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Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3403 Brief Article.doc).

Title: Incidence and characteristics of HBV reactivation in haematological malignant patients in Egypt South

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The manuscript has been improved according to the suggestions of reviewers and below is point by point answer to three reviewers:-

Reviewer 1:
The authors have focused on the reactivation of HBV in patients with HBsAg negative before receiving chemotherapy for hematological malignancies in Egypt.
They observed that 9.4 % of HBsAg-negative patients experienced the HBV reactivation. They observed escape mutation in four out of five. All HBV reactivation were observed in anti HBc positive patients. They claimed the significance of ant-HBc screening to identify high-risk patients of HBV reactivation. Their findings are significant and beneficial for the readers.

Minor points;
References should be appropriately described.

1. No information was shown about [Attia.,1998 and Khattab et al.,2010] in line 21 of page 4.
   A1. Corrected in page 4, line 21

2. [year] after author are unnecessary in line 11, 15 and 18 of page 10 & in line 24 and 1 of page 11 and 12, respectively.
   A2. Corrected and year deleted after authors in the draft

Reviewer 2:

1. The Title is too 'big'; the article contained only 53 patients from a hospital, and the data can not represent those in 'Egypt'.

A1. Thank you very much for your informative comment. The authors agree with reviewers about the small number of the studied population. However, this study is considered is the first in Egypt which investigated the HBV reactivation in Egypt and presented general outlines regarding this clinical issue. In the light of point that in Egypt monitoring of patients with malignancy is only done by liver function, the authors pointed to the importance of screening of those patients for HBV infection at least with anti-HBc before the commencement of chemotherapy. However, the title changed to be **Incidence and characteristics of HBV reactivation in haematological malignant patients in Egypt South in** page 1

In the mean time, the authors recommend for further studies with more studied population size in page 13, line 21.

2. The Abstract and Discussion are too lengthy.
   The abstract is reformatted to be suitable for brief article. The discussion is corrected.

3. There are numerous inappropriate expressions throughout the manuscript, such as
   a. "co infected" should be "co-infected"

   A3-a. Corrected
   b. "Denovo" …"De novo"
      A3-b: Corrected
   c. "anti-HBsAb"… "anti-HBs"
      A3-c: Corrected
   d. "anti-HBcAb"… "anti-HBc"
      Corrected
   e. Many others

4. The P values, rather than 'NS', should be presented in Table 1.

A4. Corrected and *P* values included in table 1 in page 17.

5. The detailed data about the HBV reactivation are absent:
   A. How long had each patient been treated with chemotherapy before he/she experienced HBV reactivation?
   A5. The duration between the start of the anti-cancer therapy and HBV reactivation development was described in Table 2, page 18

   B. Did the Patients 1, 3, and 5 in Table 2 ever have abnormal liver function tests?
      Data regarding the total bilirubin levels at the time of reactivation are added in Table 2, page 18. None of the 5 patients had abnormal levels of total bilirubin at the time of reactivation.

♦ Have the Patients 2 and 5 in Table 2 recovered from elevated ALT? how long?

Only patient 2 recovered from elevated ALT twice for one month before the patient died after overall survival for 13 months. Case 5 had normal ALT level. The outcomes of the patients are included in Table 2, page 18.

- Did the reactivated patients receive anti-HBV therapy?
  None of the reactivated patients received anti-HBV therapy. A statement of this data are included in page 8, lines 11, 12

- How about the short-term and long-term outcomes in the 5 reactivated patients?
  Outcomes of the patients were explained in table 2 in page 18.

5. Figure 1 may be more compacted: Anti-HBc—α-HBc, …, positive—(+), negative—(-), etc.
   A5. Corrected

7. Figure 2 does not provide important information, and may be omitted.
A7. Thank you very much for your comment. Figure2 demonstrated clearly two important points; First; provide a strong evidence of reactivation of occult HBV infection in immune compromised patients. Second; the application of the sequence and phylogentic analysis in demonstration the development of reactivation from an occult HBV infection. The authors believe that figure 2 is essential part of the story of the draft and cannot be omitted.

Reviewer 3

1. The authors stated that it is a prospective study in the title. However, no descriptions indicating 'prospective nature of the study' are seen in the manuscript.

