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RESEARCH

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Comparative study between ultrasound and MR enterography in evaluation of Crohn's disease

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Abstract

Background Crohn's disease is a chronic inflammatory bowel disease that can affect any part of the gut. Endoscopy is the gold standard for diagnosis, but it only assesses mucosal lesions. Magnetic resonance enterography (MRE) can assess disease presence and activity, but it has limitations such as motion sensitivity, long scan time, and high cost. Bowel sonography has been introduced as a non-invasive, practical, safe, and low-cost technique to assess disease activity and complications. In our study we aim to assess the comparability of ultrasound to MRE in evaluation of patients with Crohn's disease, and its complications.

Results Twenty-five patients with 38 Crohn's disease affected segments were evaluated by bowel ultrasound (BUS) and MRI enterography (MRE), where BUS and MRE showed equivalent diagnostic performance for disease detection and localization (97.4%, 100%), for sensitivity and specificity of both modalities. Peri-mural fluid (89.5%, 94.4%) and mural stratification loss (100%, 100%) showed high sensitivity and specificity by BUS compared to MRE, while for assessment of mural vascularity, BUS showed high sensitivity and specificity for high grade vascularity (100%, 83.3%), but low sensitivity and high specificity for low and moderate vascularity (0%–62.5%, 81.8%) compared to MRE. Complications including fistulae and abscessed were all correctly identified in BUS compared to MRE.

Conclusion BUS showed comparable results to MRE for identification, localization, assessment of findings related to disease activity, and complications in cases of Crohn's disease rendering it a viable alternative to MRE.

Keywords Intestinal ultrasound, Bowel ultrasound, Magnetic resonance enterography, MRE, Crohn's disease, IUS, BUS

Background

Crohn's disease (CD) is a chronic transmural inflammatory bowel disease (IBD) which can affect any part of the gut with predilection to the terminal ileum and ascending colon. The inflammation is characteristic for its asymmetrical, transmural nature with segmental skipping. Also, complications like fistulas, abscesses, intestinal stricture with pre-stenotic dilatation are common [1].

Since CD is a chronic condition with periods of remissions and exacerbations, it is essential to assess disease activity via safe, cheap, and widely available method [2].

Currently, the gold standard for diagnosing CD is endoscopy, despite being invasive, and limited for assessing mucosal lesions lacking extramural evaluation [3]. This requires the additional use of magnetic resonance enterography (MRE), which can identify disease presence, and location through presence of mucosal thickening, mural edema, enhancement, comb sign, perimural edema, and local lymphadenopathy; also it can identify

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complications like abscess, fistulous tracts, and strictures [4].

However, MRE has many limitations including motion sensitivity, long scan time, extensive preprocedural patient preparation, use of oral contrast material, intravenous contrast administration besides the high cost [4].

This calls for development of a robust, cheap, safe, and readily available imaging tool; hence, bowel sonography was introduced as a non-invasive and practical technique to assess CD activity and complication [5], with the main advantages being the wide availability, low cost, lack of contrast administration, ionizing radiation nor patient preparation, as compared to MRE. The aim of this study is to assess the comparability of transabdominal bowel ultrasound to MRE in evaluation of Crohn's disease including activity and its complications.

Methods

The study was conducted as a comparative observational prospective study, after acquiring ethical approval from the institutional review board (IRB) of the Faculty of Medicine of Ain Shams University (code FMASU MD 191/2022).

The study initially enrolled 31 potential patients with Crohn's disease recruited from the IBD clinic of our faculty, proved by combined endoscopic, clinical, biochemical, and pathological assessment, either complaining of recent onset of diagnosis, presence of disease activity with complications or for following up of response to treatment, over a period of 24 months. Patients unable to perform MRE including those with reduced eGFR (< 30 ml/min/1.73 m^2), suffering from claustrophobia, or unable to withstand MR imaging were excluded from the study. However, 6 patients were subsequently excluded from the study. The reasons for exclusion were as follows: 2 patients had not undergone recent ileocolonoscopy, 3 patients were deemed unsuitable for magnetic resonance enterography (MRE) assessment due to inadequate preparation, and 1 patient had undergone MRE more than 2 weeks post-ultrasound, resulting in a total of 25 patients included in the study (Fig. 1).

Informed consent was obtained from all subjects prior to the study procedures including the ultrasound and MRE, after fully explaining the study procedures to all patients.

