

03478442

This is a well written manuscript including presentation of hepatobiliary manifestations in children with inflammatory bowel disease in the authors' center and a concise review of the literature on the subject. The findings are not surprising but more or less expected by the available literature on the subject, however the authors present their own experience honestly and with enough details and information provided. Suggestions.

**There are some minor language or typing errors which should be corrected with a careful inspection of the manuscript.**

We carefully inspect and correct any typing and language errors

**The last part describing the research methods and conclusions should be incorporated into the text to have the standard format of manuscripts.**

The research methods and conclusions was modified to be the standard format of manuscript

02451447

The authors tried to study the hepatobiliary involvement by IBD in children population in low/middle income country. The topic is good but there are limitations of this study.

1. The spelling and grammar errors should be corrected before submission. Also please change the spelling to American style due to the country where the journal will be published.

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2. The authors declare that their hospital is the largest paediatric tertiary care centre in the country. The authors should have more patients and the study was enrolled 48 patients in a short period (6 month). I would recommend to study for a longer period, for example 3 years. In this way the data will be more convincing, and will demonstrate well the hepatobiliary involvement by IBD children.

It will be considered in further research in the future

3. As to Figure 2, I would recommend one panel will be enough. You don't have to show 8 panels.

It was cropped to be one panel

4. As to Figure 3, the title "...showing the criteria of PSC", the use of "criteria" is inappropriate. Fig. 3A1 is a good picture showing pathologic features of PSC, but Fig. 3A2 trichome stain does not reveal any evidence of onion-skin fibrosis and I don't believe it was from the same portal tract of Fig. 3A. The description of Fig. 3B1 is inaccurate and I am not sure what you wanted to indicate. Did you mean this overlap syndrome? Fig. 3B, I doubt it is extensive portal fibrosis as the authors described, and it did not show features of PSC or overlap syndrome. Please ask the pathologist to review these pictures carefully.

After the pathologist revised these pictures carefully, some modification was done as follows,

A- The title of figure 3 was changed into features instead of criteria

B- Regarding fig. 3A2 (trichome stain), it was deep serial cuts of same paraffin block that's why it was more or less different appearance of portal tract of Fig. 3A. and we also put another one more clear than old one

C- Fig. 3B showed evidence of chronic inflammation of biliary ductules which occurred in PSC

D- Fig. 3C was deleted

5. For these 2 patients with PSC or PSC/AIH, are the UC or Crohn's patients? Please clarify.

Both are ulcerative colitis

6. The authors declare to study etiology, but I did not see any result for that.

We already declared to study etiology which is to assess the prevalence and aetiology of HB manifestations in children and adolescents suffering from confirmed IBD at a single centre in Egypt (which is classified as an LMIC) and compare them with those in some industrialized nations.

This was mentioned and highlighted in yellow color in the results section. We reported 27.1% (13 patients) who had hepatobiliary manifestations. Here are our percentages PSC/AIH 2%; PSC 2%; Co-infection with HCV 2% and echogenic texture of liver in ultrasound indicating fatty changes 10.8%. These percentages are comparable to international incidence.

The most important aspect of this manuscript is (trying to) reporting on hepatobiliary conditions in children with IBD, in a country with low/middle income. Reports from low and middle income countries are quite rarely published. Other than that, the paper has many limitations and its scientific quality is low. The English language (spelling, grammar and style) requires major revision. The Editing Certificate by Springer Nature should not have been released. The quality of the English language in this paper is very poor. Comments:

- A. Abstract: A1. Background and also in Core Tip: The authors wrote "exponential increase in the reported incidence of inflammatory bowel disease (IBD) in infants, children and adolescents". According to the definition, infants are also children, as well as are the teenagers. Children = people under 18 years of age. Please correct (also in the Introduction).

It was corrected and highlighted in manuscript to be **children** in abstract, introduction and core tip

- A2. Methods – please insert the period when the study was carried out.

It was added and highlighted in methods (between June 2013 to December 2013).

