

# World Journal of *Gastrointestinal Pharmacology and Therapeutics*

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Editorial board member of *World Journal of Gastrointestinal Pharmacology and Therapeutics*, Dr. Shehata (MD) has been on the Faculty of Medicine at Mansoura University in Egypt since 2000 and was promoted to Professor of Internal Medicine, Gastroenterology & Hepatology Unit in 2010. She was named Director of the Specialized Medical Hospital in 2016, through which she founded the Inflammatory Bowel Disease (IBD) team and clinic. Her special interest in IBD research has led to the publication of more than 50 peer-reviewed articles and underlies her work as editor and peer reviewer for several international journals and as supervisor of 30 Master's and MD thesis students. Her dedication to sharing and increasing knowledge on IBD continues through her work in arranging national and international workshops as well as hands-on training of gastrointestinal endoscopic practices at Emergency Hospitals and Specialized Medical Hospital, Mansoura University. (L-Editor: Filipodia)

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Case Control Study

# Shared changes in angiogenic factors across gastrointestinal vascular conditions: A pilot study

Atiyekeogbebe R Douglas, Grainne Holleran, Sinead M Smith, Deirdre McNamara

**ORCID number:** Atiyekeogbebe R Douglas 0000-0001-8300-1057; Grainne Holleran 0000-0001-8845-4952; Sinead M Smith 0000-0003-3460-3590; Deirdre McNamara 0000-0003-2324-3382.

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**Atiyekeogbebe R Douglas, Grainne Holleran, Sinead M Smith, Deirdre McNamara,** TAGG Research Centre, School of Medicine, Trinity College Dublin, Dublin D24, Ireland

**Corresponding author:** Atiyekeogbebe R Douglas, MSc, Research Scientist, TAGG Research Centre, Trinity Centre, Tallaght Hospital, School of Medicine, Trinity College Dublin, College Green, Tallaght, Dublin D24, Ireland. [douglar@tcd.ie](mailto:douglar@tcd.ie)

## Abstract

### BACKGROUND

Neovascularisation is common to a variety of gastrointestinal (GI) disorders with differing aetiologies and presentations; usually affecting adults above 60 years. Shared angiogenic factors modulated by disease specific elements could be a common denominator and represent novel diagnostic and therapeutic targets. As yet, assessment of angiogenic factors across several GI vascular disorders associated with recurrent bleeding and anaemia has not been reported.

### AIM

To assess serum levels of angiogenic factors in several intestinal vascular disorders.

### METHODS

A case control study was performed in Tallaght University Hospital in patients with endoscopically proven small bowel angiodysplasia (SBA), portal hypertensive gastropathy (PHG), gastric antral vascular ectasia (GAVE) and non-bleeding, non-anaemic controls. Using enzyme-linked immunosorbent assay, concentrations of Angiopoietin 1 (Ang-1), Ang-2 and vascular endothelial growth factor (VEGF) were measured from 2 serum tubes of blood following informed consent. The relative expression of Ang-1 and Ang-2 and Ang-1/2 ratio was calculated and compared between groups. Statistical analysis was applied using a *t*-test, and a *P* value of < 0.05 was considered significant.

### RESULTS

To date 44 samples were tested: 10 SBA, 11 PHG, 8 GAVE and 15 controls. Mean age 60 (range 20-85) years and 20 (45%) were males. Controls were significantly younger (49 years *vs* 66 years, *P* = 0.0005). There was no difference in VEGF levels between the groups (*P* = 0.6). SBA, PHG and GAVE Ang-1 levels were similar and were significantly lower than controls, (*P* = 0.0002, 95%CI: 241 to 701). Ang-2 levels were statistically higher in PHG and GAVE groups compared to controls (*P*

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= 0.01, 95%CI: 77.8 to 668) and as a result, also had a lower Ang-1/2 ratios compared to controls. While SBA Ang-2 levels were higher than controls, this did not reach statistical significance. Neither age nor haemoglobin level, which was similar between disease groups, could explain the difference. In addition, the median Ang-1/ Ang-2 ratio for all patients was found to be significantly lower compared to controls, 8 *vs* 28 respectively,  $P = 0.001$ , 95%CI: -27.55 to -7.12.

## CONCLUSION

Our novel pilot study suggests common alterations in Ang-1 and Ang-2 levels across several GI vascular disorders. Differences in Ang-1/ Ang-2 ratios among vascular disorders compared to controls suggest disease-specific modulation.

**Key words:** Gastric antral vascular ectasia; Portal hypertensive gastropathy; Angiodysplasia; Angiopoietins; Angiogenic factors; Recurrent bleeding

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**Core tip:** This is the first study to look at key angiogenic factors across several distinct intestinal vascular disorders. Our novel study suggests a common alteration in Angiopoietin 1 (Ang-1) levels, a vascular factor associated with vessel stabilization and maturation, across a variety of gastrointestinal vascular disorders. VEGF appears not to play a significant role in these conditions. Serum elevation in Ang-2 levels and lower than normal Ang-1 levels are associated with clinically significant disease and warrant further investigation as potential biomarkers and therapeutic targets. This offers a potential final common pathway which could be of use diagnostically and therapeutically across several vascular conditions.

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## INTRODUCTION

Vascular abnormalities can affect any part of the gastrointestinal (GI) tract and present with bleeding and anaemia<sup>[1]</sup>. Abnormal neovascularization, with friable, dilated superficial blood vessels, is common to a variety of GI disorders with differing etiologies and presentations including small bowel angiodysplasia (SBA), gastric antral vascular ectasia (GAVE) and portal hypertensive gastropathy (PHG)<sup>[2]</sup>. While associated with different clinical conditions and different locations these lesions are all common causes of recurrent or chronic intestinal bleeding especially in adults above 60 years. Reported rates of significant bleeding are; SBA (30%-50%), GAVE (2%-4%) and PHG (3%-26%)<sup>[3-5]</sup>. GI vascular malformations and bleeding in SBA have been associated with varying disturbances in angiogenesis, however, the precise mechanisms underlying these conditions remain unclear<sup>[3]</sup>. Our hypothesis is that localised aberrant neovascularisation develops in different conditions in response to a variety of upstream triggers including portal hypertension, hypoxia and inflammation, which may be regulated at a local level by common angiogenic regulators.

Angiogenesis is mediated by several angiogenic factors including the two major angiopoietin factors: Angiopoietin-1 (Ang-1) and Ang-2 and vascular endothelial growth factor (VEGF), a sub-family of the platelet-derived growth factor (PDGF). Ang-1 is involved in the maturation of blood vessels and has vascular protective properties, whereas Ang-2 promotes endothelial permeability. Both angiopoietins utilize their angiogenic regulatory effects via the tyrosine-kinase receptor (Tie-2)<sup>[6]</sup>. A more recent study has confirmed an imbalance in Ang-1/Ang-2 ratios in SBA patients with bleeding<sup>[3]</sup>. VEGF appears to play an essential role in promoting angiogenesis and vascular development; however, it can also cause vascular permeability and tissue oedema by working in synergy with Ang-2<sup>[2]</sup>. To date, the exact aetiology and pathophysiology of these conditions and the role of angiogenic factors is not yet fully



established.

Due to a limited understanding of the role of angiogenic factors in common non-hereditary GI vascular disorders, no specific treatments targeting the angiogenic pathway are currently available. The management of these conditions is primarily endoscopic which can both diagnose and treat the source of bleeding. Despite this, these conditions frequently recur, and patients are often hospitalized for management of bleeding episodes, which has a significant health and financial impact. There is a need for identification of biomarkers as potential diagnostic and novel therapeutic targets, which could dramatically improve disease outcome for affected patients<sup>[7]</sup>. As yet, assessment of angiogenic factors across common GI vascular disorders has not been reported. Shared angiogenic factors modulated by disease specific elements could be a common denominator and represent novel diagnostic and therapeutic targets.

The aim of this study is to assess serum levels of angiogenic factors in different intestinal vascular disorders associated with recurrent bleeding and anaemia.

## MATERIALS AND METHODS

Ethical approval was obtained from our institutions research and ethics committee and any patient over the age of 18 years undergoing an endoscopic procedure with a known diagnosis of SBA, PHG or GAVE and confirmed on endoscopy were invited to participate. Gender matched controls with negative surveillance colonoscopies, with two sequential negative faecal immunological tests were identified and invited to participate. Controls with significant dyspepsia, or evidence of GI bleeding or anaemia defined as haemoglobin (Hb) of < 11.5 g/dL in females and < 13 g/dL in males along with a serum ferritin of < 14, known chronic liver disease or non-liver related portal hypertension were excluded. Similarly, cases and controls with chronic renal failure, active malignancy or severe cardiac or respiratory failure were excluded. Recruitment continued until a minimum of 8 patients was included in each group.

Following informed consent, 2 serum separator tubes (3.5 mL each) of blood were taken on the same day using standard phlebotomy techniques. All relevant clinical data including basic demographics and relevant tests were recorded and filed on an encrypted database. Plasma samples were analysed routinely for Hb level and serum samples were left to clot for 30 min before undergoing centrifugation for 15 min at 1000 rpm. The resultant supernatant was extracted and stored in aliquots at -80 °C for batch analysis.

Serum levels of Ang-1, Ang-2 and VEGF were measured using commercially available solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) kits (R and D systems, Minneapolis, MN, United States). Samples were prepared in duplicate and results were read at 450 nm absorbance. The intra-assay coefficients of variation (CV) were calculated as an average of all of the individual CVs for the sample concentration duplicates analysed by ELISA.

### Statistical analysis

Categorical data was compared with a Chi<sup>2</sup> test. Serum levels of angiogenic factors were expressed as a mean and compared between groups using the Student *t*-test. Results were controlled for patient demographics including age, gender and haemoglobin level. In addition, the relative expression of Ang-1 and Ang-2, expressed as a ratio, per individual was calculated and compared between groups. A *P* value of < 0.05 was considered significant.

## RESULTS

### Population

Overall, 44 patients were recruited, 45% (*n* = 20) were males, with a mean age of 60 years (range 20-85), 15 controls, 10 with SBA, 11 PHG and 8 with GAVE. Subjects in the control group were significantly younger, 49 years (range 20-74) than cases, 65 years (range 38-85), *P* = 0.0005, 95%CI: 8 to 26. There was no difference in mean age among patient groups; 68 years (53-79) SBA, 60 years (38-81) PHG and 68 years (58-85) GAVE. There was no statistical difference in gender between controls and cases overall or by group with 47% (*n* = 7/15), 50% (*n* = 5/10), 27% (*n* = 3/11) and 50% (*n* = 4/8) being male respectively. A current haemoglobin level was available in 29 /44 (66%) subjects and was similar among cases. Mean Hb levels by group were; SBA 11.4 g/dL

(range 7.2-13.8), PHG 11.2 g/dL (9.5-12.2), GAVE 11.5 g/dL (8.3-15.3). While as expected the mean haemoglobin for controls was higher, this did not reach statistical significance 12.5g/dL (11.5 -13.2). However, more patients in the disease groups were anaemic compared to controls 1/5 (20%) *vs* 14/24 (59%),  $P < 0.05$  (Table 1).