   Thank you very much for your support and informative comments to improve the manuscript.
   In Egypt, in clinical practice, monitoring of patients during the chemotherapy is done by the ALT testing only. HBsAg and HBV DNA are tested in patients with elevated liver enzymes. For the purpose of this study, serum samples were collected before the commencement of chemotherapy and throughout the chemotherapy course at 4 weeks interval. The collected sera were stored at -80ºC for future examination of HBsAg, anti-HBs, and anti-HBc. However, the authors changed the title of manuscript to "**Incidence and characteristics of HBV reactivation in haematological malignant patients in Egypt South".** In addition, more detailed description of the nature of the study was explained in page 5, lines 4-13

2. In this study, testing for HBV DNA and HBsAg seemed to be done only in patients showing ALT elevation.
How could they detect HBV reactivation in the 3 patients with normal ALT levels (case 1, 3, and 5 in Table 2)?.
With such monitoring strategies, can the authors explain the higher incidence of HBV reactivation in this study, when compared to those in other studies (Hui et al., Matsue et al., and other relevant studies)?
A2 In clinical practice in Egypt, patients who are receiving cancer chemotherapy are monitored by liver function tests. Only patients with elevated liver enzymes are tested for HBsAg and HBV DNA. For the purpose of this study, the testing for the HBV DNA and HBsAg was done to all patients in the studied cohort not only the patients with elevated ALT.
Based on detection of the HBVDNA either qualitatively or quantatively in these 3 patients after receiving chemotherapy, the authors consider these cases as cases with potential HBV reactivation.
Possible explanations for the discrepant in the incidence of HBV reactivation between the current study and previous studies (1) the inclusion of HBV DNA appearance after chemotherapy as a criteria of HBV reactivation. These criteria of including cases with detectable HBV DNA after cancer chemotherapy as a sign of HBV reactivation was not used in defining cases with HBV reaction in related studies.
(2) The difference in the predominant HBV genotype among different populations. Different genotypes means different virological and clinical characteristics (3) The variation in the number of the studied cohort among different studies couldn't be ignored as possible factor that is maybe implicated in such discrepancy.
However, this study is the first in Egypt which concern the HBV reactivation in Egypt and the authors recommended further studies to explore this critical clinical issue. These explanations were stated in the discussion section in page 10, lines 25-29 and page 11, line 1-2

It seems that frequent monitoring would have been done during therapy to detect reactivation?.
What are the median time and interval for monitoring of HBV DNA or HBsAg in the patients?
In Egypt, in clinical practice monitoring of patients who are receiving the chemotherapy is done monthly and only by liver function test. However, when there is elevated liver enzyme, HBsAg and HBV DNA are tested every six months. The time between the start chemotherapy and development of reactivation are included in table 2, page 18

3. Table 2: The authors would provide the quantitative data (IU/ml or copies/ml) of HBV DNA levels, rather than the qualitative results (for both before and after treatment).
Corrected and these data are included in Table 2 in page 18.

- A brief description on St. Jude protocol should be added (possibly as a footnote to the table).
  A description of the St.Jude protocol was added as footnote in Table 2

- Are there test results for HBeAg/Ab in the patients?
  The HBeAg/Ab were not tested in these patients

4. Discussion, the second para in page 11: The analysis of HBV genomic characterization would be informative, but not all reactivation cases can be explained with viral genomic analysis. Based on previous publications on HBV reactivation, it can potentially occur in any patients receiving immunosuppressive therapy, irrespective of geographical area, HBV genotypes or mutations. Also in this study, only 2, 1, and 3 cases showed genomic variants in the core promoter, precore and S gene product, respectively. The results should be interpreted with caution, without a direct comparison with data in patients with pre-existing mutants before treatment but without reactivation during/after treatment.
This section in the discussion was omitted. However in the conclusion section, the authors recommend further *in vivo* and *in vitro* studies to investigate whether specific genomic mutations are implicated in *de novo* hepatitis in this subset of patients infected with HBV genotype D1, page 11, lines 26-29

5. Reference styles and some grammatical errors in the manuscript need to be corrected.
5. Corrected

3 References and typesetting were corrected

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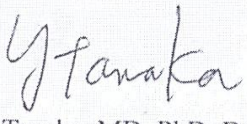
Sincerely yours,



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