Peer Review File

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Reviewer A

In this interesting study, the authors assessed the association and correlation of elevated transaminases with disease severity in celiac disease (CD) patients and whether elevated transaminases could be used as a surrogate marker to predict the severity of the histologic disease. They performed a retrospective chart review of all children ages 6 months to 17 years. Results demonstrated no statistical significance between serum transaminase levels and histologic severity of disease, however, their results may suggest a trend toward more advanced disease or longer-standing enteropathy at presentation as shown by a lower IgG value (p=0.02). Results also supported a correlation between histological disease severity and patients who experienced diarrhea.

They concluded that no association between histological disease severity and the degree of elevated transaminases was found and that future studies are needed to determine better predictors of histological severity to prompt sooner endoscopic assessment and treatment.

The study is of interest and of clinical significance since non-invasive serological markers of prognostic significance might help the proper management of CD diagnosis and follow-up. I have only minor comments to further improve the manuscript:

Comment 1: When they stated that "Initial screening for CD with serum tissue transglutaminase (tTG) immunoglobulin A (IgA) levels is the current recommendation by most consensus authorities", they should recall the important point recommended by all current international guidelines suggesting that anti-tTG IgA antibodies must be searched for together with total IgA serum levels to exclude an IgA deficiency potentially causing a false-negative anti-tTG IgA result, as recently reported in a comprehensive review comparing all current international guidelines (Current guidelines for the management of celiac disease: A systematic review with comparative analysis. World J Gastroenterol. 2022 Jan 7;28(1):154-175).

Reply 1: *Thank you for this comment. We have acknowledged this point and added in this reference to our introduction.*

Changes in text: *We added to our intro on line 90 "*Additionally, current international guidelines suggest that together with anti-tTG IgA antibodies, a total IgA serum level must also be measured to exclude an IgA deficiency that could cause a false-negative anti-tTG IgA result" *and added in the reference into our citations.*

Comment 2: A very important piece of literature data is the well-recognized role of "hypertransaminasaemia of unknown origin" as the sole manifestation of silent celiac disease, as previously demonstrated in a pivotal study (Anti tissue

transglutaminase antibodies as predictors of silent coeliac disease in patients with hypertransaminasaemia of unknown origin. Dig Liver Dis. 2001 Jun-Jul;33(5):420-5.).

Reply 2: *Thank you for this comment. In our discussion section on line 313 we did mention the same finding that elevated transaminases are sometimes the only manifestation of CD in children, which also conveys this important finding. We have also added in this reference into our results to include this additional finding as well.*

Changes in text: *We added in this reference in addition to our findings to strengthen the results section. On line 315 we added "*In support of this idea, additional studies have also reported cases where the sole manifestation of silent celiac disease is hypertransaminasemia of unknown origin". *This reference was also added in our citations.*

Comment 3: Regarding the clinical relevance of serological markers of mucosal damage severity, the authors should recall the clinically relevant previous study describing the very high specificity of anti-actin IgA antibodies as very reliable serological markers of villous atrophy as previously demonstrated (Anti-actin IgA antibodies in severe coeliac disease. Clin Exp Immunol. 2004 Aug;137(2):386-92).

Reply 3: *Thank you for this comment. This isn't an antibody that we test for as part of our work up at this institution. This test is less readily available and not routinely tested. Clinicians looking to add this test may need to check with their lab to see if they can collect it. Maybe future studies need a composite score using this antibody as well, but we don't have this data to investigate at this time.*

Changes in text: *We have taken this comment into consideration and added it in to our methods section on line 160. We explained that this is not a test currently offered at our institution. We also added this into the discussion for future studies to consider when looking for noninvasive methods to determine disease severity and triage patients for biopsy. Please see line 356. This paper has also been added to our references.*

Comment 4: The last very important point is related to the potential pitfall of anti-tTG IgA. The authors properly stated that "Although having positive tTG IgA level is both sensitive (99%) and specific (75%) for CD, the precise tTG IgA value does not correlate with the extent of luminal or extra-luminal disease severity accordingto93 the published literature". In this regard, the authors should also recall the cause of false positivity of anti-tTG IgA such as giardiasis which is characterized by villous atrophy and may present anti-tTG IgA false positivity as previously demonstrated (Antitransglutaminase antibodies and giardiasis. Am J Gastroenterol. 2004 Dec;99(12):2505-6.).