### Serum levels of angiogenic factors

**Ang-1/Ang-2:** Mean Ang-1 levels were significantly higher in controls  $53115 \pm 17506$  ng/mL than patients  $29559 \pm 18084$ ,  $P = 0.0002$ , 95%CI: -35040 to -12072. While mean serum SBA, PHG and GAVE Ang-1 levels were all similar; 35696 ng/mL (range 12466-54338), 23111 ng/mL (range 1950-72445), 30753 ng/mL (range 9773-72609) respectively. As with Ang-1, mean Ang-2 levels were also similar among SBA, PHG and GAVE groups; 2803 ng/mL (range 125-13141), 4298 ng/mL (range 1299-8702) and 4232 ng/mL (range 1253-6081) respectively. Contrary to Ang-1 findings which were lower, the mean Ang-2 levels,  $3764 \pm 2763$  ng/mL for patients were higher than controls,  $1899 \pm 772$  ng/mL,  $P < 0.01$ , 95%CI: 389 to 3342.

The relative expression of Ang-1 and Ang-2 was calculated for each group. Controls as expected had a higher ratio;  $35696/2803$  ng/mL = 13 for SBA,  $23111/4298$  ng/mL = 5 for PHG,  $30753/4232$  ng/mL = 7 for GAVE *vs*  $53114/1899$  ng/mL = 28 for controls. However, the difference between SBA and control Ang-1/Ang-2 ratios did not reach statistical significance which was not unexpected as SBA Ang-2 levels while higher, was not statistically different from controls (Table 2).

In addition, the median Ang-1/Ang-2 ratio for all patients was found to be significantly lower compared to controls, 8 *vs* 28 respectively,  $P = 0.001$ , 95%CI: -27.55 to -7.12.

Age has been previously reported to affect angiopoietin levels<sup>[8]</sup>. In our cohort only SBA cases showed a variation in Ang-1 levels by age, with older age (> 70 years) associated with higher levels. Mean Ang-1 SBA < 70 years =  $28306 \pm 11479$  ng/mL *vs* > 70 years =  $46780 \pm 5251$  ng/mL,  $P = 0.02$ , 95%CI: 4142 to 32805. While mean Ang-1 levels by age for PHG and GAVE were 21661 ng/mL *vs* 24194 ng/mL,  $P = 0.9$  and 29027 ng/mL *vs* 32415 ng/mL,  $P = 0.8$  respectively. There was no difference in Ang-2 levels among cases by age. SBA 3562 ng/mL *vs* 1665 ng/mL, PHG 3555 ng/mL *vs* 3646 ng/mL and GAVE 4338 ng/mL *vs* 3055 ng/mL (Figure 1). Of note neither haemoglobin level or the presence of anaemia affected our findings.

**VEGF:** Again, mean serum VEGF levels were similar among cases; SBA = 443 ng/mL (range 273-964), PHG = 316 ng/mL (range 51-600) and GAVE = 435 ng/mL (range 288-637). Unlike angiopoietin levels, VEGF did not differ significantly between cases and controls. Mean VEGF for the control group was 421 ng/mL (range 250-678) (Table 2).

## DISCUSSION

To our knowledge, this is the first study to look at key angiogenic factors across several distinct intestinal vascular disorders. In our pilot study subjects with endoscopically documented sporadic small bowel angiodysplasia, portal hypertensive gastropathy and gastric antral vascular ectasia all shared a common dysregulation in serum angiopoietin profile, with reduced levels of Ang-1 and higher levels of Ang-2 and resultant lower Ang-1/Ang-2 ratios. Adding weight to the hypothesis that dysregulated neovascularisation driven by Ang-2 excess is a key factor in the development of these conditions irrespective of upstream drivers. This offers a potential final common pathway which could be of use diagnostically and therapeutically across several vascular conditions.

Ang-2 is a circulating antagonist of the endothelial-specific Tie-2 receptor and has been identified as an important modulator of angiogenesis. While Ang-1 acts as a growth and maturation factor promoting development and stabilization of mature normal vessels in vivo by mediating endothelial cell interactions. Ang-2 antagonises Ang-1 and prevents Tie-2 receptor activation by competitively binding Tie-2. Ang-2 is known to be expressed at the site of vascular remodelling and its antagonism of Ang-1 actions therefore promotes vessel destabilization, possibly leading to the development of abnormal friable, leaky immature vessels, a hallmark of all these conditions<sup>[8,9]</sup>.

Of interest, in contrast to previous studies which have documented elevated VEGF levels in GI disease and bleeding, VEGF levels were similar among controls and all disease groups in our cohort<sup>[10,11]</sup>. These findings mirror those we have previously demonstrated in a larger sporadic SBA cohort<sup>[8]</sup>. The role of VEGF and angiopoietins in blood vessel development and maturation is complex and their specific roles may

**Table 1 Characteristics of study population**

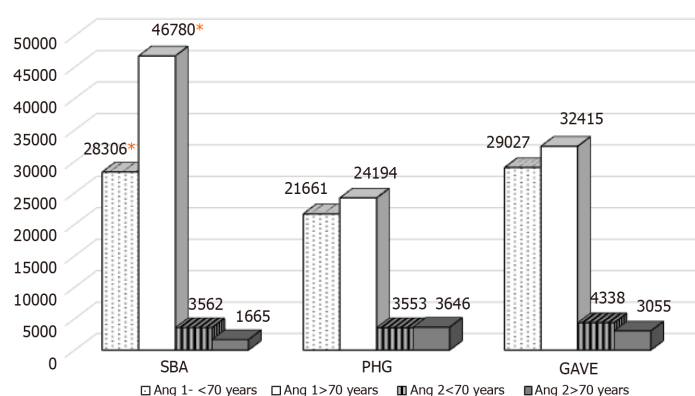
Factor	Controls	SBA	PHG	GAVE	Controls vs cases
Number	15	10	11	8	
mean age (range)	49 (20-74)	68 (53-79)	60 (38-81)	68 (58-85)	$P < 0.0005$
Male gender, $n$ (%)	7 (47)	5 (50)	3 (27%)	5 (63)	NS
mean haemoglobin, g/dL	12.5	11.4	11.2	11.5	NS
Anaemia, $n$ (%) <sup>1</sup>	1 (20)	4 (40)	6 (75)	3/5 (60)	$P < 0.05$

<sup>1</sup>Anaemia defined as Hb < 11.5 g/dL in females and < 13 g/dL in males. SBA: Small bowel angiodysplasia; PHG: Portal hypertensive gastropathy; GAVE: Gastric antral vascular ectasia; NS: Not significant.

**Table 2 Levels of serum angiogenic factors according to group**

	SBA	PHG	GAVE	Control
Ang-1 (ng/mL), mean (range)	35696 (12466-54338)	23111 (1950-72445)	30753 (9773-72609)	53115 (28624-82066)
$P$ value vs controls	0.01	0.0003	0.01	
Ang-2 (ng/mL), mean (range)	2803 (125-13141)	4298 (1299-8702)	4232 (1253-6081)	1899 (742-3693)
$P$ value vs controls	0.4	0.0008	0.003	
VEGF (ng/mL), mean (range)	443 (273-964)	316 (51-600)	435 (288-637)	421 (250-678)
$P$ value vs controls	0.8	0.08	0.8	
Ang-1/ Ang-2 ratios by group	13	5	7	28
Median Ang-1/ Ang-2 ratios	8			28
$P$ value vs controls	0.001			

SBA: Small bowel angiodysplasia; PHG: Portal hypertensive gastropathy; GAVE: Gastric antral vascular ectasia; VEGF: Vascular endothelial growth factor; Ang-1: Angiopoietin 1; Ang-2: Angiopoietin 2.



**Figure 1 Mean angiopoietin 1 and angiopoietin 2 levels in ng/mL by age and group.** \*Statistically significant increase in Ang-1 in > 70 years  $P = 0.02$ , 95%CI: 4142 to 32805. SBA: Small bowel angiodysplasia; PHG: Portal hypertensive gastropathy; GAVE: Gastric antral vascular ectasia.

explain our findings. VEGF is a central mediator of early phase angiogenesis<sup>[12]</sup>. The initial phase of angiogenesis is characterized by VEGF-dependent formation and sprouting of blood vessels. Local concentrations of VEGF determine whether normal or aberrant angiogenesis is induced<sup>[13]</sup>. High local concentrations of VEGF result in vascular malformations and lesions resembling the chaotic architecture of angiodysplasias, thin-walled fragile vessels lacking smooth muscle cells that are susceptible to rupture<sup>[14,15]</sup>. To mature, primitive vascular complexes require remodelling during which vessels acquire a smooth muscle layer, and become less



permeable and friable, a process dependant on other angiogenic factors including Ang-1<sup>[16]</sup>. As such, it remains a possibility that high local tissue levels of VEGF are responsible for initiation of angiogenesis triggered by a variety of pathological processes. While high circulating levels of Ang-2 interferes with vessel remodelling and maturation, resulting in the abnormal friable, permeable vasculature, with a propensity for bleeding, common to these conditions. Confirmatory studies exploring local tissue VEGF expression would be required to confirm this hypothesis, which is beyond the scope of this pilot study.

While our initial results are of interest and warrant further investigation, there are significant limitations with our study. This is only a small, pilot study and findings will need to be confirmed in a larger cohort. Our sample size prevents effective subgroup analysis including the potential effects of age and other potential confounding factors including recent active bleeding events, although haemoglobin level did not affect expression in our cohort. We did control for gender and excluded conditions known to be associated with disturbances in angiogenic levels, which adds weight to our findings. In addition to a larger sample size, repeated testing at different times would be advantageous and help to establish whether these factors are predictive of disease severity, bleeding events and long-term outcomes. Similarly, our small cohort included patients with symptomatic disease only requiring endoscopic intervention which could represent a bias as potentially less severe forms may have a different angiogenic profile. However, clinical application is likely only to be relevant to just such a symptomatic cohort.

As mentioned previously, circulating angiogenic factors may not reflect local tissue activity and should be investigated to confirm the potential key role of angiopoietins and possibly VEGF across several GI vascular conditions. However, as a potential diagnostic or prognostic biomarker serum sampling is preferable and would be more useful clinically.

In conclusion, this is the first study to show common changes in angiogenic factors across several distinct intestinal vascular disorders. Serum elevation in Ang-2 levels and lower than normal Ang-1 levels are associated with clinically significant disease and warrant further investigation as potential biomarkers and therapeutic targets.

