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Dear Professor Lian-Sheng Ma,
Company Editor-in-Chief
Baishideng Publishing Group

17th October 2021

**RE: Manuscript ID: 70918**

**Title: The yield of testing for micronutrient deficiencies associated with pancreatic exocrine insufficiency in a clinical setting: an observational study**

Thank you for your response to our manuscript. Following the advice from the editorial board and the three reviewers, we have revised our manuscript to address all the comments and requests for clarification.

As requested, we include:
1. Marked-up version with line numbering and all changes visible
2. Clean copy without visible track changes and no line numbering
3. Point-by-point reply to all queries raised by the reviewers

Thank you for re-considering our manuscript.

Kind regards

Dr M Jalal
Corresponding Author

**Reviewer #1:**

**1. The small sample size in this study may lead to inadequate power of the research.**

Author response

Thank you. We have now acknowledged this as a limitation in the discussion section.

Page 13 line 333

*"…. our sample size in this study may have led to it having inadequate power"*

**2. The majority of patients with PEI were identified as "idiopathic PEI", which may lead to limitation for generalization in clinical practice. More investigations, e.g. EUS, may be needed to identify the etiology of PEI.**

Author response

We did perform EUS in patients with idiopathic PEI and no morphological features of CP on CT. We did include it in the study as we did not perform it in all the other patients with CP and it was not part of the original study protocol however we have now included this in the results as requested.

**Page 8 Line 197**

*The aetiology of PEI was attributed to alcohol-induced chronic pancreatitis, (n=9), idiopathic chronic pancreatitis (n=4), pancreatic atrophy (n=9), when no CP changes or identifiable cause was identified this was termed 'idiopathic PEI', these 19 patients also underwent EUS with no diagnostic criteria for CP reached (range 0-3 EUS features; median 1).*

**3. Most patients were not evaluated for fat soluble vitamins, which are the major concerns in patients with PEI.**

Author response

Thank you for this comment. Our study was performed in a clinical setting to enable the data to be transposed into clinical practice. The micronutrients tested in this study were those that have been previously associated with PEI and clinically available to us at the time of design. Although we agree that fat soluble vitamins are a concern in PEI, our aim was to identify the yield of testing for those micronutrients that had previously been identified to be deficient in patients with PEI.

Vitamin D has not been shown to be associated with PEI due to its prevalence in the general population and therefore not included. Vitamin A was not included as it was not available to us clinically and therefore to reflect a clinical setting we indirectly represented Vitamin A with RBP levels (the carrier

for retinol in the blood) which has been shown to predict vitamin A deficiency with high sensitivity and specificity. Vitamin E was made available during the study but limited in patient numbers.
We have therefore clarified further the reasons why patients were not evaluated for Vitamin D the alternative option for vitamin A and the limited number of Vitamin E in patients recruited but also acknowledged this in the limitation discussion.

*Page 4 line 108*

*Vitamin D is one of the most common markers studied in patients with PEI and CP however a sole link to PEI has not been demonstrated. A high prevalence of vitamin D deficiency has been reported in control groups and healthy individuals [1, 2], therefore vitamin D is not associated with PEI.*

*Page 5 line 148*

*At the time of FEL-1 testing, PEI associated micronutrient serum levels were obtained for prealbumin, RBP, copper, zinc, selenium, magnesium and later in the study lipid adjusted vitamin E. Vitamin A testing was not available, however as this was a clinical study we indirectly represented this with RBP levels (the carrier for retinol in the blood) which has been shown to predict vitamin A deficiency with high sensitivity and specificity [3, 4].*

Page 13 line 329

*Fat-soluble vitamins are a major concern in PEI, however in this study their assessment is limited due to our clinical availability of vitamin E (giving lower numbers), vitamin A (using RBP levels as an alternate) and not including those without a significant association (vitamin D).*

**4. Some micronutrients, e.g. Zn, RBP, Cu, are affected by inflammation. In this case, CRP should be evaluated.**

Author response

Given the aim of our study the CRP results that we had available on all patients were not included, they also gave no additional information; however thank you for raising this important point. We have now included the CRP results in the result section as below as requested.