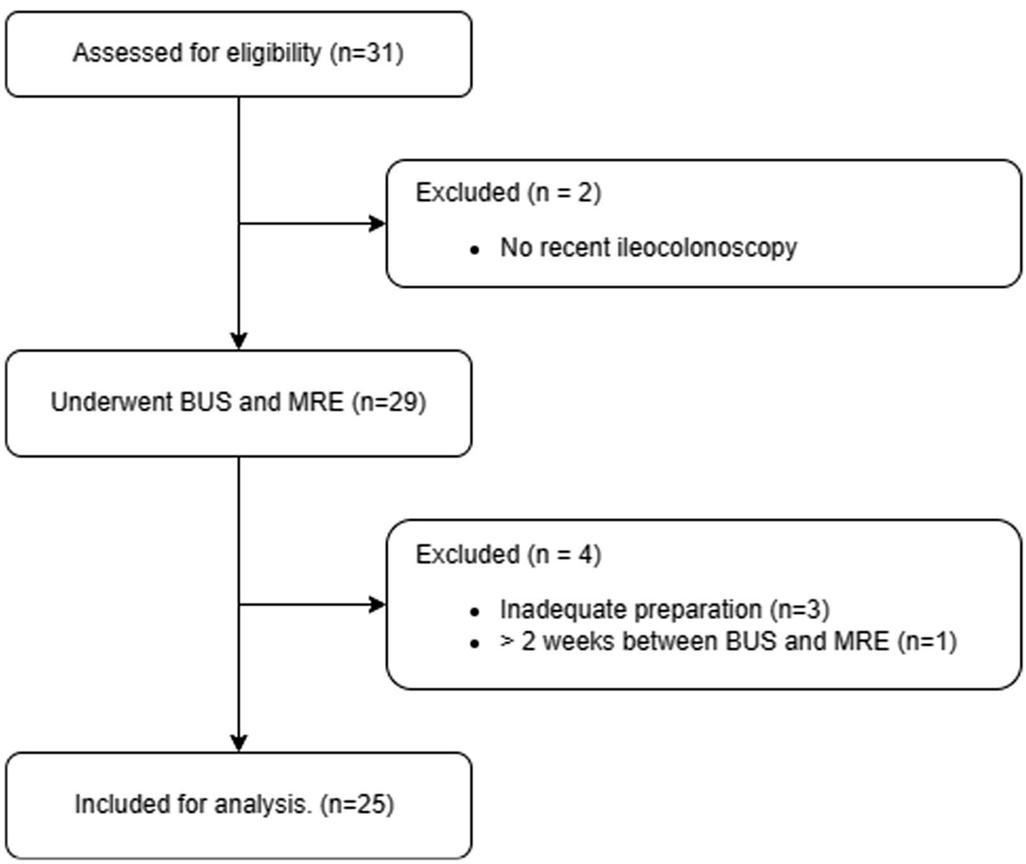

Fig. 1 Diagrammatic presentation for patients' enrollment in the study

History taking, full clinical, and laboratory assessment of all study subjects were carried out prior to enrollment by an experienced gastroenterologist, followed by detailed explanation of the imaging procedures including the ultrasound and MR scans. For each patient, transabdominal sonographic assessment was performed initially followed by the MRE procedure within a period of 2 weeks.

Bowel ultrasound (BUS)

Prior to the scan, all 25 patients were instructed to fast for 6 h, with no additional required preprocedural preparation. Sonographic examination was performed by an expert radiologist with an experience of 16 years in ultrasound imaging, using an ultrasound machine Logic P9 (GE healthcare medical system, USA). Initial scanning was carried by low-frequency (3–7 MHz) curvilinear probe for general bowel survey, and examination of deep structures, using "lawn mowing" technique starting at the right iliac fossa and ending at the left iliac fossa, followed by linear high frequency (4.5–13 MHz) 12L-RS probe scanning for focused imaging of diseased segments. Procedure duration was 17–30 min [median (IQR) = 21(7) min].

Magnetic resonance enterography (MRE)

All enrolled patients underwent MRE, after preprocedural preparation including low residue diet for 24 h and fasting for 6 h prior to scan. All patients were instructed to ingest an osmotically active oral contrast of 115 gm polyethylene glycol (Macrogol 3350 MW—Prepawest ®) dissolved in 1500 ml water, divided over 150 ml doses (150 ml/10 min).