## ARTICLE HIGHLIGHTS

### Research background

Neovascularisation is a common feature of gastrointestinal (GI) vascular disorders with differing aetiologies and presentations; including small bowel angiodysplasia (SBA), gastral antral vascular ectasia and portal hypertensive gastropathy. These lesions are all common causes of recurrent or chronic intestinal bleeding especially in adults above 60 years. GI vascular malformations and bleeding in SBA have been associated with varying disturbances in angiogenesis, however, the precise mechanisms underlying these conditions remain unclear. Our hypothesis is that response to a variety of upstream triggers including portal hypertension, hypoxia and inflammation, which may be regulated at a local level by common angiogenic regulators including Angiopoietin 1 (Ang-1), Ang-2 and vascular endothelial growth factor (VEGF) may be responsible for these conditions.

### Research motivation

At present, the precise mechanisms underlying these conditions remain unclear and assessment of angiogenic factors across several GI vascular disorders associated with recurrent bleeding and anaemia has not been reported. In addition, there is currently no specific treatment for these vascular conditions, the development of which is limited by a deficient knowledge of the underlying pathophysiology.

### Research objectives

The overarching aim of our work is to identify angiogenic factors associated with the condition which may be useful both as diagnostic and prognostic markers and as future treatment targets.

### Research methods

Using enzyme-linked immunosorbent assay, concentrations of Ang-1, Ang-2 and VEGF were measured from 2 serum tubes of blood using standard phlebotomy techniques. Categorical data was compared with a Chi<sup>2</sup> Test. Serum levels of

angiogenic factors were expressed as a mean and compared between groups using the Student *t*-test. Results were controlled for patient demographics including age, gender and haemoglobin level. A *P* value of < 0.05 was considered significant.

### Research results

We observed a common reduction in Ang-1 levels and elevation in Ang-2 levels across several GI vascular disorders compared to controls. Differences in Ang-1/Ang-2 ratios among vascular disorders compared to controls suggest disease-specific modulation. This warrants further investigation as potential biomarkers and therapeutic targets.

### Research conclusions

Our novel pilot study shows the common alteration in Ang-1 and Ang-2 levels across a variety of GI disorders. This suggests that the modulation of these angiogenic factors may play a vital role in these GI vascular conditions. This shows the value of these factors as potential biomarkers and therapeutic targets.

### Research perspectives

Targeting these angiogenic factors could potentially serve as diagnostic or prognostic biomarkers and therapeutic targets in a clinical setting.

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Observational Study

# Hepatobiliary manifestations in children with inflammatory bowel disease: A single-center experience in a low/middle income country

Mortada HF El-Shabrawi, Sara Tarek, Maha Abou-Zekri, Safa Meshaal, Afaf Enayet, Engy Adel Mogahed

**ORCID number:** Mortada HF El-Shabrawi 0000-0002-1995-4213; Sara Tarek 0000-0002-5132-4019; Maha Abou-Zekri 0000-0002-9917-2085; Safa Meshaal 0000-0003-0370-3820; Afaf Enayet 0000-0002-8202-1678; Engy Adel Mogahed 0000-0001-9348-7409.

**Author contributions:** El-Shabrawi MHF designed the study; Abou-Zekri M, Mogahed EA, Meshaal S and Tarek S participated in the acquisition, analysis, and interpretation of the data, and drafted the initial manuscript; Enayet A participated in statistical analysis and drafting the initial manuscript; El-Shabrawi MHF wrote and revised the article critically for important intellectual content.

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**Mortada HF El-Shabrawi, Sara Tarek, Maha Abou-Zekri, Afaf Enayet, Engy Adel Mogahed,** Department of Paediatrics, Kasr Alainy School of Medicine, Cairo University, Cairo 11562, Egypt

**Safa Meshaal,** Department of Clinical Pathology, Kasr Alainy School of Medicine, Cairo University, Cairo 11562, Egypt

**Corresponding author:** Mortada HF El-Shabrawi, MD, Professor, Department of Paediatrics, Kasr Alainy School of Medicine, Cairo University, 1 Gamaa Street, Giza, Cairo 11562, Egypt. [melshabrawi@kasralainy.edu.eg](mailto:melshabrawi@kasralainy.edu.eg)

## Abstract

### BACKGROUND

There has been a worldwide increase in the reported incidence of inflammatory bowel disease (IBD) in children over the past 2-3 decades. The hepatobiliary (HB) manifestations of IBD have been well-studied in children in industrialized and developed countries but are infrequently reported in low- and middle-income countries (LMIC) such as Egypt.

### AIM

To determine the prevalence of the HB manifestations in a cohort of Egyptian children with IBD.

### METHODS

This cross-sectional observational study was carried out over a period of 6 mo (between June 2013 to December 2013) at the Paediatric Hepatology and Gastroenterology Units of Cairo University Children's Hospital, which is the largest paediatric tertiary care centre in the country.

### RESULTS

The study included 48 patients with confirmed IBD based upon clinical, laboratory, endoscopic and histopathological features, 29 (60.4%) were male. Twenty-four patients (50%) had ulcerative colitis (UC), 11 (22.9%) had Crohn's disease (CD) and 13 (27.1%) had unclassified-IBD (IBD-U), which was formerly known as indeterminate colitis. The mean age of the patients at the time of presentation was 8.14 ( $\pm$  SD 4.02) years and the mean age at the time of study enrolment was 10.16 ( $\pm$  SD 4.19) years. All patients were screened for HB manifestations by physical examination, liver function tests, imaging and liver

report.

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biopsy when indicated. HB disorders were confirmed in 13 patients (27.1%). Transaminases were elevated in 3 patients (6.3%). Two patients (4.2%) had elevated biliary enzymes (one was diagnosed as primary sclerosing cholangitis (PSC) and the other was diagnosed with PSC/autoimmune hepatitis overlap syndrome and the third patient had hepatitis C virus infection. Ten patients (20.8%) had bright echogenic liver on ultrasound suggesting fatty infiltration as a sequel of malnutrition or medication toxicity.

## CONCLUSION

The commonest HB disorders in Egyptian children with IBD were abnormal liver function tests, fatty infiltration and PSC. These HB manifestations in paediatric patients in LMIC may be relatively more common than in industrialized countries. Therefore, IBD patients in LMIC should be meticulously screened for liver disease to allow prompt diagnosis and management.

**Key words:** Children; Crohn's disease; Egypt; Elevated liver enzymes; Hepatobiliary; Inflammatory bowel disease; Ulcerative colitis

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**Core tip:** The incidence of inflammatory bowel disease (IBD) in children has increased recently worldwide. Similarly, the reported incidence of the hepatobiliary (HB) manifestations of IBD in developed countries is rising, while in low- and middle-income countries, there are no much available reports especially in the paediatric age group. In this cohort of Egyptian children with IBD, all patients were screened for HB disorders by physical examination, liver function tests, imaging and liver biopsy when indicated. The most frequently reported HB disorders in these children with IBD were abnormal liver function tests, fatty infiltration and primary sclerosing cholangitis.

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## INTRODUCTION

Inflammatory bowel disease (IBD) in childhood includes Crohn's disease (CD), ulcerative colitis (UC) and unclassified-IBD (IBD-U), which was formerly known as indeterminate colitis<sup>[1]</sup>. In the past few years, the incidence of IBD in children in industrialized and developed countries has increased<sup>[2-4]</sup>. However, although in the low- and middle-income countries (LMICs) there have been few reports, the incidence of IBD in children may also be increasing<sup>[5]</sup>.

The characteristics of IBD in the Egyptian population are similar to the patterns seen in Asian and African populations, and the disease behaviour is milder than that reported in Western countries<sup>[6]</sup>. Extra-intestinal manifestations (EIMs) of IBD involving the skin, eyes and joints usually parallel the disease activity in the gut; however, diseases involving the hepatobiliary (HB) and pulmonary systems typically do not correlate with the disease activity of bowel inflammation<sup>[7]</sup>. The reported prevalence of liver and biliary tree disorders in IBD ranges from 3% to more than 50%, depending on whether the definition of the disease includes only definite and persistent conditions or transient alterations of liver function<sup>[8,9]</sup>.

HB complications can be a result of the primary IBD process, medication toxicity, or due to an underlying primary hepatic disorder unrelated to IBD, such as hepatitis caused by viruses, drugs or toxins which are more prevalent in LMICs<sup>[10,11]</sup>. Some HB manifestations, such as cholelithiasis and portal vein thrombosis, can occur due to the effect of chronic inflammation and the severity of bowel disease with bouts of severe diarrhoea and dehydration<sup>[7]</sup>. Non-specific hepatomegaly is commonly reported in IBD and is strictly related to fatty infiltration in more than 30% of patients<sup>[12]</sup>, and it does



not seem to be related to the patient's sex or type of IBD<sup>[8]</sup>.

The management of these disorders often requires the expertise of a multidisciplinary team to achieve the best outcome<sup>[13]</sup>.

The aim of the present study was to assess the prevalence and aetiology of HB manifestations in children and adolescents with IBD at a single centre in Egypt (which is classified as a LMIC) and compare them to those in some of the industrialized nations.

## MATERIALS AND METHODS

### Study design

This observational study was carried out at the Paediatric Hepatology and Gastroenterology Units of Cairo University Children's Hospital, Cairo, Egypt, over a 6-mo-period (between June 2013 and December 2013). Forty-eight children with an established diagnosis of IBD were included in the study. The diagnosis of IBD was based upon clinical, laboratory, endoscopic and histopathological features.

### Ethical considerations

The study protocol was approved by the Research Ethics Committee of the Paediatrics Department, Faculty of Medicine, Cairo University, Egypt. The research was conducted in accordance to the Helsinki Declaration. All patients were enrolled in the study after an informed consent was obtained from their parents/guardians.

### Participants

All patients < 18 years of age of both sexes with an established diagnosis of IBD were included. The clinical and laboratory data were retrospectively retrieved from 44 patients' files, and 4 newly diagnosed patients were prospectively enrolled during the study period. Patients were excluded if any crucial data was missing in their files.

### Assessment and evaluation

Detailed history taking was carried out, including: (1) Age at the time of presentation; (2) Age at the time of IBD diagnosis; (3) Meticulous family history of IBD in other siblings or parents; (4) History of comorbid conditions other than IBD; (5) Symptoms suggestive of HB manifestations [jaundice, abdominal distension, pruritus, gastrointestinal (GI) bleeding]; (6) Age at the start of treatment and age at the time of onset of HB manifestations; and (7) Medication history [aminosalicylate, azathioprine, corticosteroids, methotrexate, infliximab and/or drugs for associated diseases such as colchicine for Familial Mediterranean fever (FMF)].

The patients' anthropometric measurements (weight and height) were plotted on Egyptian growth curves (Standard Egyptian Growth, 2008)<sup>[14]</sup>, and the corresponding Z-scores were obtained.