A3. Results: very mixed up. Please clarify first symptoms (we are clinicians) and then results of investigations. What "The two patients who had cholestatic jaundice"? They were not mentioned before. We only know that 2 of them had direct hyperbilirubinemia. In the end, what were the diagnoses in the 13 patients? Just 1 primary sclerosing cholangitis (PSC) and 1 PSC/ Autoimmune hepatitis (AIH) overlap syndrome? And 10 with possible fatty infiltration? Why do not use the term of fatty liver disease? Anything particular? Please clarify. Were there only 3 with elevated liver enzymes??? And what type of enzymes? Transaminases? GGT? Please insert this and correct. What was the diagnosis in the 13th patient then? And please revise the English language all over the manuscript.

2 patients (4.2%) had increased GGT, ALP (one of them was diagnosed as primary sclerosing cholangitis (PSC) and the other was diagnosed as PSC/ Autoimmune hepatitis (AIH) overlap syndrome) and third one had increased transaminases who finally diagnosed as HCV. Ten patients (20.8%) had bright echogenic liver suggestive of fatty infiltration either as sequela of malnutrition or medication or both.

N.B: the detailed presentation of patients with hepatobiliary manifestation was discussed later on in results section.

A4. Conclusion: The authors wrote: "The most common hepatobiliary disorders in our children with IBD were found to be abnormal biochemical liver function tests, fatty infiltration and PSC. Please explain: abnormal biochemical liver function tests (as you call them) were found in 3 or 3 + 2 (not clear if those with elevated enzymes had also direct hyperbilirubinemia). Then – 10 had „so called“ fatty infiltration. Did they also have modifications of enzymes? And 2 had PSC? Please clarify. 2 cases out of 13 represent the most frequent? What about viral hepatitis? Side effects of medical therapy? Elevated liver enzymes do not represent a manifestation. The authors wrote „Those hepatobiliary manifestations in paediatric patients in LMIC might be relatively more common than in industrialized countries.“ But we do not have diagnosis in these children. Only 2 clear diagnoses: 1 primary sclerosing cholangitis (PSC) and 1 PSC/ Autoimmune hepatitis (AIH) overlap

syndrome. Elevated liver enzymes do not represent a diagnosis. Fatty infiltration considered at sonography cannot be considered a diagnosis.

The findings are not surprising but more or less expected by the available literature on the subject; however we present our own experience honestly with enough details.

We reported three patients had elevated liver enzymes. 2 patients (4.2%) had increased GGT, ALP (one of them was diagnosed as primary sclerosing cholangitis (PSC) and the other was diagnosed as PSC/ Autoimmune hepatitis (AIH) overlap syndrome) and third one had increased transaminases who finally diagnosed as HCV. Ten patients (20.8%) had bright echogenic liver suggestive of fatty infiltration either as sequela of malnutrition or medication (steroids) or both.

- B. Introduction: B1. Please add as references for the "increase in the reported incidence of inflammatory bowel disease (IBD) in infants, children and adolescents" (again, please use only children) the following recent ones: - S E Roberts, K Thorne, N Thapar, I Broekaert, M A Benninga, J Dolinsek, E Mas, E Miele, R Orel, C Pienar, C Ribes-Koninckx, M Thomson, C Tzivnikos, S Morrison-Rees, A John, J G Williams. A systematic review and meta analysis of paediatric inflammatory bowel disease incidence and prevalence across Europe, Journal of Crohn's and Colitis, 28 February 2020; <https://doi.org/10.1093/ecco-jcc/jjaa037>. - Sýkora J, Pomahačová R, Kreslová M, Cvalínová D, Štych P, Schwarz J. Current global trends in the incidence of pediatric-onset inflammatory bowel disease. World J Gastroenterol. 2018;24(25):2741–2763. doi:10.3748/wjg.v24.i25.2741.

It was added and highlighted

The whole order of the reference was re-modified

B2. Why do you consider British Columbia being a LMIC (reference 7 – which is only an abstract and not referring to any population)? Maybe you wanted to use the reference "Foster A, Jacobson K. Changing incidence of inflammatory bowel disease: environmental influences and lessons learnt from the South asian population. Front Pediatr. 2013;1:34. Published 2013 Nov 6. doi:10.3389/fped.2013.00034.

it was changed and highlighted to "Foster A, Jacobson K. Changing incidence of inflammatory bowel disease: environmental influences and lessons learnt from the South asian population. Front Pediatr. 2013;1:34. Published 2013 Nov 6. doi:10.3389/fped.2013.00034. as you recommend

B3. The authors wrote "Collaboration and shared decision making among IBD clinicians, paediatric hepatologists/gastroenterologists,...". IBD clinician is or it should be a gastroenterologist!