Page 10 line 243

*As micronutrient levels can vary with inflammation a C- reactive protein (CRP) was also measured in all patients with no significant differences in mean CRP values between patients with PEI (5.6 ± 8.2) and those without PEI (5.1 ±7.7), or those with a PEI-MD (7.3 ± 10.7) and those without a PEI - MD (4.4 ± 5.4).*

**Reviewer #2:**

**Line180-There is not enough clinical data, which should be completed: Steatorrhea, jaundice, pain, etc.**

**Author response**

Thank you. We have now added more information about the clinical symptoms of our cohort to the end of table 1:

**Table 1: Demographics of patients with suspected or proven benign pancreatic disease investigated with Faecal Elastase-1(FEL-1)** ***including documented symptoms at time of investigation.***

| | PEI **(FEL-1<200µg/g)**<br>n=41<br>Mean ± (SD) or n (%) | No PEI **(FEL-1>200µg/g)**<br>n=71<br>Mean ± (SD) or n(%) |
|---|---|---|
| Age (years) | 58.2 (13.2) | 57.4 (5) |
| Female | 14 (34%) | 44 (61%) |
| BMI (kg/m$^2$) | 26.1 (5.9) | 29.9 (7.1) |
| Type 1 Diabetes Mellitus<br>Type 2 Diabetes Mellitus | 9 (22.0%)<br>13 (31.7%) | 11 (15.4%)<br>26 (36.6%) |
| Alcohol (unit/week) | 17.7 ± (22.9) | 12.3 ± (10.5) |
| Smoking (pack years) | 22.3 ± (21.5) | 21.6 ± (20.5) |
| Morphological changes of CP* | 22/41 (53.7%) | 10/41 (24.3%) |
| *Clinical Symptoms*<br>*Abdominal pain*<br>*Diarrhoea*<br>*Steatorrhoea*<br>*Bloating*<br>*Weight loss* | <br>*21*<br>*9*<br>*2*<br>*5*<br>*4* | <br>*38*<br>*19*<br>*0*<br>*11*<br>*3* |

**Line 182 – Why was the 30 patients without a CT scan enrolled in this study? And what is their definitive diagnosis?**

Author response:

The patients recruited to the study were patients referred to our centre with maldigestion symptoms investigated with FEL-1 for suspected or proven benign pancreatic disease. As this was a clinical study as part of a clinical pathway patients underwent further investigations including CT imaging when there was a clinical suspicion of CP, a new diagnosis of PEI made on the stool sample, recurrent pancreatic type pain (epigastric abdominal pain radiating to back with or without previous acute pancreatitis attacks) or weight loss

This resulted in 30 patients tested with FEL-1 without PEI and without the earlier symptoms that did not qualify for a CT scan; we have clarified this in the discussion. We have also added the information about the final diagnosis of this 'no-PEI and no CT' group to results section.

Page 8 line 210

*In total 30 patients that were recruited and tested with FEL-1 without PEI and without the earlier symptoms that did not qualify for a CT scan. The final diagnosis made in patients identified with no PEI and no CT scan was most commonly found to be due to non-specific (functional or unexplained) abdominal pain (n=15), irritable bowel syndrome (n=5), gall stone disease (n=4), or gastritis and peptic ulcer disease (n=6)*

**Line 193- Were there any cases of pancreatic duct stenosis in the patients with CP?**

Author response

We have reviewed our CT images and confirm that there were no cases of pancreatic duct stenosis in our cohort.

**Line 195- Were there any cases in the patients with idiopathic PEI that suspected early chronic pancreatitis (JPS 2009 criteria)?**

Author response

We have reanalysed this group with according to the Japanese revised clinical diagnostic criteria for chronic pancreatitis[5], 2 patients from the idiopathic PEI group would fit the 'early CP' criteria this was due to the presence of PEI, abdominal pain, raised alcohol and 3 EUS features but did not have CP according to our criteria used in this study.

We have added this information to the discussion section

Page 14 line 365

*Using an alternative criteria to look for early CP, the Japanese revised clinical diagnostic criteria for chronic pancreatitis[30], we identified 2 patients from the 19 idiopathic PEI group that would fit the 'early CP' criteria. This was due to the presence of PEI, abdominal pain, raised alcohol intake and 3 EUS features but did not have CP according to the Cambridge criteria used in this study.*

**Line 226- Did the accuracy of using the significant micronutrients identified in your results as a predictor of CP in your cohort show?**

Author response

We have recalculated the accuracy of using the most significant micronutrients deficiencies as a predictor for CP rather than PEI and displayed the findings to the table below. The results are quite poor. We feel that this table would distract form the aim of the study and add little to the results and conclusions therefore we have not included it in the revised manuscript, however, if the reviewer or editorial board would like to include this additional data it is displayed below:

**Table 3 Assessment of significant micronutrient deficiency to predict (a) PEI (FEL-1 <200µg/g) and (b) chronic pancreatitis.**

| | Prealbumin | Selenium | Magnesium |
|---|---|---|---|
| (a) Pancreatic exocrine insufficiency | | | |
| Sensitivity | 19.5% [CI=8.8-34.9] | 14.6% [CI=6.6-33.7] | 9.8 % [CI=3.3-21.4] |
| Specificity | 97.2% [CI=90.2-99.7] | 98.5% [CI=92.1-99.7] | 98.6% [CI=92.4-100] |
| PPV | 80.0% [CI=47.1-94.7] | 85.7% [CI=42.1-99.6] | 83.3% [CI=37.6-97.7] |
| NPV | 67.7% [CI=64.2-71] | 66.7% [CI=56.8-75.6] | 60.3% [CI=58.1-62.2] |
| Accuracy | 68.8% [CI=59.3-77.2] | 67.9% [CI=58.4-76.3] | 61.5% [CI=52.2-70.1] |
| (b) Chronic pancreatitis | | | |
| Sensitivity | 18% (CI=7.2-36.4) | 15.6% (CI=5.3-32.8) | 9.4% (CI=2-25) |
| Specificity | 96% (CI=86.3-99.5) | 64.9% (CI=89.4-99.9) | 96% (CI=86.3-99.5) |
| PPV | 75% (CI=39.2-93.3) | 83.3% (CI=38-97.6) | 60% (CI=21-89.5) |
| NPV | 64.9% (CI=60.8-68.8) | 64.5% (CI=60.9-67.9) | 62.3% (CI=59.4-65.2) |
| Accuracy | 65.9% (CI=54.6-76) | 65.9% (CI=54.6-76) | 62.2% (CI=50.8-72.7) |

**Reviewer #3:**

**1. Please add a Table reporting the final diagnosis of the 112 patients enrolled into the study.**

We have now given a the presenting symptoms and the final diagnosis for the 112 patients in responses to earlier reviewers remarks regarding the manuscript. To save repetition we have avoided a further table but included the entries below where the diagnoses are listed: this included 41 patients with PEI, 30 patients without both CP and PEI. We would be happy to replace this with a table at the editorial team's discretion.

Page 8 Line 197

*The aetiology of the 41 patients with PEI was attributed to alcohol-induced chronic pancreatitis (n=9), idiopathic chronic pancreatitis (n=4), pancreatic atrophy (n=9), and when no CP changes or identifiable cause was identified this was termed 'idiopathic PEI', these 19 patients also underwent EUS with no diagnostic criteria for CP reached (range 0-3 EUS features; median 1). Patients with 'idiopathic PEI' were noted to have a history of smoking in 10/19 and alcohol consumption in 11/19 (mean 25.1 ± 28.3 units per week). There was no significant difference in the number of patients with steatorrhoea or weight loss when PEI was present. 10 patients with CP had did not have PEI, but CP was felt to be the likely cause of the patients pain.*

*The remaining 31 patients who underwent CT for significant symptoms without PEI and no evidence of CP on the CT had a final diagnosis of nonspecific abdominal pain (functional or unexplained) (n=14), biliary dyskinesia (n=8), recent acute pancreatitis with no residual morphological changes (n=4), dyspepsia secondary to gastritis (n=3), and weight loss due to chronic fatigue syndrome (n=1) and consequences of medication from human immunodeficiency virus (n=1).*

*In total 30 patients that were recruited and tested with FEL-1 without PEI and without the earlier symptoms that did not qualify for a CT scan. The final diagnosis made in patients identified with no PEI and no CT scan was most commonly found to be due to non-specific abdominal pain (n=15), irritable bowel syndrome (n=5), gall stone disease (n=4), or gastritis and peptic ulcer disease (n=6)*

**2. How many patients has fecal elastase concentration less than 100 mcg/g?**

Author response

There were 23 patients with a FEL-1 <100µg/g, we have highlighted this in the results section and compared the micronutrient deficiency yield for <100 µg/g and >100 µg/g.

Results page 10 line 237

*There was a significantly higher prevalence of patients with a micronutrient deficiency if severe PEI (FEL-1 0-100µg/g) was present: 56.5%(13/23), compared to when moderate PEI (FEL-1=100-200µg/g) was present: 22.2%(4/18) (p=0.05).*

**3. How many patients had symptoms related to steatorrhea? Please add a Table reporting the possible relationships between PEI assessed by fecal elastase concentrations and clinical symptoms (Pezzilli R, et al. Pancreas. 2020 Jul;49(6):793-798.).**

Author response

We have now added all relevant patient symptoms including steatorrhoea to table 1. We acknowledge the relationship between PEI and symptoms but given the single patient with steatorrhoea and similar weight loss numbers we have been unable to show a relationship.