Scanning was performed using a 1.5 T MRI (Philips Achieva scanner, Healthcare, Netherlands) with a body phased array coil according to the examination protocol in our institute. Sequences included; axial TSE T2 weighted image (section thickness, 6 mm; TR msec/TE msec, 583/80; field of view, 420 mm), axial SPIR T2 weighted image (section thickness, 6 mm; TR msec/TE msec, 1000/80; field of view, 420 mm), coronal TSE T2 weighted image (section thickness, 6 mm; TR msec/TE msec, 700/80; field of view, 288 mm), axial diffusion weighted images (echo-planar imaging; *b* values, 200, 400, and 800 s/mm^2; section thickness, 6 mm; TR msec/TE msec, 1700/73; field of view, 420 mm), pre-contrast axial T1 weighted image (section thickness, 6 mm; TR msec/TE msec, 454/46; field of view, 420), and post-contrast axial fat-saturated T1 THRIVE acquiring enteric 40 s, and delayed 180 s phases after injection of 0.1 mmol/kg of IV injected gadolinium contrast (section thickness, 6 mm; TR msec/TE scanning performed using a 1.5 T MRI (Philips Achieva scanner, Healthcare, Netherlands) with a body phased array coil).

Image interpretation

For BUS, each diseased segment was identified (mural thickening > 3 mm), localized anatomically, thickness measured for all segments, and length measured for ileal disease, also mural stratification (preserved or lost), presence of mural penetration/fistula, degree of mural vascularity using Limberg scoring system into grades 1 (no vascular signal), grade 2 (spotty mural signal), grade 3 (linear mural signal), and grade 4 (mural and peri-mural signal), peri-mural fluid, and localized collections or abscesses (presence and size) were all performed.

For MRE, images were surveyed and analyzed by a specialized abdominal imaging radiologist with an experience of 8 years in abdominal imaging blinded to the BUS results for the corresponding ultrasound parameters, including disease presence (mural thickening), localization, measuring mural thickness and length for diseased small bowel segments, assessment of mural stratification, peri-mural edema/fluid, localized collections or abscess on T2 SPIR sequence, mural vascularity assessed via mural enhancement on the post-contrast T1WI and graded into grade 1 (similar to normal bowel), grade 2 (slightly higher but significantly below vascular signal), grade 3 (significantly higher than bowel but below vascular signal), and grade 4 (approaching vascular signal), locoregional LNs.

Data management and analysis

The collected data for the bowel sonography and MRE were revised, coded, tabulated, and analyzed using Statistical Package for Social Science (SPSS, Chicago, IL) version 23. Quantitative data were represented by the mean and standard deviation (SD) for normally distributed data and by the median and interquartile range (IQR) for non-normally distributed data. Comparative analysis was done using Pearson's correlation for continuous variables and cross-tabulation for ordinal variables calculating the diagnostic indices. Measurement for agreement was taken using the Cohen's Kappa coefficient, with values 0.21–0.40 as fair, 0.41– 0.60 as moderate, 0.61–0.80 as substantial, and 0.81–1.00 as almost perfect agreement.

Results

Our study was carried out as an observational prospective study, on a total of 25 subjects (12 males, 13 females) with a mean age (± SD) of 25.8 (± 6.1) years and an age range of 23 years (17–40 years), where a total of 38 diseased segments were identified, localized, and assessed for disease severity by ileocolonoscopy (patient demographics summarized in Table 1).

Table 1 Demographics of patients included in the study

Gender	Female	Male	Total
Number	13	12	25
Age			
Mean	25	26.7	25.8
SD	6.6	5.6	6.1
Range	7–40		
Disease location by ileocolonoscopy			
Terminal ileum	7	9	16
Distal ileum	5	7	12
Ascending colon	0	1	1
Transverse colon	2	1	3
Descending colon	2	1	3
Sigmoid Colon	1	2	3
Total	17	12	38
Disease severity by ileocolonoscopy			
Mild	4	2	6
Moderate	9	15	24
Severe	4	4	8

For comparative analysis, ileocolonoscopy was utilized as the reference standard for identification of disease presence and localization of diseased segments compared to both BUS and MRE results, whereas MRE was utilized as the reference standard for measurement of length of diseased segments, transmural disease characteristics (mural thickness, stratification, vascularity, peri-mural fluid), and assessment of complications (fistulae and collections).