Reply 4: *Thank you for this comment. We agree that a false positive could have come from giardia which is also characterized by villous atrophy, although, our pathologist confirmed that there were no cases of giardia in any of the samples we*

had for this study. We feel confident that because we investigated other causes of villous atrophy in our samples that we did not include any false positives in our sample.
Changes in Text: *We appreciate this valid note on what could cause a false positive for our results. In our paper, we added a sentence in the methods to explain that giardia was also tested for in each of the samples so there is no confusion for future readers. We added the following comment to our methods section as suggested on line 174; "*….and confirmed there were no cases of giardia in any of the samples, which could have led to a false positive diagnosis of CD."

Reviewer B
Some comments and questions:

Comment 1: Am I correct that you do not routinely look for transaminase values in patients that are referred to your specialised GI Unit? You may have missed patients with transaminase elevation in CD and none-CD patients or other disease.Therefore it remains a bit unclear how representative the real numbers /percent of these patients are.
Anyhow since liver enzyme elevation in CD patients occurs without beeing understood it was worth looking at this parameter and to look for a potential correlation with severity of CD.
Reply 1: *Thank you for this comment. For clarification, we do routinely look for transaminase values in patients that are referred to the unit now. This study is retrospective, and as such, this was not common practice in the past. This is why we have a smaller sample size as few patients were referred to the unit with liver enzyme abnormalities measured. As a result of this finding, we have changed our practice so that this is measured for all patients suspected of CD. Hopefully this clears up this confusion. You will also see on line 282 "*Both the retrospective nature, the size of the patient population, and the missing information due to variation in investigative practice at time of diagnosis amongst the clinician group limited our ability to compare certain variables of interest to histological severity" – *This point also demonstrates that there has been a change in practice as a result of this finding. We have also added in another line to make sure it is not confusing for others.*
Changes in text: *We have noted this confusion and added "*As this is a retrospective study, the authors discovered that investigative practices in the past did not routinely check for transaminase values when patients are suspected of CD, and as such current practice has changed to include measuring liver enzymes as standard practice for all patients moving forward." *On line 284.*

Comment 2: Do you have the possibility to find out wether there were differences in dietary intake/choice of Food in different patients groups (Can you exlude that diet had an effect on liver enzyme Elevation Independent of the

occurance of CD?
Reply 2: *Thank you for this interesting comment. Our records unfortunately do not have data on diet specifically for each of these patients that we would be able to investigate for differences. Therefore, we would be unable to comment on whether other foods were responsible for liver enzyme elevation.*
Although, we agree with you that dietary intake/choice of food could have an impact on liver elevation, specifically through the development of fatty liver disease. Fatty liver disease risk factors include being overweight, having high blood fat levels and high cholesterol, all of which can be caused by a high fat diet. You are correct in suggesting that dietary choices like these could influence someone's liver enzyme levels. For this study however, no patients in this study sample were overweight and we also checked for fatty liver disease and subjects were excluded if they had any other causes of liver injury or disease. In our methods section, we explained the exclusion criteria. We feel confident that because we removed other causes of elevated liver enzymes that diet/ choice of food was controlled for.
Changes in text: *We agree with your comment that dietary choice/food intake could influence liver enzymes. In this study we also feel that this was controlled for through our exclusion criteria. In our methods section, we added "fatty liver disease" to the conditions that would cause liver injury along with other examples that we included prior, just to make sure that there is no confusion for future readers. We hope this makes it more clear. Please see line 150 where this was added in.*

Comment 3: The conclusion that you always should look für enzyme elevation in patients with abdominal pain is a trivial.
Reply 3: *Thank you for this comment. We hope to clarify that we are suggesting that a full set of liver enzymes should still be included in the work up for patients that are suspected of CD and are presenting with abdominal pain and loose stools, not just in all patients with abdominal pain. You will see in our abstract as well as our conclusion we had the line " Based on this, current work up for children with abdominal pain and loose stools should still include serum liver transaminases , but elevated values may not be indicative of CD disease severity…" we had hoped that it was clear that a liver enzyme profile should be done in these cases specifically. After reading this comment however, we have decided to change this sentence slightly so that there is no confusion.*
Changes in text: *On line 369 you will see we changed the wording here so that it says " children suspected of CD and presenting with abdominal pain and loose stools" and on line 370 we changed serum liver transaminases to read " full set of liver enzymes (ALT, AST, GGT and ALP)".*