All patients were subjected to meticulous clinical examination focusing on the signs of hepatic complications (jaundice, palmar erythema, spider nevi, lower limb oedema, and abdominal examination for organomegaly and ascites).

All patients were evaluated by liver chemistry, including the following: (1) Total and direct serum bilirubin; (2) Alanine aminotransferase (ALT); (3) Aspartate aminotransferase (AST); (4) Alkaline phosphatase (ALP); (5) Gamma glutamyl transpeptidase (GGT); (6) Serum albumin; and (7) Prothrombin time (PT) and concentration and international normalized ratio.

Abdominal ultrasound was performed in all patients using a greyscale device (FUKUDA-DENSHI; FF Sonic-400 Tokyo, Japan) to assess the liver size, texture, and echogenicity, dilatation of the intra-hepatic biliary radicals, gallbladder size, content and wall thickness, focal lesions, hepatic vasculature, and the presence of ascites. Splenic size was also assessed.

Serum immunoglobulin G (IgG), antinuclear antibody (ANA), smooth muscle antibody (SMA), anti-liver kidney microsomal 1, anti-neutrophil cytoplasmic antibody and anti-*Saccharomyces cerevisiae* antibody testing was performed in selected cases suspected of having autoimmune hepatitis (AIH) and/or primary sclerosing cholangitis (PSC). Viral markers were assessed for patients suspected of having hepatitis B virus (HBV) or hepatitis C virus (HCV) infections.

Diagnostic and follow-up upper and lower GI endoscopic examinations were performed for all patients to confirm the diagnosis of IBD and to monitor the treatment response. The gross endoscopic appearance as well as the histopathological

examination of mucosal biopsy specimens withdrawn at the time of GI endoscopy was the cornerstone for IBD diagnosis in our patients. Endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography (MRCP) was performed in cases suspected of having PSC or overlap syndrome (AIH and PSC).

### Statistical analysis

The data were tabulated and statistically analysed. Qualitative data were expressed as numbers and percentages, and were compared by the chi-square test or Fisher's exact test when appropriate. Quantitative data were expressed as the mean  $\pm$  SD, median and interquartile range, minimum and maximum, and were compared by the Student's *t*-test or the Mann-Whitney U test. In all tests, a probability (*P*) value was considered significant if less than 0.05.

## RESULTS

### Demographic characteristics

Based on the classic clinical findings, gross endoscopic appearance and microscopic histopathological features, we recruited 48 patients with IBD during this relatively short period from a large tertiary referral centre for all of Egypt. Twenty-nine (60.4%) patients were male; 24 (50%) had UC, 11 (22.9%) had CD, and 13 had IBD-U. The mean age at the time of presentation was  $8.14 \pm 4.02$  years, while the mean age at the time of study enrolment was  $10.16 \pm 4.19$  years.

The most common clinical presentation was recurrent abdominal pain in 47 patients (97.9%), followed by chronic diarrhoea with tenesmus and rectal bleeding (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 other affected siblings. Twelve patients (25%) had other associated diseases: 11 had FMF, and 1 patient had systemic lupus erythematosus (SLE).

Regarding the symptoms suggestive of HB manifestations of IBD, 2 patients had jaundice associated with dark-coloured urine. Eighteen patients (37.5%) presented with abdominal distension, which may also have been attributed to IBD itself. One of our patients presented with pruritus, and none had manifestations of portal hypertension. Upper GI endoscopy confirmed the absence of oesophageal varices. The mean age at the onset of hepatic complications in our patients diagnosed with IBD was  $12.10 \pm 3.51$  years.

The median (range) weight and height by Z score were -1.0 (-5.0-3.0) and -1.2 (-6.6-1.7), respectively. Sixteen patients (33.4%) were on the 3<sup>rd</sup> percentile or lower in weight, while 13 (27.1%) had short stature.

Abdominal examination showed that 16 patients (33.3%) had abdominal distension, 6 of whom (12.5%) had only hepatomegaly, with mean  $\pm$  SD size of the liver in the right midclavicular line and midline of  $4.83 \pm 1.72$  and  $5.33 \pm 2.94$  cm, respectively. Two patients (4.2%) had splenomegaly and none had ascites.

With regard to administered medications for the treatment of IBD at the time of study enrolment: (1) Thirty-five patients (72.9%) were receiving mesalamine; (2) Twelve (25%) received salazopyrine; (3) Twenty-six patients (54.2%) were receiving corticosteroids; (4) Twenty patients (41.7%) received azathioprine; (5) Four patients (8.35%) received methotrexate; and (6) Only one patient received infliximab.

In addition, 11 patients (22.9%) who were diagnosed with FMF received colchicine.

### Outcome data

Liver chemistry revealed that 3 patients (6.25%) had elevated liver enzymes, 2 patients (4.2%) had direct hyperbilirubinaemia, and 10 patients (20.8%) had hypoalbuminaemia that may also have been attributed to IBD, as none of the patients had coagulopathy (Table 2). Abdominal ultrasonography revealed that 10 patients (22.7%) had a bright echogenic liver (Figure 1), which may suggest fatty infiltration. Two patients (4.5%) had dilated intrahepatic biliary radicals. Two patients (4.5%) had thickened gallbladder walls, while 4 patients (9.1%) had splenomegaly. None had ascites.

Therefore, the overall frequency of HB manifestations in this study was 27.1% (13 patients). Of these 13 patients, 2 had cholestatic jaundice, elevated transaminases and elevated ALP and GGT, and both were confirmed to have PSC by MRCP (Figure 2) and percutaneous liver biopsy (Figure 3). One of these 2 patients had elevated serum IgG levels, positive ANA and positive SMA, and was diagnosed with PSC/AIH overlap syndrome. Another patient with elevated liver enzymes was diagnosed with

**Table 1** The presenting symptoms of children with inflammatory bowel disease in the study group (n = 48)

Complaint	n	Percentage
Abdominal pain	47	97.9
Diarrhoea	43	89.6
Tenesmus	36	75
Bleeding per rectum	35	72.9
Chronic fatigue	19	39.6
Weight loss	13	27.1
Low grade fever	13	27.1

**Table 2** The liver biochemical profile of children with inflammatory bowel disease in the study group

Parameter	Result	Range
ALT in median (IQR) (up to 45 U/L)	16.5 (13-24.8)	8-184
AST in median (IQR) (up to 75 U/L)	27 (20.5-34)	6-226
TB in median (IQR) (up to 1.4 mg/dL)	0.4 (0.3-0.5)	0.1-3.4
DB in median (IQR) (up to 1.4 mg/dL)	0.1 (0.1-0.1)	0-1.7
ALP in median (IQR) (up to 640 U/L)	161 (88-239)	50-439
GGT in median (IQR) (up to 50 U/L)	15 (12.3-19.8)	8-331
Albumin in mean $\pm$ SD (3.5-4.5 g/dL)	3.75 $\pm$ 0.78	1.3-5.3
PT in mean $\pm$ SD (s)	13.1 $\pm$ 0.59	11.7-14.8
PC in mean $\pm$ SD	92.8 $\pm$ 7.41	76-100
INR in mean $\pm$ SD	1.02 $\pm$ 0.05	1-1.2

ALT: Alanine aminotransferase; AST: aspartate aminotransferase; ALP: Alkaline phosphatase; DB: Direct bilirubin; IQR: interquartile range; GGT: Gamma-Glutamyl transferase; INR: International normalized ratio; PC: Prothrombin concentration; PT: Prothrombin time; TB: Total bilirubin.

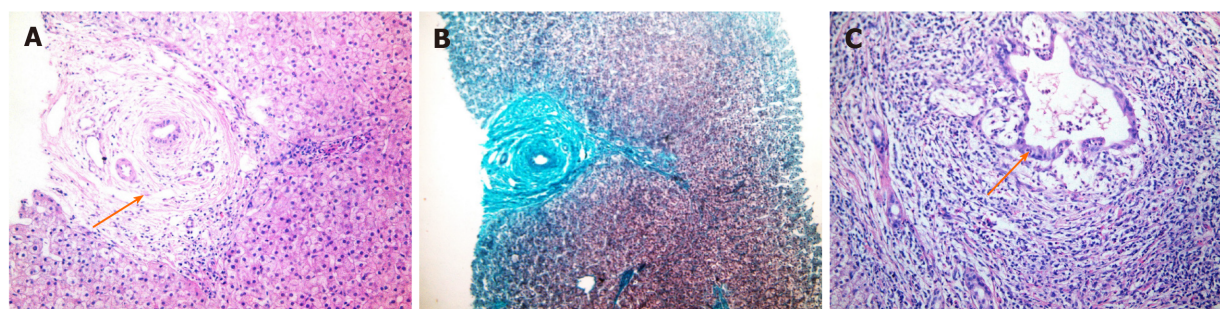

**Figure 1** Abdominal ultrasound of a patient with inflammatory bowel disease showing increased liver echogenicity.

SLE and had a concomitant HCV infection. He was recently treated for HCV with oral direct acting antivirals (sofosbuvir/ledipasvir) and achieved sustained virological response followed by normalization of his liver functions. Ten patients had echogenic liver by ultrasound: 3 of them were receiving steroids, 3 patients had malnutrition with poor growth, and 4 patients had both risk factors.

Regarding the 6 patients (12.5%) who had hepatomegaly, two had PSC, 3 had fatty liver, and the remaining patient had HCV infection.



**Figure 2** Magnetic resonance cholangiopancreatography of a patient with inflammatory bowel disease and primary sclerosing cholangitis showing dilatation of the intra- and extra-hepatic biliary tree with multiple strictures (arrows).



**Figure 3** Histopathological findings of a liver biopsy specimen from a patient with inflammatory bowel disease showing the features of primary sclerosing cholangitis. A: Diffuse bile ductular proliferation "Onion skin" fibrosis around affected ducts, concentric collagen fibre deposition; B: Same findings by Masson's trichrome stain which imparts a blue color to type 1 collagen against a red background of hepatocytes; C: Aggression of inflammatory cells towards the biliary epithelium and the portal area is markedly expanded with lymphoplasmacytic inflammatory cellular infiltrate (all photos H&E, Original magnification  $\times 400$ ).

## DISCUSSION

IBDs, including CD, UC, and IBD-U, are chronic and relapsing intestinal disorders<sup>[15]</sup>. In the Asia-Pacific region, a marked increase in the number of cases of IBD was found, which was compatible with a similar trend worldwide<sup>[16-18]</sup>. Our current study included 48 Egyptian children with IBD. The most common clinical presentation in our patients was recurrent abdominal pain, which was present in 97.9% of patients, followed by chronic diarrhoea. These findings are consistent with those of other studies where the most common presenting symptoms of IBD in children were abdominal pain (87.5%) and diarrhoea (75.0%)<sup>[14]</sup>.

