

**Name of Journal:** *World Journal of Hepatology*

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*Observational Study*

**Title:** Characteristics of escape mutations from occult hepatitis B virus infected patients with hematological malignancies in South Egypt

The authors appreciate the careful revision of the manuscript also the informative comments by the reviewers.

Below are answers point by point to reviewers' comments.

The major changes are underlined in the main manuscript

**Reviewer 1 (No. 00225318): COMMENTS TO AUTHORS**

The work addressed by Abeer Elkady et al it is no to original and overall the information provided has been reported in multiple previous studies. In this sense it is well known the problems of detection of the variants studied in this report such as the corresponding to positions 120 and 143 of the MHR region of the HBV genome. This reports only confirms that the methodology used did not adequately detect these variants and this does not represent an important input. The authors confirm that this is a phenomenon of no detection while not due to a lower HbsAg production, but this fact do not contributes too much originality to the study.

**Q1.** The authors should have used alternative methods to detect HBsAg in order to know if any of them is able to detect HbsAg in the presence of such variants and recommend its possible use in the population they present.

**A1.** Thank you very much for your careful revision of the manuscript. The authors appreciate your informative comments in order to improve the draft. The authors used 2 different methods to examine the HBsAg simultaneously.

**Q2.** The only interest of this study is the high prevalence of these mutations in patients with hematologic diseases, but the authors do not provide any possible explanatory mechanism for this phenomenon.

**A2.** This point is discussed in page 13, line 25-29 and page 14, line 1-4

**Q3.** Surprisingly, the authors get amplify the whole HBV genome from samples with very low viral loads, and they must and provide more experimental data to confirm this. Perhaps this viral loads are underestimated by the methodology they use. They should confirm these viral load with

commercial methodology they use. They should confirm these viral load with commercial available and well validated methods, validated for HBV DNA quantification.

**A3. Thank you very much for your informative comment. In this study, the authors harvested DNA eluted with 50ul buffer from 400ul of serum. And we used highly sensitive RTD-PCR method with specific primers targeting the conserved S gene genomic sequence of the genomic HBV 2 or 3 times one sample. In conclusion, viral load was confirmed in these 6 patients by TaqMan PCR (Roche). In full sequence analysis, we set the primers to analyzed short length of HBV sequence divided into 8 area, and tried to read repeatedly.**

**Q4.** Despite that the authors state that there were no differences in the extracellular HbcAg levels between mutated or WT genomes, Figure 2 seems to suggest that the extracellular HbcAg in cell culture technique studied by Fujirebio is lower in mutated than WT genomes which contrast with the absence of differences in HBV-DNA levels. The authors do not give any explanation for this phenomenon that might have some interest.

**A4. Thank you very much. We agree with the reviewer the difference of expression of HBcrAg by the mutant compared to the Wild clones. However, this difference did not reach significant value by Mann-Whitney U-test.**

#### **Reviewer 2 (No. 02945187): COMMENTS TO AUTHORS**

In this manuscript, Abeer Elkady et al. argue characteristics of escape mutations from occult hepatitis B virus infected patients with hematological malignancies in Egypt. The authors concluded that occult HBV infection is associated with P120T and S143L mutations and 120T mutation impairs the detection of HBsAg by CLEIA. The aim of this study might be interesting and important. There are several comments to be addressed.

Minor comments

**Q1.** In page 7, line1, authors described that “All patients received chest computed tomography and ultrasonography of abdomen as an initial evaluation.” In this study, the imaging findings are not discussed. Authors should remove this sentence.

**A1. Thank you very much for your careful revision of the draft. The statement is deleted from the draft.**

**Q2.** In table 2, diagnosis is lack coherence. For example, authors described that one patient has NHL-small cell-low grade in detail. But authors described that another patient has malignant lymphoma simply. Authors should bring unity in diagnosis in table 2.