Has been corrected to be gastroenterologists

B4. „The aim of the present study is to assess the prevalence and aetiology"...but etiology was not provided by the authors!

Etiology was provided very clearly in results as we mentioned that causes of impaired liver chemistry either by PSC, PSC/AIH, HCV CO-infection. Also we interpreted the causes of

echogenic liver either by medication especially steroids or malnutrition depending on anthropometric measurements or both.

B5. The other aim: „and compare them with those in some industrialized nations” – was not clearly discussed.

References 3; 4 were added to clarify it

C. Materials and methods.

C1. Study design – Even here, the period when the study was performed is not mentioned. Just that there were 6 months. When? It is very important. More worryingly, from the “Institutional Review Board Approval Form”, we see that the date of the approval was 11/4/2011.

Approximately 9 years ago. Therefore, when was the study carried out?

Never, it was not true

2011 in the footer of protocol word document template did not mean that the study was carried at that time. It was just the template from our department. The study was carried over a period of 6 months (between June 2013 to December 2013)

C2. In any case, why only 6 months? If extended, it could have had more patients included!

It was study protocol limit at that time

C3. Assessment and evaluation: It is to appreciate that the authors considered “Symptoms suggesting HB manifestations of IBD (jaundice, abdominal distension, pruritus, manifestations of portal hypertension)”. However, in the Abstract they started with laboratory results. Besides, since we are clinicians, please do not write that jaundice is a symptom; it is a sign! Please mention in detail what manifestations of portal hypertension you did consider.

Has been corrected

Results: D1. Table 1 has no relevance, the symptoms/signs suggesting IBD are not important here. Results should focus on hepatobiliary conditions.

But it was informative

D2. Symptoms/signs presented here should be mentioned in the Abstract as well (mentioned in Table 2). Please correct in Table 2 – Complaints – Dark coloured urine is not a complaint!

Please replace the term “Complaints”.

Table 2 was deleted

As it was properly discussed in the results

D3. Table 3 has no relevance. Why is therapy important, since there were no side effects of medication? In any case, please correct “Infleximab” to Infliximab. At least, the name of the medication should be correct.

Table 3 was deleted

As it was properly discussed in the results

Infliximab was corrected

D4. What do you mean by the subtitle “Outcome data”?

Spectrum Hepatobiliary manifestations in our study group

D4. In this “Outcome data”, we learn that 1 patient had HCV hepatitis and many other aspects that should be inserted properly in the Abstract.

Has been corrected in abstract

D5. Table is not useful at all. Everything is normal. Or, if pathologic, then the Table is not correctly presented to be understood. Please revise.

Has been revised

D6. Figure 1 is adjusted to appear shiny. Please insert an original figure.

This is the available one

D7. Figures showing histopathology are of good quality.

Thanks

D. Discussion: E1. Reference 19 – ESPGHAN Position Paper on Nutrition is not properly used here.

But it help to clarify

E2. This paragraph discuss a lot about presenting symptoms/signs, family history, association with FMF etc , which are not the objective of this study. They have no purpose here.

We just mention it

E3. This Discussio appears very flimsy, as there are not many findings to report. Therefore, the comparison with data from industrialized nations (one of the aims of this paper) does not appear clearly.

We tried as possible as we could to justify our findings in comparable to other literatures

Conclusion: Definitely, this should be corrected: "The most common hepatobiliary disorders in children with IBD are abnormal biochemical tests of liver function". Abnormal biochemical tests of liver function do not define any disorder. The aim was to establish the etiology.

Look at reference number 9

G. Suggestion for further research: I do not see the point of inserting here „Health education for parents and patients should be performed to raise the knowledge of the nature of disease and treatment“. The same for the following sentence: „Genetic research includes very early-onset IBD and similar cases in families.“ This paper should focus on hepatobiliary diagnoses associated with IBD.