*Page 8 Line 201*

*There was no significant difference in the number of patients with steatorrhoea or weight loss when PEI was present.*

**Table 1: Demographics of patients with suspected or proven benign pancreatic disease investigated with Faecal Elastase-1(FEL-1) including documented symptoms at time of investigation.**

| | PEI **(FEL-1<200µg/g)** n=41 Mean ± (SD) or n (%) | No PEI **(FEL-1>200µg/g)** n=71 Mean ± (SD) or n(%) |
|---|---|---|
| Age (years) | 58.2 (13.2) | 57.4 (5) |
| Female | 14 (34%) | 44 (61%) |
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| Type 1 Diabetes Mellitus<br>Type 2 Diabetes Mellitus | 9 (22.0%)<br>13 (31.7%) | 11 (15.4%)<br>26 (36.6%) |
| Alcohol (unit/week) | 17.7 ± (22.9) | 12.3 ± (10.5) |
| Smoking (pack years) | 22.3 ± (21.5) | 21.6 ± (20.5) |
| Morphological changes of CP* | 22/41 (53.7%) | 10/41 (24.3%) |
| Symptoms<br>Abdominal pain<br>Diarrhoea<br>Steatorrhoea<br>Bloating<br>Weight loss | <br>21<br>10<br>1<br>5<br>4 | <br>38<br>19<br>0<br>11<br>3 |

**4. The authors stated that the prevalence of micronutrient deficiencies was significantly higher in patients with PEI than those without (7%) which confirms the association from previous studies. Please add references.**

Author response

Thank you for pointing this out, the previous studies are now referenced

Page 11 line 268

*The prevalence of micronutrient deficiencies was significantly higher in patients with PEI than those without which confirms the association from previous studies [6-9]*

**5. The discussion contain a repetition of data already present in the results: Please modify.**

As requested, we have removed the following data from the discussion which is already in the results:

**Page 11**

Line 267 ~~*(with 42% of patients showing at least one deficiency when PEI is present*~~)

line 268 ~~*(7%)*~~

line 181 ~~*(32/112)*~~

line 296~~*(20% in PEI compared to 3% without PEI)*~~

**6. Please reference the methods used to evaluate the micronutrients.**

We have now expanded the methods used to evaluate the micronutrients

**Page 5 line 145**

*PEI associated micronutrient serum levels were obtained for prealbumin, RBP, copper, zinc, selenium, magnesium and later in the study lipid adjusted vitamin E. Vitamin A testing was not available, however as this was a clinical study we indirectly represented this with RBP levels (the carrier for retinol in the blood) which has been shown to predict vitamin A deficiency with high sensitivity and specificity [24, 25]. Serum samples were analysed with standard methods using calibrated ranges noted in Table 2. Lipid adjusted Vitamin E was calculated as a ratio of Vitamin E and total cholesterol and triglycerides in a fasting blood sample. Prealbumin and RBP levels were measured by nephelometry.*

**7. It is not clear as the authors selected only prealbumin, selenium and magnesium among the various micronutrients they evaluated.**

Author response

We apologise that this is not clear, and would like to clarify the reasoning for the selection.

The 3 micronutrients we tested to predict PEI (prealbumin, selenium, and magnesium) were selected because they had been found to have a significantly higher deficiency prevalence in patients with PEI.

We have clarified this further:

Results Page 10 line 249

*Using the 3 micronutrient deficiencies that were found to have a significantly higher deficiency prevalence in patients with PEI (prealbumin, selenium, and magnesium: table 2) we calculated their accuracy as a test for PEI in our cohort;…*

**8. The authors suggest that the accuracy of using the significant micronutrients identified in the cohort as a predictor of PEI showed a positive predictive value of 80-85.7%% (95% Confidence interval (CI:38-100%) and a low sensitivity of 9.8-19.5% (95% CI: 3.3-34.9%). For this reason probably the substances tested may be used as guide to select those patients that must be supplemented.**

Author response

Thank you for your valuable comment. We agree and have added this observation to the discussion section

Page 13 line 343

*The higher PPV but lower sensitivity of the most significant micronutrients (prealbumin, selenium, and magnesium: table 2) could be used as a guide in clinical practice to identify those with PEI and who would benefit from PERT.*

References


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