A positive family history remains the strongest recognizable risk factor for the development of IBD and is reported in approximately 8%-12% of IBD patients<sup>[19]</sup>. Genetic factors, such as nucleotide-binding oligomerization domain containing 2 (NOD2) and other autophagy-related genes, such as ATG16L1 (autophagy-related 16-like 1), have been implicated in the aetiopathogenesis of IBD<sup>[20]</sup>. NOD2, a cytosolic protein expressed in monocytes, functions as an intracellular receptor for a bacterial product and is also a component of the innate immune system. CD is associated with frameshift mutations in NOD2, resulting in a truncated and non-functioning protein<sup>[21]</sup>. Autophagy, the major lysosomal pathway for degrading and recycling cytoplasmic material, constitutes an important homeostatic cellular process. Of interest, single-nucleotide polymorphisms of ATG16L1, a key component in the autophagic response to invading pathogens, have been associated with an increased risk of developing CD<sup>[22]</sup>. In the current study, nine patients had a positive history of IBD in their siblings. This relatively higher prevalence of positive family history in our study is mostly related to the deeply rooted Egyptian cultural traditions of consanguineous marriages and a large family size<sup>[23]</sup>. Therefore, we recommend meticulous genetic studies in our patients in Egypt and other LMICs who have a high prevalence of consanguinity.



The most common extra-intestinal manifestation of IBDs, particularly CD, in childhood and adolescence is reported to be impaired physical growth<sup>[24]</sup>. This was found in the present study, where almost one-third of our patients was on the 3<sup>rd</sup> percentile or lower for body weight, while 27.1% had short stature<sup>[25]</sup>.

In the present study, 11 patients (22.9%) were also diagnosed with FMF confirmed by genetic testing. This observation agrees with the study by Salah *et al*<sup>[26]</sup> conducted at our institute, which reported a significant association between *MEFV* gene mutation and IBD in Egyptian children, especially in patients with indeterminate colitis (IBD-U)<sup>[26]</sup>.

HB disorders are common in patients with IBD. Persistently elevated transaminases are observed in approximately 20%-30% of these patients<sup>[10]</sup>. An Italian study reported that abnormal liver chemistry was detected in 20.9% of IBD patients<sup>[12]</sup>. In this study, the frequency of overall HB manifestations was 27.1% (13 patients). Liver chemistry revealed that only 3 patients (6.25%) had elevated liver enzymes. Of the two patients who had cholestatic jaundice, one was diagnosed with PSC, and the other was diagnosed with PSC/AIH overlap syndrome. This finding was similar to that reported by Broomé *et al*<sup>[27]</sup>, who found that 1.4%-7.5% of patients with IBD eventually developed PSC during the course of their disease. Patients with PSC usually present with jaundice<sup>[28]</sup>, which was the case in our patients. The disease onset of PSC is typically insidious, but it has been increasingly diagnosed at the asymptomatic stage, likely because of the widespread availability of ERCP and MRCP for evaluating elevated serum ALP levels<sup>[10]</sup>.

AIH/PSC overlap must be considered in any patient with IBD, particularly UC, and PSC exhibits elevated transaminases, polyclonal hyper-gamma-globulinaemia and a liver biopsy suggestive of periportal hepatitis<sup>[28]</sup>. Autoimmune liver disease is reported in up to 7.8% of children with IBD<sup>[29]</sup>. In the present study, only one patient suffered from AIH/PSC overlap syndrome.

Corticosteroids may alter the hepatic lipid metabolism and induce hepatic steatosis<sup>[30]</sup>. Malnutrition is another factor that is highly associated with hepatocyte fatty infiltration. Furthermore, protein synthesis is decreased during protein-calorie malnutrition, which plays a role in triglyceride export into the blood<sup>[31]</sup>. Thus, triglycerides are retained within the hepatocytes resulting in fatty liver. Increased import of free fatty acids into the liver as a result of abdominal lipolysis, which develops during the weight-loss stages, may add to this process<sup>[32]</sup>. This finding is in agreement with our study, in which 10 patients had echogenic liver by ultrasound; 3 of them were receiving steroids, 3 patients had malnutrition with poor growth according to the Egyptian growth curves<sup>[25]</sup>, and 4 patients had both risk factors. Although the diagnosis of fatty liver needs to be confirmed histologically, screening can be accomplished *via* analysis of serum aminotransferases, GGT, triglyceride levels and ultrasonographic appearance of a bright echotexture of the liver<sup>[8]</sup>. On the other hand, a previous study suggested a complex, multifactorial relationship between the IBDs and the development of non-alcoholic fatty liver disease (NAFLD) beyond the scope of current pharmacological intervention<sup>[33]</sup>.

As immunosuppressive drugs are being used more frequently in IBD, concerns about viral reactivation are increasing. The impact of immunosuppressive therapy for IBD on HCV remains controversial, as steroids can promote viral replication. Egypt has the highest prevalence of HCV infection in the world<sup>[34]</sup>. The prevalence of HCV infection has increased in IBD patients in comparison to the general population both in Spain and France in studies over the last decade of the 20<sup>th</sup> century<sup>[35]</sup>. Similarly, one of the patients (2%) in the current study had associated chronic HCV infection. This patient had multiple risk factors for HCV acquisition, including blood transfusion, recurrent hospitalizations, and repeated endoscopies with intestinal biopsies. However, other studies have demonstrated a similar prevalence in the general population<sup>[36,37]</sup>.

The drug armamentarium for IBD is very wide and might be frequently complicated by hepatotoxicity, adding to the spectrum of liver disease in IBD. However, drug therapy for the associated liver disease itself might be limited, and appropriate timely diagnosis might be all that is offered to the patients, but that issue is beyond the scope of this study.

The prognosis of IBD patients with liver disease is worsening, and close collaboration between IBD clinicians, paediatric hepatologists/gastroenterologists, and primary care providers should be encouraged for early diagnosis, shared decision making and optimal outcome before end-stage liver disease sets in<sup>[10]</sup>. Liver transplantation (LTx) is not readily available in LMICs. Moreover, recipients with comorbidities at the time of LTx, including those with IBD, have increased morbidity and mortality. Due to the association between IBD and PSC, the frequency of LTx in



patients with IBD has increased<sup>[10]</sup>. Patients with a longer duration of UC and extensive colonic involvement are known to have an increased risk of colorectal cancer. Routine colorectal cancer surveillance should continue for patients with IBD who have had LTx, even in the setting of clinically quiescent disease<sup>[37]</sup>.

In conclusion, the prevalence of HB manifestations in paediatric patients with IBD in Egypt, as one of the LMICs, is relatively high compared to industrialized countries. Investment in research for confounding factors in LMICs might be justifiable, including family genetic studies that might diagnose early cases of IBD and work-up for very early-onset IBD. These patients should be screened for liver disease to allow prompt diagnosis and the offer of available treatment. The most common HB disorders in children with IBD are abnormal liver tests, fatty infiltration and PSC.

### **Suggestion for further research**

Screening for HB manifestations of IBD is mandatory, as many of these complications could be initially asymptomatic. HBV and HCV should not be overlooked and should be screened for in patients with IBD, as these patients have multiple risk factors for HCV acquisition including endoscopies, blood transfusion and recurrent hospitalizations. Therefore, it is necessary to implement mass screening for HCV in these high-risk groups especially in an endemic country such as Egypt. Health education for parents and patients should be carried out to increase knowledge on the nature of the disease and treatment options. Genetic research on the diagnosis of very early-onset IBD and family screening should be emphasized.

## **ARTICLE HIGHLIGHTS**

### **Research background**

Inflammatory bowel disease (IBD) is a systemic illness that can present with multiple extra-intestinal manifestations. Hepatobiliary (HB) disorders associated with IBD are not uncommon. From an aetiopathogenic perspective, there are multiple layers of evidence suggesting that these disorders are not isolated entities and may share mechanistic pathways. All of these disorders usually manifest with abnormal hepatic biochemical tests. Of the HB complications of IBD, primary sclerosing cholangitis (PSC) carries the most significant clinical implications and remains a highly challenging disease to manage.

### **Research motivation**

Recently, the reported incidence of IBD in paediatrics has increased dramatically. The HB manifestations of IBD have been well studied in children in the industrialized and developed countries. On the other hand, there is a paucity of studies on IBD in low- and middle-income countries (LMICs) such as Egypt.

### **Research objectives**

The main objective was to determine the frequency of HB manifestations in Egyptian paediatric patients with IBDs, to achieve an early diagnosis in order to obtain prompt treatment. Moreover, the risk factors associated with the occurrence of HB complications in patients with IBD were determined.

### **Research methods**

This study was carried out at the Paediatric Hepatology and Gastroenterology Units of Cairo University Children's Hospital over a 6-mo-period. Patients younger than 18 years of age of both sexes were included. The available data were retrieved from the files of patients diagnosed with IBD as well as newly diagnosed patients. The collected data included a full medical history such as name, age, sex, residence, meticulous family history of IBD and other associated autoimmune illnesses; history of jaundice; abdominal distention; pruritus; bleeding; a history of blood transfusion; and a detailed medication history. A meticulous clinical examination was performed, including the assessment of anthropometric measures and a general and detailed abdominal examination. Laboratory investigations, such as CBC, complete liver functions (ALT, AST, total and direct bilirubin, ALP, GGT, albumin and PT) were performed in all patients. In addition, serum immunoglobulin G, antinuclear antibody, smooth muscle antibody, anti-liver kidney microsomal 1, anti-neutrophil cytoplasmic antibody and anti-*Saccharomyces cerevisiae* antibody were performed for selected cases suspected of having autoimmune hepatitis (AIH) and/or PSC. Viral markers were assessed in

patients suspected of having hepatitis B virus (HBV) or hepatitis C virus (HCV) infections. Abdominal ultrasound was performed in all patients. Endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance cholangiopancreatography was performed for cases suspected of having PSC. Percutaneous liver biopsy was performed when indicated.

### Research results

We recruited 48 paediatric patients with IBD. Twenty-nine (60.4%) patients were male. Twenty-four (50%) had UC, 11 (22.9%) had CD, and 13 patients had IBD-U. The median (range) of the weight and height by Z score were -1.0 (-5.0-3.0) and -1.2 (-6.6-1.7), respectively. Sixteen patients (33.4%) were on the 3<sup>rd</sup> percentile or lower for weight, while 13 (27.1%) had short stature. The most common clinical presentation was recurrent abdominal pain, which was present in 47 patients (97.9%), followed by chronic diarrhoea with tenesmus and rectal bleeding. None of our patients presented with ulcerating perianal disease. There was a positive family history of IBD in 9 patients (18.75%). Twelve patients (25%) had other associated diseases: 11 had FMF, and one patient had systemic lupus erythematosus. Therefore, the overall frequency of HB disorders in this study was 27.1% (13 patients). Two patients had cholestatic jaundice, one of them was diagnosed with PSC, and the other patient had PSC/AIH overlap syndrome. Three (6.3%) patients had elevated liver enzymes. Ten (20.8%) patients had echogenic liver suggesting fatty changes.