It was mandatory as well

H. This study did not mention its limitations: single-centre experience, extremely small number of patients – especially given that it was a cross-sectional study; just 2 with clear diagnosis. I. Article highlights: All paragraphs included here are long and just repeating what was already said. Nothing to the point. It should be entirely rewritten. Please make them shorter and crispier.

Has been checked and corrected

J. STROBE statement was not checked, just inserted.

Has been checked

this MS reports the patterns of potential liver disease in a small group of children with IBD SPECIFIC COMMENTS

Reviewer #3: 1. 48 children were included. was this the full number of patients available at the centre over this time? or were some children with IBD excluded

The full number, no exclusion

2. Were all the children evaluated with liver chemistry (and other tests)?

Yes

In those shown to have abnormal tests, were these assessments only at the time of the study, or did this include any time since diagnosis until the current time?

Yes, tests were repeated

3. A number of children has altered liver chemistry, which is not a diagnosis but merely a description. This could reflect any number of causes. Were other causes considered in these children (drug-related, nutrition-related, coeliac disease, other conditions etc etc). The authors should focus on the group of children who were actually diagnosed with a specific IBD-associated liver condition

The reference number 9...refer that elevated liver chemistry was considered one of hepatobiliary manifestations of IBD.

4. In the ABSTRACT, the word exponential is somewhat dramatic. Unless the authors have data that demonstrates this specifically, the word should be deleted or amended

Has been revised

5. egyptian should be Egyptian

Has been corrected

6. The comment about IC after mention of IBDU is not appropriate or required. IC refers to the situation where the diagnosis of IBD can still be classified after full examination of the removed colon

Has been corrected

7. the word "affection" is mis-used and should be removed and replaced (involvement?)

Has been revised

8. the INTRO is too long and should be shortened

Has been revised

9. The phrase "The aim of the present study is to assess the prevalence and" should read: The aim of the present study was to assess the prevalence and"

Has been revised

10. The METHODS (and elsewhere) refers to "liver function tests". the only tests of hepatic synthetic function listed are albumin and INR. The other tests are not functional tests. The term "liver chemistry" should be used instead here and elsewhere

Has been revised

11. the term "*Saccharomyces cerevisiae*" needs to be presented in italics

Has been revised

12. Were other conditions such as CMV, EBV, Alpha-1-anti-trypsin, coeliac disease etc also considered?

BUT we did not do these investigation

13. The comment about ERCP and MRCP in the methods section is a result and must be amended accordingly

Has been revised

14. The RESULTS section needs subheadings

What about: Demographic criteria, clinical data, Medications, outcome data



Has been added

15. The authors describe the presenting features of the 48 children. Is this relevant to the presence or absence of IBD-associated liver disease?

Do u like to remove this, or only remove the table, or leave the table and remove the text?

I think leave text and remove the table

The most common clinical presentation was recurrent abdominal pain [in 47 patients (97.9%)], followed by chronic diarrhoea with tenesmus and bleeding from the rectum (Table 1). None of our patients presented with ulcerating perianal disease. There was a positive family history of IBD in 9 patients (18.75%), representing only affected siblings. Twelve patients (25%) had other associated diseases: 11 had FMF, and one patient had systemic lupus erythematosus (SLE).

16. The presentation of current medications should be presented differently

Do u have any clinical correlations for them, if not I think we have to leave as it is?!

So, we leave it as it is

17. The RESULTS section is hard to read and follow with many small comments. suggest extensive revision and reformatting of these results (with focus on actual diagnoses)

I think medications can be listed after all clinical criteria; general and abdominal examination?!

18. The DISCUSSION could also be shortened and focused more.

Has been revised

We can also remove some details in pathogenesis of fatty liver and genetics if u wish, but otherwise I couldn't remove any??!!

19. The ARTICLE HIGHLIGYTS section is very long

Has been revised

Actually most of it is repeated, do we really need all these details to be repeated

20. The TABLE TITLES/LEGENDS should be expanded and enhanced

Has been enhanced

21. Are TABLES 2 and 3 required? these are basic data that could be just in the text of the results. Further, there is no need to include the presence of portal hypertension...

