of pre-S deletion with HBV genotypes comprehensively, and found differential patterns of pre-S deletion between HBV/B and HBV/C infected carriers, which may be associated with the progression of liver diseases. The paper was well written, but some specific points should be addressed.

**Specific points**

1. Page 1, Data sharing statement: All sequence data should be submitted to a database such as GeneBank to have accession numbers.

   **Responses:** As suggested by the Reviewer, all sequence data will be submitted to a database such as GeneBank in the future.

2. Page 11, line 20. "in three functional sites (the nucleocapsid binding site, the start codon of M, and site for viral secretion)". The author should use abbreviations NBS or VS, because the terms appears here not for the first time.

   **Responses:** As suggested by the Reviewer, the statement was revised. (**Page 12, lines 23-24**)

3. Page 12, lines 15-17. "An interesting finding that one type of N-terminus pre-S1 deletion mutants including the start codon of the L protein was frequently found in HBV/C group (20.9% vs. 9.3%, *P*=.228)." The difference was not significant, was it?

   **Responses:** Yes, the difference was not significant.

4. In Table 1, the age of those with wild type pre-S 45.39±6.6 should be rounded off to 45.4±6.6. Total number of those with wild type pre-S who had CH, LC, NC-HCC or LC-HCC is not equal to the number of those who had progressive liver diseases. Please indicate the number of ASC.

   **Responses:** As suggested by the Reviewer, the age was rounded off to 45.4±6.6, the number of those who had progressive liver diseases was revised, and the number of ASC was indicated (Table 1). (**Page 24**)

5. In Table 4, P value for difference in d183 M between genotypes B and C (0% vs 8.3%)

among LC-HCC patients is shown to be 0.04. Is it right? In Table 4, P values 0.000 should be shown to be <0.001.

**Responses:** The P value for difference in d183 M between genotypes B and C (0% vs 8.3%) among LC-HCC patients is 1.000, the mistake (P value 0.004) was corrected. As suggested by the Reviewer, the P values 0.000 in Table 4 were revised. (**Page 27**)