### Research conclusions

HB manifestations in paediatric patients with IBD in LMICs are more common than those in the industrialized countries. Investment in research for confounding factors in LMICs is cost-effective, including family genetic studies that can offer an early diagnosis of IBDs in general and specifically for very early-onset IBD. The importance of awareness of the implications and causes of abnormal hepatic biochemical tests in IBD patients is due to the wide range of possible complications and risks associated with the medications used to treat and manage them. Hepatic biochemical tests should be routinely performed in these patients. When abnormalities are detected, a prompt step by step diagnostic approach should be followed until an aetiology is reached. The frequency of HB manifestations in our patients was not low and may be affected by the type of treatment modality and malnutrition. The highest proportion was found in those suffering from an echogenic liver by abdominal ultrasound, and many of these patients received corticosteroids.

### Research perspectives

Screening for HB manifestations of IBD is mandatory, as many of these complications may be initially asymptomatic. Complete liver function tests and abdominal ultrasound should be performed on a regular basis. A high index of suspicion of PSC should be raised in patients who present with an unexplained cholestatic jaundice or elevated ALP levels. An echogenic liver detected by abdominal ultrasound requires thorough assessment of the patient's growth and medication history. Liver biopsy may be needed in selected cases for the definitive diagnosis of NAFLD. Drugs used to treat IBD should be administered cautiously, as a majority of them can potentially cause hepatotoxicity. HBV and HCV should be screened for in patients with IBD especially in endemic areas such as Egypt. Patients with IBD have multiple risk factors for HCV and HBV acquisition, including repeated endoscopies, transfusion of blood products and recurrent hospitalizations. Genetic research should include the diagnosis of very early-onset IBD and screening of similar cases in families.

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Prospective Study

## Tongue thickness in health vs cirrhosis of the liver: Prospective observational study

Manish Tandon, Harshita Singh, Nishant Singla, Priyanka Jain, Chandra Kant Pandey

**ORCID number:** Manish Tandon 0000-0003-0087-6216; Harshita Singh 0000-0001-7620-0365; Nishant Singla 0000-0003-3424-4006; Priyanka Jain [orcid.org/0000-0002-3374-882X](https://orcid.org/0000-0002-3374-882X); Chandra Kant Pandey 0000-0003-4472-0547.

**Author contributions:** Tandon M conceived and wrote the manuscript and performed the literature search and review; Singh H collected and compiled data and reviewed the literature; Singla N collected L3 skeletal muscle index data, trained others on ultrasonography measurements, and supervised ultrasonography measurements; Jain P performed statistical analysis of the data; Pandey CK reviewed the manuscript, and provided intellectual input.

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**Clinical trial registration statement:** The study titled "Study of thickness of tongue by ultrasound and its relation with severity of disease in patients with cirrhosis of liver: Prospective Study", is registered with clinical trials registry of India vide No.

**Manish Tandon, Harshita Singh, Chandra Kant Pandey,** Formerly at Department of Anesthesia, Institute of Liver and Biliary Sciences, New Delhi 110070, India

**Nishant Singla,** Formerly at Department of Intervention Radiology, Institute of Liver and Biliary Sciences, New Delhi 110070, India

**Priyanka Jain,** Formerly at Department of Research, Institute of Liver and Biliary Sciences, New Delhi 110070, India

**Corresponding author:** Manish Tandon, MD, Doctor, Ex Additional Professor Anaesthesia, Department of Anaesthesiology, Institute of Liver and Biliary Sciences, D-1, Vasant Kunj, New Delhi 110070, India. [manishtandon25@rediffmail.com](mailto:manishtandon25@rediffmail.com)

### Abstract

#### BACKGROUND

Malnutrition affects 40%-90% of patients with cirrhosis of the liver. L3 skeletal muscle index (L3SMI) is presently accepted as the most objective and quantitative measure available for sarcopenia, a surrogate marker of malnutrition. L3SMI application is, however, limited by non-availability of computed tomography scanning in remote areas, cost, need for extensive training, and the risk of exposure to radiation. Therefore, an alternative dependable measure with wider availability is needed. Malnutrition causes sarcopenia not only in skeletal muscles but also in other muscular structures such as the psoas muscle, diaphragm and tongue. We therefore hypothesised that the tongue, being easily accessible for inspection and for measurement of thickness using ultrasonography, may be used to document sarcopenia.

#### AIM

To measure and compare tongue thickness in healthy individuals and in patients with cirrhosis of the liver and to study its correlation with conventional prognostic scores for patients with cirrhosis of the liver.

#### METHODS

Tongue thickness was measured using ultrasonography. One hundred twenty subjects of either gender aged 18 to 65 years were studied, with 30 subjects in each group. The tongue thickness was compared between groups based on "Child Turcotte Pugh" (CTP) scores. The correlations between measured tongue thickness and "Model for end stage liver disease" (MELD) score and between age



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and measured tongue thickness were also assessed.

## RESULTS

Mean tongue thickness (mean  $\pm$  SD) in patients with CTP class A, B and C was  $4.39 \pm 0.39$  cm,  $4.19 \pm 0.53$  cm, and  $3.87 \pm 0.42$ , respectively, and was  $4.33 \pm 0.49$  cm in normal healthy individuals. Significant differences were seen in tongue thickness between patients with CTP class C and those with CTP class A and B ( $P < 0.05$ ). Patients with CTP class C also had a significantly reduced tongue thickness than normal individuals ( $P < 0.05$ ). However, no significant difference was seen in tongue thickness between patients with CTP class A and B and normal individuals. A statistically significant, negative correlation was found between MELD score and tongue thickness ( $r = -0.331$ ) ( $P < 0.001$ ). No correlation was observed between L3SMI and MELD score ( $r = 0.074$ ,  $P = 0.424$ ). L3SMI (mean  $\pm$  SD) in healthy subjects was  $39.66 \pm 6.8$  and was  $38.26 \pm 8.88$  in patients with CTP class C, and the difference was not significant. No significant correlation was found between age of the patients and tongue thickness. Intra-class correlation coefficient was used to determine the reliability of the tongue thickness measurements. The intra-class correlation coefficient was 0.984 (95%CI: 0.979-0.989) and was indicative of good reliability.

## CONCLUSION

Tongue thickness measured by ultrasonography, correlates significantly with the severity of liver disease, as assessed by CTP and MELD scores. The patients with a CTP score  $\geq 10$  have significantly reduced tongue thickness as compared to normal individuals and those with less severe liver disease and CTP scores of 5-9. No significant difference in tongue thickness was found between healthy individuals and CTP class A and B patients.

**Key words:** Sarcopenia; Malnutrition; Cirrhosis of the liver; Child Turcotte Pugh class; Model for end stage liver disease score; Ultrasonography

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**Core tip:** Sarcopenia has implications for the management and outcome of patients with cirrhosis of the liver and is therefore included in prognostication. However, the only objective and reproducible measure for sarcopenia using computed tomography (CT) scanning which measures skeletal muscle thickness, is the L3 skeletal muscle index (L3SMI). However, the application of L3SMI is restricted by the need for CT scanning. Compared with CT scan measured L3SMI, tongue thickness can easily be measured in a reproducible manner and accurately with minimal training using ultrasonography and as suggested by this study, is a more sensitive indicator of sarcopenia. Further studies are required to validate these findings and propose tongue thickness as a tool to diagnose sarcopenia.

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## INTRODUCTION

Malnutrition has been estimated to affect 50%-90% of patients with cirrhosis of the liver<sup>[1]</sup>. However, malnutrition is frequently overlooked, in part because nutritional assessment can be difficult in patients with cirrhosis due to fluid retention and because patients with cirrhosis may develop simultaneous loss of skeletal muscle and gain of adipose tissue, culminating in the condition of "sarcopenic obesity"<sup>[1]</sup>. Sarcopenia is characterised by the loss of muscle mass and is a surrogate marker of malnutrition<sup>[2]</sup>. Severity of liver disease is assessed using "Model for end stage liver disease" (MELD)

score, which is calculated by an online calculator using values for serum creatinine, bilirubin and the international normalised value for prothrombin time. The MELD score is also used to decide upon organ allocation for liver transplant<sup>[3]</sup>, and currently does not include a measure of sarcopenia. Researchers have suggested changes in the MELD score calculation to include a measure of sarcopenia, considering its prognostic implications<sup>[4]</sup>. Similarly, the “Child Turcotte Pugh” (CTP) score, a conventional scoring system for severity of liver disease based on values of serum bilirubin, albumin, international normalised value for prothrombin time, and measures of encephalopathy and ascites, do not include any measure of sarcopenia<sup>[5]</sup>. L3 skeletal muscle index (L3SMI) is presently accepted as the most objective and quantitative measure of sarcopenia<sup>[6]</sup>. However, non-availability of computed tomography (CT) scanning in remote areas, cost, and exposure to radiation are limitations to the use of L3SMI; besides, the use of CT for documenting and quantifying sarcopenia can be justified only in patients who have an indication for CT as part of their standard medical care due to risks of radiation exposure. An optimal index for sarcopenia in terms of availability, reproducibility, practicality, and of prognostic significance is therefore needed and remains a challenging issue.

Malnutrition causes sarcopenia in several muscular structures besides the much-studied lumbar muscles, such as the diaphragm, psoas muscle and tongue<sup>[7-9]</sup>. In the quest for ultrasonography (USG)-based, bedside targets for documenting sarcopenia, we hypothesised that the tongue being a muscular structure affected by sarcopenia with easy access for inspection and measurement, may also be used to quantify and document sarcopenia. Therefore, a prospective study was conducted with the primary objective to measure and compare tongue thickness in healthy individuals and in patients with cirrhosis of the liver. The secondary objective was to determine the correlation between tongue thickness and conventional prognostic scores for patients with cirrhosis of the liver.

## MATERIALS AND METHODS

The study was performed at a tertiary care institution after approval by the institutional ethics committee. Consent for the study protocol was obtained from the study subjects. To study 30% difference with power of 80 and type II error of 5%, we needed to study 30 subjects in each group. A total of 120 subjects, who satisfied the inclusion criteria were enrolled and studied from May 2017 to October 2018.