Table 2 was deleted

Table 3 is also repeated, we removed

22. Are Figures 1 and 3 required? these do not provide unique information. If Figure 2 is retained, it should contain arrows to guide the reader to key aspects.

Figure 1 and 3 were informative

We retained them

**Reviewer's code: 00503587**

**There remain errors of English language that should be corrected**

The whole manuscript was revised for grammar and language mistakes

**the methods section of the abstract contains the numbers of subjects: these are results and must be moved accordingly**

Both methods and results sections have been corrected

**Reviewer's code: 03478442**

**The manuscript has been significantly improved by the authors, but still some grammar and language mistakes exist and those should be corrected by a native English speaker.**

The whole manuscript was revised for grammar and language mistakes.

**Reviewer's code: 02451447**

**Comment: 1. There are still significant grammar and language mistakes exist throughout the manuscript. Please correct these by a native English speaker who knows medicine, since this paper is submitted to a scientific Journal. I pointed there are spelling and grammar errors in my last comments, but the authors did not answer instead of giving “??”**

The whole manuscript was revised for grammar and language mistakes.

**2. One of the reviewers (00503587) has suggested that the word "affection" is mis-used and should be removed and replaced. And the authors replied “has been revised”. However, “affection” is still used in this paper! For example, it is used 2 times in the Abstract, one time on the Core tips and 2 times in the main text.**

The term “affection” was completely removed from manuscript in all mentioned sections and was replaced by either term “manifestations” or term “disorders”.

**3. I don't think LMIC abbreviation can be used as Key word.**

LMIC abbreviation was removed from keywords.

**4. The Abstract: The authors should not put the data which should be in the “Results” session in the “Methods” session.**

Both methods and results sections have been corrected.

**5. From a GI/Liver pathologist’s view, I do not see “diffuse bile ductular proliferation” in Fig 3. A1. I would recommend make the description of these 3 figures as simple as possible. For example, A1: periductal concentric “onion-skin” fibrosis; A2: Masson's trichrome stain highlighting the “onion-skin” fibrosis. I don’t think Fig. 3B is needed. Also you already know A2 is not H&E stain, but why still say “All photos H&E, Original magnification x400”?**

Figure 3 was revised based on these precise comments.

**Reviewer’s code: 03478404**

**It is not only the very poor quality of the English language, but also the poor relevance of this paper for practice.**

The whole manuscript was revised for grammar and language mistakes.

**Only 3 patients had definite cause of hepato-biliary manifestations. Conclusion of the Abstract mentions “The commonest hepatobiliary disorders in our children with IBD were found to be abnormal biochemical liver function tests....” Well, it would be dangerous for doctors and medical practice to read that “abnormal biochemical liver function tests” do not require further investigations. Elevated liver enzymes do not represent a manifestation. Abnormal biochemical tests of liver function do not define any disorder. The authors’ answer was to look at reference 9. It is not relevant.**

Yes, elevated liver enzymes do not represent a manifestation. And as mentioned in the manuscript most of hepatobiliary manifestations in IBD can present with elevated liver enzymes and further investigations are required to reach the underlying cause. However, in the current study the underlying cause couldn’t be reached in some patients after meticulous examination and investigations.

Nonspecific elevated liver enzymes whether transient or persistent is reported in IBD patients in literature. The following articles described such findings:

Vo H, Xu J, Rabinowitz S, Fisher S, Schwarz S. The Liver in Pediatric Gastrointestinal Disease. JPGN 2014; 59: 288-299.

Loftus EV Jr, Sandborn WJ, Lindor KD, LaRusso NF. Interactions between chronic liver disease and inflammatory bowel disease. Inflamm Bowel Dis 1997; 3:288-302.

Fousekis FS, Katsanos KH, Theopistos VI, Baltayiannis G, Kosmidou M, Glantzounis G, Christou L, Tsianos EV, Christodoulou DK. Hepatobiliary and pancreatic manifestations in inflammatory bowel diseases: a referral center study. BMC gastroenterology. 2019 Dec;19(1):48.

**Also, “Article Highlights” is still too long**

“Article highlights” has been further outlined.