Disease presence and localization

For all patients, a total of 38 diseased segments were identified by ileocolonoscopy, the most frequent of which was the terminal ileum ($n=16$), followed by the distal ileum ($n=12$), while the least frequent was the ascending colon ($n=1$).

MRE successfully detected a total of 37 out of the 38 diseased segments on ileocolonoscopy, of which the terminal ileum was the most frequently affected ($n=15$), followed by the distal ileum ($n=12$). On the other hand, BUS successfully identified 37 of the 38 diseased segments, with the terminal ileum being the most frequently affected ($n=16$), followed by the distal ileum ($n=11$).

Comparative analysis of BUS and MRE to ileocolonoscopy for disease detection and localization, both showed the same diagnostic accuracy with a sensitivity, specificity, and overall accuracy of 97.4%, 100%, and 97.4%, respectively, while both MRE and BUS showed almost perfect agreement for the disease location ($\kappa=0.927$, $p<0.001$) (disease localization data summarized in Table 2).

Table 2 Frequency distribution of the localization of the affected bowel segments in the study group

		Ileocolonoscopy	BUS	MRE
Diseased segment	Terminal ileum	16	16	15
	Distal ileum	12	11	12
	Ascending colon	1	1	1
	Transverse colon	3	3	3
	Descending colon	3	3	3
	Sigmoid colon	3	3	3
	Total number of segments	38	37	37
	Ileocolonoscopy and BUS	**Ileocolonoscopy and MRE**	**BUS and MRE**	
Correlation	$\chi^2=190$ ($p<0.01$) $r=0.981$ ($p<0.01$)	$\chi^2=190$ ($p<0.01$) $r=0.995$ ($p<0.01$)	$\chi^2=190$ ($p<0.01$) $r=0.976$ ($p<0.01$)	
Agreement	$\kappa=0.963$ ($p<0.01$)	$\kappa=0.963$ ($p<0.01$)	$\kappa=0.927$ ($p<0.01$)	
Sensitivity	97.4%	97.4%		
Specificity	100%	100%		
Accuracy	97.4%	97.4%		

Diseased segment length, mural characteristics, and complications

On MRE, the mean (± SD) mural thickness for all segments was 5.4 (± 2.2) mm, with the length of the ileal affected segments being 14.9 (± 11.7), whereas for BUS, the mean (± SD) mural thickness of all affected segments was 5.4 (± 1.8) mm, with the length of the ileal affected segments being 13.8 (± 10.7) cm.

Regarding MRE mural characteristics, 31 segments showed lost mural stratification, 12 segments showed hypervascularity approaching vascular signal, 3 segments with faint enhancement, and 8 segments showing moderate enhancement, and peri-mural fluid was detected in 19 segments. Seven abscesses were detected with a median (IQR) volume of 9.8 (58) ml, and 9 fistulae were detected with the most frequent being enteroenteric ($n=4$).

On the other hand, for BUS, 31 segments showed lost mural stratification, 16 segments showed marked (grade 4) hypervascularity, 8 moderate (grade 3), and 6 mild (grade 2) hypervascularity using Limberg Doppler scoring system, and peri-mural fluid rim was identified for 18 segments. BUS detected 7 abscesses, with a median (IQR) volume of 8 (56) ml and 9 fistulae, of which enteroenteric was the most frequent ($n=4$). (The distribution of the diseased segment thickness, length and mural characteristics, for MRE and BUS is summarized in Table 3.)

Comparative analysis of BUS to MRE, in terms of the characteristics of the diseased segments and complications, showed excellent correlation between BUS and MRE for mural thickness ($r=0.96$, $p<0.001$) and good correlation for the length of the affected ileal segments ($r=0.81$, $p<0.001$).

