Response letter

Journal title: World Journal of Clinical Cases

Manuscript NO: 54622

Title: Gilbert's syndrome coexisting with hereditary spherocytosis is not rare

Response to reviewer 1

Question 1. From the individual prevalence rates (GS, 3-10%; HS, 1 per 2,000 persons), the calculated rate of coexistence of GS and HS is 15 to 35 per million births. However, HS can be masked in patients with GS owing to the high prevalence rates and similar symptoms of GS and HS. I agree with the authors' opinion that there are a large number of overlooked cases owing to the lack of awareness and techniques. There must be several more cases of coexistence of GS and HS than those actually diagnosed. However, the authors describe in the title that the coexistence of GS and HS is not rare. It seems to be too early to assert that it is not rare, because the authors encountered 6 patients (4 patients in real cases because 2 family cases were included) for only 6 months. It is possible that no case has been encountered before this period and none will be encountered in the coming years. Statistical evaluations are required to demonstrate that the coexistence of GS and HS is not rare.

Response *：T**he scrupulous attitude of the reviewer deeply moved us. We had to admit that we overemphasized the novelty and attractiveness of the title, but lacked the thorough consideration of its scientificity. According to the reviewer's pertinent suggestions, we changed the title from "is not rare " to " might not be rare", and modified it accordingly in the discussion section.*

It is worth further explaining here that the six cases of GS coexisting with HS we

reported this time were unexpectedly discovered no long after May 1st, 2018, the study of CO breath test applied in the differential diagnosis of isolated unconjugated hyperbilirubinemia in adults (Note: including hemolytic and non-hemolytic disease).

The study is still in progress. Up to now, we have collected 37 (16+21) cases of hemolytic hyperbilirubinemia, of which only 16 (16/37, 43.2%) cases of simple hemolytic disease, but 21 cases (56.7%) of GS coexisting with hemolytic disease, including seven cases of HS, four cases of G-6-PD deficiency, four cases of chemotherapy-induced hemolysis, two cases of thalassemia, and one case of megaloblastic anemia, one case of secondary erythrocytosis, one case of Evans syndrome, and one case of hemolysis after cardiac valve implantation. The results suggest that the incidence of GS coexisting with the hemolytic disease might not be high in the estimation of population incidence, but in the subset population like isolated hyperbilirubinemia, it may not be rare. We will detail it in a forthcoming paper.

Question 2. The authors described that Levitt's CO breath test was useful for the measurement of RBC lifespan to screen for the coexistence of GS and HS among patients with GS. However, although GS is not associated with overt evidence of hemolysis, a shortened RBC lifespan has been found in some affected individuals (reference: for example, Powell LW et al. Australas Ann Med 1967;16:221-225; Kang LL (author). Medicine (Baltimore) 2020;99:e19109). In my literature review, the CO breath test shows shortening of RBC lifespan in cases of hyperbilirubinemia by hemolysis and is useful for diagnosing hemolytic jaundice. However, it is necessary to establish the cut-off value of RBC lifespan, which distinguishes between simple GS and coexistence of GS and HS, to demonstrate that the CO breath test approach is a reliable screening tool to evaluate a patient with HS and GS.

Response **：** *The reviewer's good proposal is much appreciated. The report of coexistence of GS and HS was just an unexpected finding in a series of our studies of Levitt's CO breath test applied in isolated hyperbilirubinemia. As the reviewer said,*

our previous paper published in the journal Medicine reported that shortened RBC lifespan indeed, existed in a portion of persons with GS (95.4±28.9 days vs. 126 days; t=7.504, P<.01), with 30.0% below the lower limit of the normal reference range (75 days), consistent with mild hemolysis in 30% to 80% of persons with GS based on slightly shortened RBC lifespan, relative to typical values, estimated by 51Cr-labeled RBC re-transfusion tests.[Ref] Besides, we found that the RBC lifespan of 100% hemolytic hyperbilirubinemia patients was below the lower limit of a standard reference value of 75 days. The results showed that the rapid and straightforward Levitt's CO breath test could be susceptible to identify the presence of hemolysis even slight in isolated hyperbilirubinemia.

The presence of slight hemolysis would interfere with the differential diagnosis of hereditary hyperbilirubinemia and hemolytic hyperbilirubinemia using the Levitt's CO breath test. However, we found that the RBC lifespan of hemolytic hyperbilirubinemia was severely shorter than that of GS. As the reviewer suggested, we performed an ROC analysis. The result suggested that the cut-off value was 60 days, to be optimal threshold for the diagnosis of hemolytic hyperbilirubinemia, of which sensitivity was 95.1%, and specificity was 97.1% (Fig, unpublished data).
Further comparison for the RBC lifespan of GS coexisting with hemolytic disease and of merely hemolytic hyperbilirubinemia did not show significant statistical differences, implying Levitt's CO breath test could not be used for both identifications (Fig, unpublished data). However, as reported in this article, we found that hyperbilirubinemia inconsistent with the level of hemoglobin was an essential feature of GS with hemolytic disease, which would be much helpful to distinguish it from simple hemolytic hyperbilirubinemia.