**A2. We corrected table 2.**

#### **Reviewer 3 (No. 00053556): COMMENTS TO AUTHORS**

Thank you very much for your careful review of the present article. The authors appreciate your valuable comments in order to improve the manuscript. Below is a point by point reply to the reviewer 3 comments. Comments to the Editor: Thanks for inviting me to review the article entitled

"Characteristics of escape mutations from occult hepatitis B virus infected patients with hematological malignancies in Egypt". Minor Comment: ? Editing revision is mandate ? The article needs revision in term of structure and grammar. ? Language evaluation: "C"

**Q1. TITLE** Reflect the major content of the article; however South Egypt is more specific rather than Egypt.

**A1. We corrected TITLE.**

**Q2. ABSTRACT** It includes important data and conclusions, but lacking background and statistical values.

**A2. In the "Aim" section of the abstract, according to guideline of WJH to the authors it should include no or minimal background information.**

**Q3. INTRODUCTION** Provides sufficient background regarding the studied topic, however, redundancy is noticed. The purpose of the study is not clearly defined at the end of this section, it is the prevalence rather than the incidence and this has to be corrected

**A3. The redundant statements is omitted from introduction and" Incidence" is corrected to "prevalence" in page 6 line16**

**Q4. MATERIALS AND METHODS** Partially full description is provided for this section and statistical methods are appropriate. Some important issues have to be considered.

**Q4A. Patients:** Inclusion and exclusion criteria were not clearly identified, whether these patients under chemotherapy, coinfection,..... ?

**A4A. The serum samples were collected consecutively from hospitalized patients in the participating institutions. The statement concerned these data is included in the draft page 6, line 24-27**

**Q4B. "All patients received chest computed tomography and ultrasonography of abdomen as an initial evaluation."** Add no value to the study and has to be deleted. ?

**A4B. The statement is deleted**

**Q4C. "Serum samples were collected and stored at -80 oC for future examination of HBsAg, anti-HBs, and anti-HBc."**What about HBV DNA detection. It was missed.

**A4C. "HBV DNA" statement is added to sentence in page 6, line 29**

**Q4D. The study does not evaluate the status of HCV co infection in such patients. Egypt is considered as one of the most countries with high prevalence for HCV infection. This is better to be evaluated particularly in OBI patients.**

**A4D.**

**The authors agree with reviewer regarding the importance of HCV infection status and its association with occult HBV infection particularly in immunocompromised patients. However, the authors believe that this study requires a different design which must include two groups of patients; (1) selected group consisting of HCV-infected patient in comparison to (2) a**

second group including non-HCV infected patients. In the present study, the samples were collected consecutively from hospitalized patients with hematological malignancy. The hepatitis virus infection status (HCV and HBV) was completely “obscured” at the time of collection of the samples. Based on the background that HBV genotype D is the prevalent in Egypt, the researchers mainly aimed to investigate the underlying HBV genomic mutations and its virological characteristics in HBV genotype D particular with the lack of studies concerned HBV genotype D in comparison to genotype C. The starting point for the study of occult HBV infection was HBsAg(-)/ Anti-HBc (+). The authors presented now preliminary data concerned HCV infection status in the studied cohort in page 10, line 10-15, and line 22.

Q4E. Organization of subheadings for the methods in use has to be maintained; serological markers, DNA extraction, PCR, Sequencing. This will make the work more reproducible.

**A4E. The organization of subheading is corrected in page 7.**

Q4F. Serological Markers of HBV Infection: Detection of HBsAg was done by either of two methods, or the same sample was evaluated by the two tests for confirmation. This has to be clarified.

**A4F. The detection of HBsAg was done by the two tests. The statement is clarified in page 7.**

Q4G. Detection of HBsAb is missing.

**A4G. We corrected it in page 7.**

Q4H. Detection and Quantitation of Serum HBV DNA The assay was performed for the total number of patients or for the OBI samples only. This has to be clarified.

**A4H. Detection of HBV DNA was done in all serum samples that were negative serologically for HBsAg and positive for anti-HBc. Statements explaining this is already present in the abstract page 4 “Serum samples negative for HBsAg and positive for anti-HBc were subjected to nucleic acid extraction and HBV DNA detection by RTD-PCR. DNA sequences spanning the S region were analyzed in cases with occult HBV infection” , and in result page 10, line 21-31 “HBV DNA was detected in 42.6% (23/54) of patients with hematological malignancies who were negative for HBsAg, but positive for anti-HBc, suggesting occult HBV infection.”**

Q4I. Cell Culture and DNA Transfection : FBS is missing its origin. ? Reference for the technique has to be added.

**A4I. We cultured cells in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (p.31, line16). Citation [19] is provided now in page 8.**

Q4J. Determination of HBV Markers HBV markers determination on supernatants/cell lysate of cell culture is missing and has to be mentioned.