Patients with cirrhosis of the liver due to any aetiology and healthy individuals aged 18-65 years and a body mass index (BMI) > 18 and < 30, visiting the hospital for reasons other than illness, were included in the study. Individuals aged less than 18 years and more than 65 years, patients with acute liver failure and those with glossitis were not included in the study. Tongue thickness was measured using USG with a 3.75 MHz convex probe while the subjects were seated in an upright position. The subjects were instructed to swallow their saliva several times to set the tongue at the resting position, and then the ultrasonic measurements were carried out. The measurement points were determined on the upper and lower surfaces of the lingual muscles in the centre of the plane, perpendicular to the ‘Frankfurt horizontal plane’ in a frontal section (Figure 1). The Frankfurt horizontal plane is formed by drawing a straight horizontal line from the top of the ear canal to the bottom border of the eye along either side of the human skull. This line is called the Frankfurt horizontal line<sup>[10]</sup>. The vertical distance was measured from the surface of the mylohyoid muscle to the tongue dorsum (Figure 2). Measurements were performed thrice in freeze-frame when the tongue was restored to the resting position after swallowing saliva, and the mean values were obtained. Tongue thickness was measured for all the study subjects, but the L3SMI was calculated from CT scans only for patients with CTP class C who were being investigated for liver transplant and for healthy individuals who were evaluated as possible organ donors. MELD scores were calculated using the online calculator at <https://www.mdcalc.com/meld-score-model-end-stage-liver-disease-12-older>. Tongue thickness was compared between the groups based on CTP scores. Correlations were also determined between measured tongue thickness and MELD score, and between age and measured tongue thickness.

### Statistical analysis

Data are presented as mean  $\pm$  SD or frequencies (percentage) and were analysed using SPSS 23.0 software. One-way ANOVA was used to test the significance of parametric data and the Kruskal-Wallis test for non-parametric data. Comparison of categorical

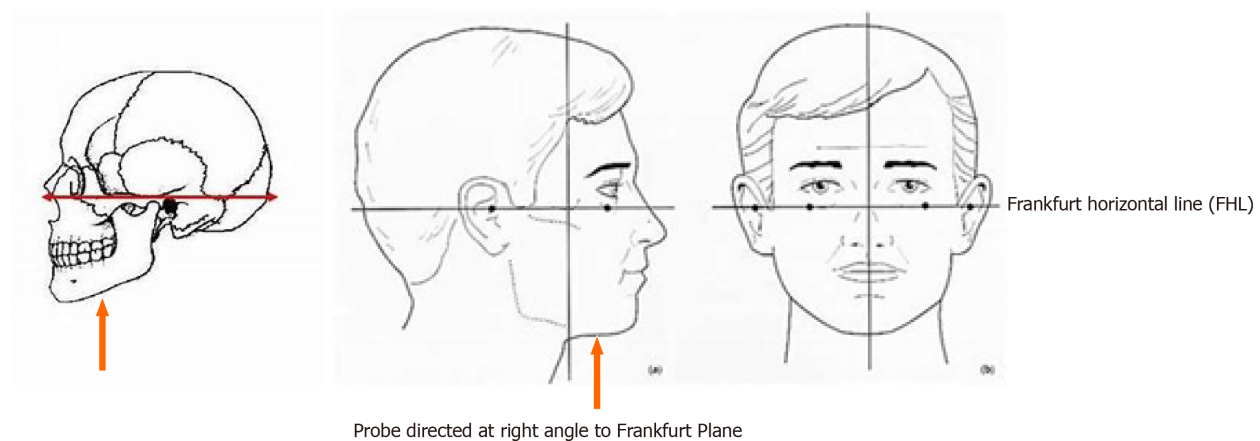


Figure 1 Ultrasonography probe position for measuring tongue thickness.

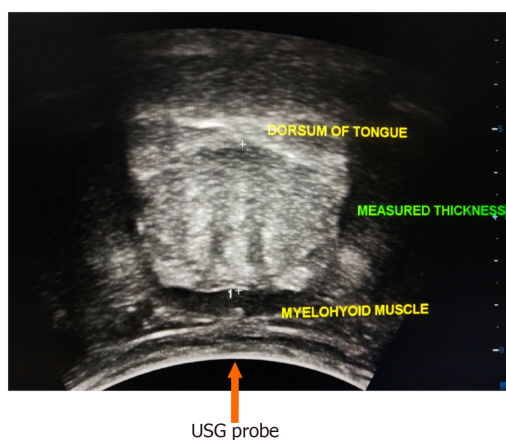


Figure 2 Vertical distance measured from the surface of the mylohyoid muscle to the tongue dorsum. USG: Ultrasonography.

data was carried out using the chi square test/Fisher's exact test. Continuous data were compared by the student *t*-test/Mann-Whitney test, as applicable. A *P* value less than 0.05 was considered significant. Intra-class correlation coefficient (ICC) was used to determine the reliability and agreement of the tongue thickness measurements.

## RESULTS

Of 120 patients, 96 were males and 24 were females with a mean age of 47.12 years. The various aetiologies of cirrhosis were ethanol-related, non-alcoholic steato-hepatitis, cryptogenic, hepatitis B virus, hepatitis C virus, autoimmune, hepatic vein outflow tract obstruction, non-alcoholic fatty liver disease, and primary sclerosing cholangitis (Figure 3).

### Tongue thickness and CTP score

Mean tongue thickness in patients with CTP class A was  $4.39 \pm 0.39$  cm (range 4.25-4.53), in patients with CTP class B was  $4.19 \pm 0.53$  cm (range 3.99-4.39), in patients with CTP class C was  $3.87 \pm 0.42$  cm (range 3.71- 4.02) and in normal healthy individuals was  $4.33 \pm 0.49$  cm (range 4.15-4.51) (Table 1; Figure 4).

A significant difference was seen in tongue thickness between patients with CTP class C and those with CTP class A and B ( $P < 0.05$ ). Patients with CTP class C also had significantly reduced tongue thickness than normal individuals ( $P < 0.05$ ). However, no significant difference was observed in tongue thickness between patients with CTP class A and B and normal individuals (Table 2).

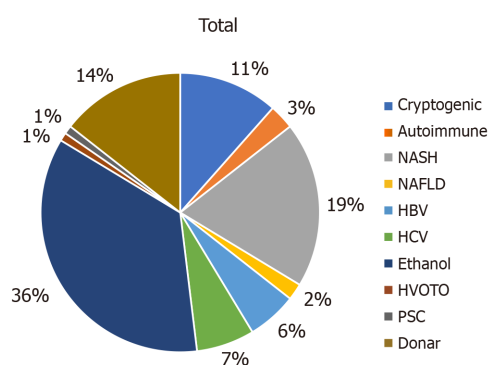
**Table 1 Tongue thickness in the study groups**

Study group	mean $\pm$ SD	Median (range)
Child class A	4.39 $\pm$ 0.39 cm	4.50 cm (range 4.25-4.53)
Child class B	4.19 $\pm$ 0.53 cm	4.15 cm (range 3.99-4.39)
Child class C	3.87 $\pm$ 0.42 cm	3.80 cm (range 3.71-4.02)
Normal (healthy) subjects	4.33 $\pm$ 0.49 cm	4.35 cm (range 4.15-4.51)

SD: Standard deviation.

**Table 2 Comparison of tongue thickness in the study groups**

(I) CTP class	(J) CTP class	Mean difference (I-J)	Std. error	Significance	95%CI	
					Lower bound	Upper bound
Class A	Class B	0.2000	0.1186	0.335	-0.109	0.509
	Class C	0.5233 <sup>a</sup>	0.1186	0.000	0.214	0.832
	Normal	0.0567	0.1186	0.964	-0.252	0.366
Class B	Class A	-0.2000	0.1186	0.335	-0.509	0.109
	Class C	0.3233 <sup>a</sup>	0.1186	0.037	0.014	0.632
	Normal	-0.1433	0.1186	0.623	-0.452	0.166
Class C	Class A	-0.5233 <sup>a</sup>	0.1186	0.000	-0.832	-0.214
	Class B	-0.3233 <sup>a</sup>	0.1186	0.037	-0.632	-0.014
	Normal	-0.4667 <sup>a</sup>	0.1186	0.001	-0.776	-0.158
Normal	Class A	-0.0567	0.1186	0.964	-0.366	0.252
	Class B	0.1433	0.1186	0.623	-0.166	0.452
	Class C	0.4667 <sup>a</sup>	0.1186	0.001	0.158	0.776

<sup>a</sup>Statistically significant. CTP class: Child Turcotte Pugh class. CI: Confidence interval**Figure 3 Aetiologies of liver cirrhosis.****MELD score**

mean ( $\pm$  SD) MELD score in patients with CTP class A, B and C was  $9.63 \pm 2.24$ ,  $13.90 \pm 2.96$  and  $25.37 \pm 7.92$ , respectively.

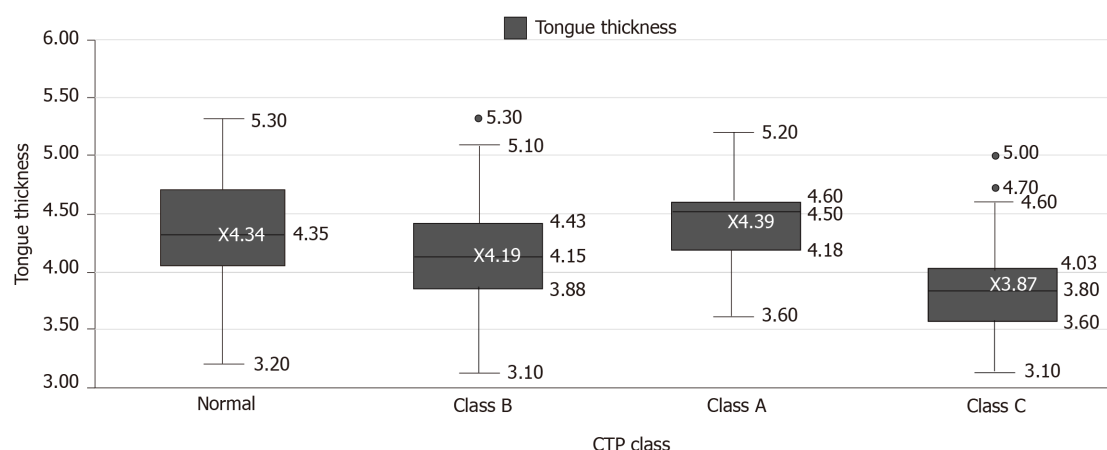
**Tongue thickness and MELD score**

A statistically significant, negative correlation was found between MELD score and tongue thickness ( $r$ : -0.331) ( $P < 0.001$ ) (Table 3).

**Table 3** Correlation between “Model for end stage liver disease” score and tongue thickness

		Tongue thickness	MELD score
Tongue thickness	Pearson correlation	1	-0.330 <sup>a</sup>
	Significance (2-tailed)		0.001
	<i>n</i>	120	90
MELD score	Pearson correlation	-0.330 <sup>a</sup>	1
	Significance (2-tailed)	0.001	
	<i>n</i>	90	90

<sup>a</sup>Statistically significant. MELD score: Model for end stage liver disease score.



**Figure 4** Box plot of the distribution of tongue thickness in different “Child Turcotte Pugh” class patients and normal healthy subjects.  
CTP class: Child Turcotte Pugh class.

### Age and tongue thickness

No significant correlation was found between age of the patients and tongue thickness by USG (Table 4).