References *Ling-Ling Kang, Yong-Jian Ma, Hou-De Zhang. Carbon monoxide breath test assessment of mild hemolysis in Gilbert's syndrome. Medicine. 2020;99(7):e19109. doi: 10.1097/MD.0000000000019109*

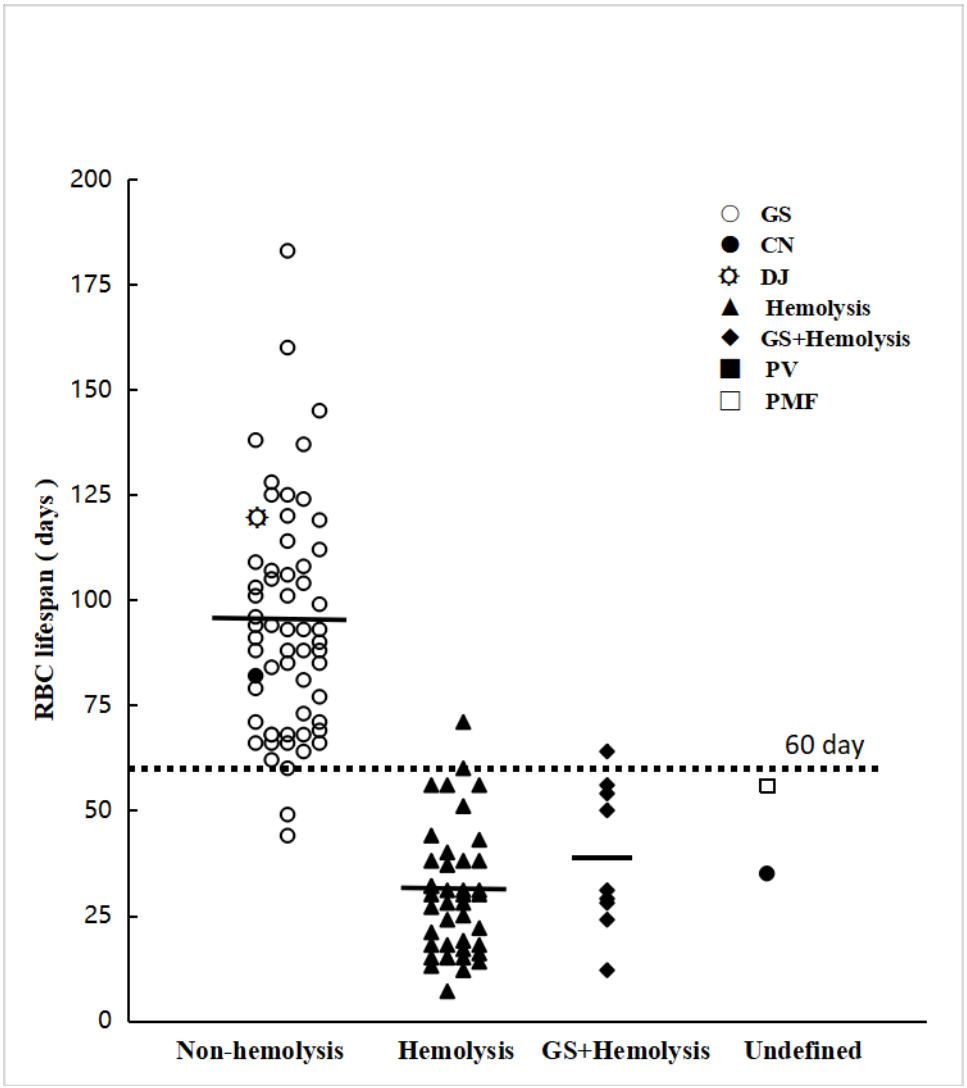

Fig. Scatter plot of isolated hyperbilirubima

Question 3: Abstract: According to the guidelines of World Journal of Clinical Cases, the abstract should be structured, and the subsections should include **Background**, **Case summary,** and **Conclusion**.

Response : *Thank the reviewer's kind indication. the structure of abstract has been corrected in the revised manuscript.*

Question 4: The description of the subsection of cases one and two is too long.

Response : *Thank the reviewer's frank remarks. Because some basic conceptions such as isolated hyperbilirubima are need to be introduced beforehand in order to let*

readers better understand the characteristics of the disease, we intend to describe first two examples in a little more detail. Some deletions have been made in the revised version.

Question 5: In the subsection of cases one and two, there are two incomprehensible descriptions; "hepatocellular injury and cholestasis, i.e. Isolated unconjugated hyperbilirubinemia" and "the average RBC lifespan of ~120 days (normal range: 70~140 days)." Please modify them for clarity.

Response : *Thank the reviewer's good suggestion. Corrections have been made in the revised manuscript.*

Question 6: References: The method of describing the number of reference citations in the main text and the description of the references in the reference section should follow the guidelines of the World Journal of Clinical Cases.

Response : *Thank the reviewer's kind remind. Corrections have been made in the revised manuscript.*

Question 7: Minor: In the main text, several expressions are unsuitable for a scientific article. **Please modify the phrases,**

"Taking his history, we learned … told needless of any treatments" (page 5, line 24),

"CO breath test, which was serviced in our Gastroenterology" (page 7, line 22),

"To the doctor's surprise" (page 7, line 24),

"To our great surprise, there is still a few smart doctors…" (page 11, line 7).

Response : *We are sorry for the mistakes and errors. Corrections have been made in the revised manuscript.*

Response to reviewer 2

Question 1: The authors should give bilirubin value in cases 1 and 2.

Response : *We thank the reviewer's suggestion. The bilirubin value was shown in the revised manuscript in addition to Table 1 .*

Question 2: There are many grammatical errors in the article.

Response : *It is hard work for a non-English speaker to write English article. Our manuscript was re-edited by a professional scientific editor at Write Science Right in the USA (editing certification enclosed). Careful check was made once more for the revised manuscript.*