**A4J. The method for determination of HBV markers is the cell supernatant and lysate is**

**already present in Method section in page 8, line 25-27 “HBsAg and HBcAg were determined by CLEIA using commercial assay kits (Fujirebio Inc., Tokyo, Japan). “**

## **5. RESULTS**

**Q5A.** Provide sufficient experimental data, however, repeating what is in tables again within the text has to be avoided. ?

**A5A.** Thank you very much for your comment. The authors believe that simple explanation of the data in the figures is mandatory particularly to the general readers of the journal.

**Q5B.** Figures & tables: Titles has to be revised and written in more plausible form? Table 2 is missing (Qty/unit) for Anti-HBs ?

**A5B.** The Qty/unit is provided in table 2. Titles of tables are revised.

**Q5C.** Supplement table 1: is better to be deleted.

**A5C.** We deleted Supplement 1.

**Q5D.** Figure 2(b) / (c):HBcAg has to be revised and written correctly ?

**A5D.** The authors measure the hepatitis B core related antigen and its abbreviation HBcrAg.

**Q5E.** Figure 3(b): It is HBc protein not HBe protein? Title of table 3 has to be in its proper site? P value is missing throughout this section and is better to be mentioned.

**A5E.** The authors measured the hepatitis B e protein not HBc protein.

The title of figure 3 is corrected.

The authors conduct statistical test and no significant correlation was detected. Since table 3 is not comparable table rather being a demonstrative one exploring the impact of different mutation in the incidence of occult HBV, the authors prefer not to include the P value in the table.

## **6. DISCUSSION:**

**Q6A.** An overall theoretical analysis of the study results is poorly covered. The first two lines in the second paragraph were not convincing and better to be deleted.

**A6A.** The first 2 lines which include the statement “The association between occult HBV infection and hematological malignancy is poorly understood” is deleted.

**Q6B.** The authors are advised to quote the following Egyptian study” High prevalence of occult hepatitis B in hepatitis C-infected Egyptian children with haematological disorders and malignancies. Ths. Said ZN, El-Sayed MH, El-Bishbishi IA, El-Fouhil DF, Abdel-Rheem SE, El-Abedin MZ, Salama II. Liver Int. 2009 Apr;29(4):518-24.

The authors have to identify the 6 patients as those with OBI ?

**A6B.** The authors identify the 6 patients as patients with OBI and detectable HBV complete genome. The statement is present in the results section page 10 ” HBV DNA was detected in 42.6% (23/54) of patients with hematological malignancies who were negative for HBsAg, but

**positive for anti-HBc, suggesting occult HBV infection. The complete genome of HBV was successfully obtained from 6 cases with occult HBV infection.”**

Q6C. It is better to add the No(%) when emphasizing the % of any case ?

**A6C. The No is added in the discussion.**

Q6D. The authors were missing important issues as false isolated anti-HBc positivity, anti-HBs level,

**A6D: These points are now discussed in page 13, line 9-24**

presence of HCV co infection especially in those with HbsAg mutation ?

HCV-RNA is previously shown to be a significant predictor for occult HBV. It is advised to quote the above mentioned paper.

**A6D. The authors agree with reviewer regarding the importance of HCV infection status and its association with occult HBV infection particularly in immunocompromised patients. However, the authors believe that this study requires a different design which must include two groups of patients; (1) selected group consisting of HCV-infected patient in comparison to (2) a second group including non-HCV infected patients. In the present study, the samples were collected consecutively from hospitalized patients with hematological malignancy. The hepatitis virus infection status (HCV and HBV) was completely “obscured” at the time of collection of the samples. Based on the background that HBV genotype D is the prevalent in Egypt, the researchers mainly aimed to investigate the underlying HBV genomic mutations and its virological characteristics in HBV genotype D particular with the lack of studies concerned HBV genotype D in comparison to genotype C. The starting point for the study of occult HBV infection was patients negative for HBsAg but positive for anti-HBc. This group was 53 patients only and of the occult hepatitis B group, 6 patients were positive for anti-HCV. Then, the authors presented now preliminary data concerned HCV infection status in the studied cohort in page 10, line 10-14, and line 20, and discussed it in page in page 13, line 4-8 and also refer the above mentioned paper.**