### Tongue thickness and L3SMI

No significant correlation was found between tongue thickness and L3SMI ( $P = 0.83$ ) (Table 5). In healthy subjects, the mean ( $\pm$  SD) L3SMI value was  $39.66 \pm 6.8$ , and was  $38.26 \pm 8.88$  in patients of CTP class C. The difference was not significant ( $P = 0.63$ ) (Table 6). Barring the outliers, in 2 healthy subjects and in 2 CTP class C patients, the median L3SMI was 40.19 (33.89-45.4) and 39.39 (32.68-45.35), respectively (Figure 5). ICC was used to determine the reliability of tongue thickness measurements. The ICC value was 0.984 (95% CI: 0.979-0.989) and was indicative of good reliability (Table 7).

## DISCUSSION

Our study indicates that tongue thickness measurement by USG correlates significantly with the severity of liver disease, as assessed by CTP scores. The study established that patients with a CTP score  $\geq 10$  have significantly reduced tongue thickness as compared to normal individuals and those with less severe liver disease with CTP scores of 5-9. Studies have shown that sarcopenia also affects other muscles besides the much studied L3SMI<sup>[7-9]</sup>. Tongue thickness has even been examined as a bedside measure of sarcopenia in patients with critical illness and has also been correlated with clinical outcome<sup>[9]</sup>.

Malnutrition in cirrhosis is secondary to a multifactorial process and is seen more often in patients with more severe liver disease. We studied the correlation between tongue thickness and MELD score and found a significant negative correlation



**Table 4 Correlation between age and tongue thickness**

		Tongue thickness	Age
Tongue thickness	Pearson correlation	1	-0.081
	Significance (2-tailed)		0.382
	<i>n</i>	120	120
Age	Pearson correlation	-0.081	1
	Significance (2-tailed)	0.382	
	<i>n</i>	120	120

**Table 5 Correlation between tongue thickness and L3 skeletal muscle index**

		Tongue thickness	L3SMI
Tongue thickness	Pearson correlation	1	-0.074
	Significance (2-tailed)		0.424
	<i>n</i>	120	120
L3SMI	Pearson Correlation	-0.074	1
	Significance (2-tailed)	0.424	
	<i>n</i>	120	120

L3SMI: L3 skeletal muscle index.

**Table 6 Comparison of L3 skeletal muscle index in Child Turcotte Pugh class C and normal healthy subjects**

CTP class	L3SMI					P value
	Mean	Standard deviation	Median	Minimum	Maximum	
Class C	38.2613	8.88428	39.3900	18.37	52.85	0.63
Normal	39.6640	6.80565	40.1900	31.24	50.25	

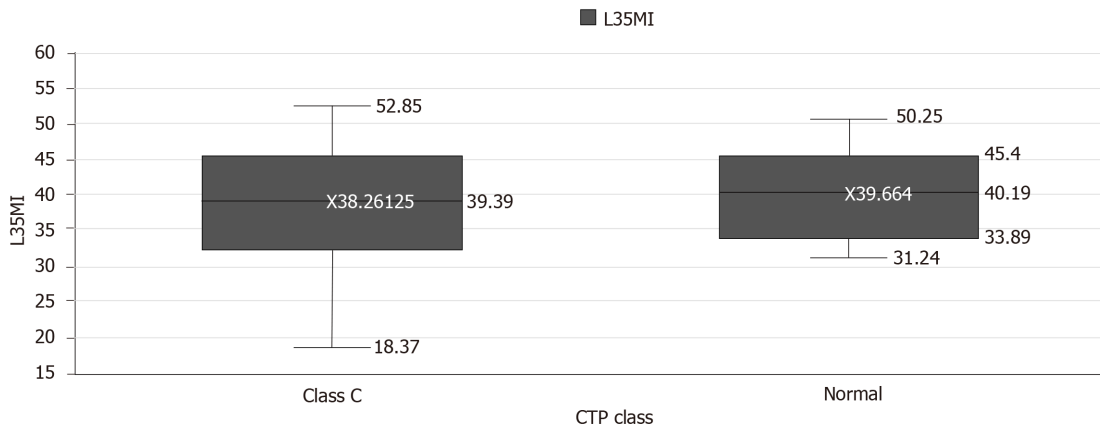
L3SMI: L3 skeletal muscle index; CTP class: Child Turcotte Pugh class.

**Table 7 Intraclass correlation coefficient for ultrasonography measurements of tongue thickness**

		95%CI		F test with true value 0			
	Intraclass correlation	Lower bound	Upper bound	Value	df1	df2	Sig
Single measures	0.954 <sup>a</sup>	0.939	0.966	63.487	119	238	0.000
Average measures	0.984 <sup>a</sup>	0.979	0.989	63.487	119	238	0.000

<sup>a</sup>Statistically significant. Sig: Significance. CI: Confidence interval.

between the two ( $r$ : -0.331,  $P < 0.01$ ), indicating that as the MELD score increased, tongue thickness decreased and may be interpreted as worsening of sarcopenia with worsening of liver disease. We also found a significant difference in tongue thickness between CTP class C patients and healthy individuals and between CTP class C patients compared to CTP class A and B patients. However, we did not find any significant difference in tongue thickness between healthy individuals and CTP class A and B patients. Apparently, an appreciable degree of sarcopenia manifests only later in the course of liver cirrhosis when a patient qualifies for CTP class C categorisation. Similar to our findings, Montano-Loza *et al*<sup>[11]</sup> studied 248 patients and found that sarcopenia was more prevalent in patients with CTP class C ( $P < 0.05$ ) and in patients



**Figure 5** Box plot of the distribution of L3 skeletal muscle index in Child Turcotte Pugh class C patients and healthy individuals. L3SMI: L3 skeletal muscle index; CTP class: Child Turcotte Pugh class.

with higher MELD scores ( $P < 0.02$ ). In other studies, Thandassery *et al*<sup>[6]</sup> and Tandon *et al*<sup>[12]</sup> also found a correlation between the prevalence of sarcopenia and disease severity, as measured by L3SMI and CTP.

However, in the present study, we did not find a correlation between tongue thickness and L3SMI. We also did not find any significant difference in L3SMI between healthy subjects and CTP class C patients. Measuring the mass of a muscle or group of muscles that predominantly have dynamic or postural functions, we believe, is flawed and the paravertebral muscles being postural muscles, are apparently only affected to an appreciable degree late in the disease course when patients may be critically ill and become bedridden. None of the patients included in this study were critically ill and/or admitted to the intensive care unit or were bedridden, and this could possibly be the reason why no difference was found between L3SMI in healthy subjects and CTP class C patients. This reasoning may also be inferred from the study of 116 patients with cirrhosis and hepatocellular carcinoma by Meza-Junco *et al*<sup>[13]</sup>. In their study, similar to our findings, the degree of sarcopenia measured using L3SMI did not correlate with CTP or MELD scores. However, in our study, tongue thickness was consistently and significantly decreased in CTP class C patients. Tongue thickness is probably a more sensitive marker of sarcopenia compared to L3SMI and requires further investigation.

A significant number of people more than 65 years old have decreased muscle mass<sup>[14]</sup>. In this study, we did not include patients more than 65 years, but we did not find a significant correlation between tongue thickness and age of the subjects included (Table 4). Sarcopenia in cirrhotic patients has been associated with increased mortality, sepsis, hyperammonemia, overt hepatic encephalopathy, and increased length of stay after liver transplantation<sup>[6]</sup>. The literature also suggests that patients with cirrhosis, poor nutritional status and sarcopenia have a higher risk of mortality, independent of the CTP and MELD scores<sup>[15]</sup>. It is therefore important to diagnose, quantify and perhaps classify the degree of sarcopenia for the medical management of patients with cirrhosis of the liver, for outcome prognosis and for planning interventions such as liver transplantation. This could be greatly helped by having a readily available and reproducible method for the diagnosis and quantification of sarcopenia.

The tongue has the advantages of direct inspection and ease of bedside measurement of thickness using USG, which is more readily available than CT scanning and is without the risk of radiation exposure, and unlike CT does not require extensive training. Tongue thickness measurement, as suggested by our findings, in addition to being objective, reproducible and easy, could be a more sensitive index for detecting sarcopenia than L3SMI. Our study was limited due to it being located at a single centre, and directed at patients with only a single disease, namely cirrhosis of the liver. Further studies exploring USG-measured tongue thickness in people of diverse ethnicity and different age groups in health and in disease, should be carried out to validate our findings and to establish this as a convenient bedside tool for diagnosing sarcopenia. In view of our study findings, we propose that tongue thickness measurement using USG should be considered for the diagnosis and quantification of sarcopenia.

## ARTICLE HIGHLIGHTS

**Research background**

Sarcopenia in patients with chronic liver disease has prognostic implications. L3 skeletal muscle index (L3SMI) calculated from computed tomography (CT) images is currently the only objective and reproducible method accepted for the quantification of sarcopenia. This study aims to determine tongue thickness measured using ultrasonography as an alternative method for diagnosing sarcopenia.

**Research motivation**

Sarcopenia in patients with chronic liver disease has prognostic implications. Wider application of L3SMI calculated from CT images is limited by cost, the need for extensive training, limited availability and due to the risk of radiation exposure. Clinical researchers have suggested the inclusion of a measure of sarcopenia in established prognostic models for patients with liver disease. A dependable and reproducible method with wider availability is therefore needed.

**Research objectives**

This study aimed to examine tongue thickness measured using ultrasonography as a dependable bedside tool for the diagnosis of sarcopenia. Significant differences were seen in tongue thickness between healthy individuals and individuals with less severe liver disease compared to patients with more severe chronic liver disease.

**Research methods**

Patients with chronic liver disease and healthy individuals who satisfied the inclusion criteria underwent tongue thickness measurement using ultrasonography. The study was observational in nature and no intervention was planned on the basis of observations made. Tongue thickness measurements were compared between healthy individuals and patients with liver disease of different severity. The imaging technique used was ultrasonography, which has wider availability and does not involve radiation exposure unlike CT scanning used to measure L3SMI.

**Research results**

Significant differences were seen in tongue thickness between healthy subjects and patients with less severe liver disease compared to patients with more severe liver disease. Tongue thickness measured using ultrasonography is therefore proposed as a bedside measure of sarcopenia. However, its application requires further validation in studies involving subjects of different ethnicity, in health and in disease.

**Research conclusions**

This study established consistent and significantly reduced tongue thickness in patients with severe liver disease compared to healthy individuals and patients with less severe liver disease. Tongue thickness measured using ultrasonography may therefore be used as a bedside tool for the diagnosis of sarcopenia, an application with wide availability and no risk of radiation exposure compared to CT-based measurement of L3SMI.

**Research perspectives**

The findings in this study require validation in a similar study of tongue thickness using ultrasonography in people of different ethnicity in health and in disease.

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