Table 3 Frequency distribution of the characteristics of the affected bowel segments in the study group

		BUS	MRE	Correlation
Mural thickness (mm)—mean (± SD)	Terminal ileum	5.1 (± 1.9)	5.2 (± 2.1)	$r=0.8$ ($p<0.01$)
	Distal ileum	5.3 (± 0.85)	4.9 (± 1.1)	
	Ascending colon	12	14	
	Transverse colon	6.3 (± 1.6)	6.1 (± 2)	
	Descending colon	5.1 (± 1.7)	5.4 (± 1.7)	
	Sigmoid colon	4.4 (± 1.6)	4.7 (± 1.6)	
	All segments	5.4 (± 1.9)	5.4 (± 2.2)	
Diseased segment length (cm)—mean (± SD)	Terminal ileum	7.3 (± 3.1)	7.6 (± 3.2)	$r=0.96$ ($p<0.01$)
	Distal ileum	23.5 (± 10.7)	24.2 (± 11.9)	
	All segments	13.8 (± 10.8)	14.9 (± 11.7)	
Mural stratification pattern	Preserved	6	6	$\chi^2=36$ ($p<0.01$) $r=1$ ($p<0.01$)
	Lost	31	31	
Perimural edema	Negative	19	19	$\chi^2=61.7$ ($p<0.01$) $r=0.91$ ($p<0.01$)
	Rim	14	15	
	Free fluid	4	3	
Mural vascularity	Grade 1	7	14	
	Grade 2	6	3	
	Grade 3	8	8	
	Grade 4	16	12	
Fistula formation	Not detected	28	28	
	Enteroenteric	4	4	
	Enterocolic	1	1	
	Enterocutaneous	3	3	
	Complex	1	1	
	Total detected	9	9	
Localized collections/Abscess	Present	7	7	
	Absent	30	30	
	Volume in ml—median (IQR)	8 (55.2)	9.8 (58)	$r=0.92$ ($p=0.004$)

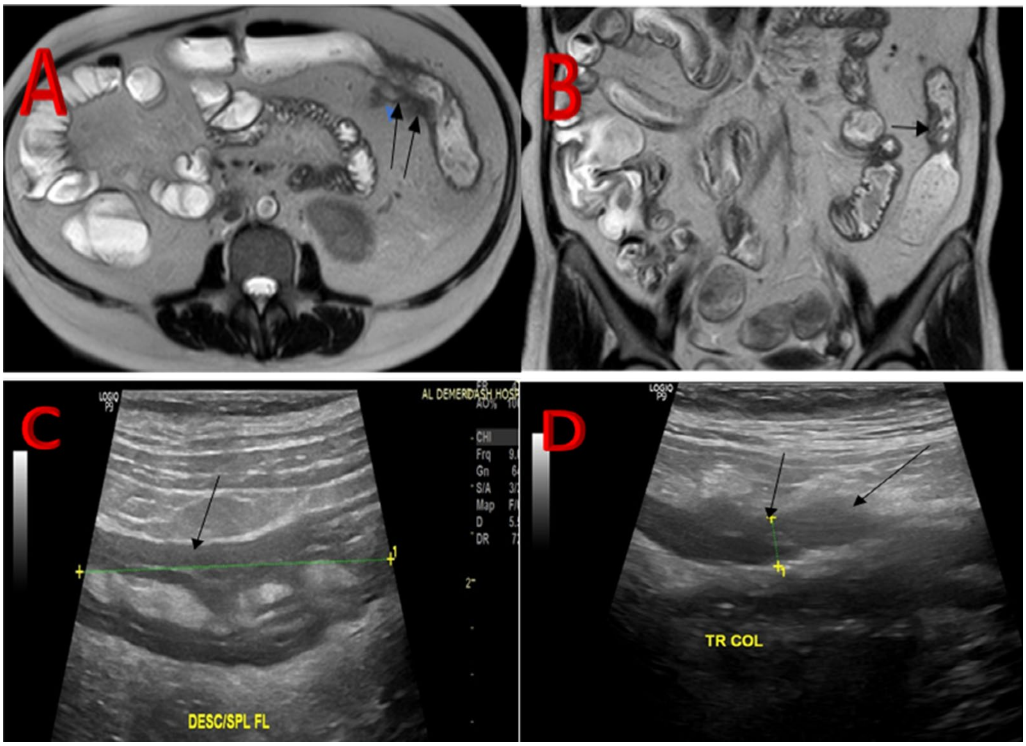

Fig. 2 A 25-year-old female diagnosed with colonic Crohn's disease presented by left-sided lower abdominal pain; MRE T2 axial & coronal images (**A**, **B**) and BUS images (**C**, **D**) show increased mural thickness of the distal transverse colon and the proximal descending colon with loss of wall stratification (black arrows)

Peri-mural fluid was identified by BUS in 18 out of the 19 segments detected by MRE, resulting in a sensitivity, specificity, and accuracy of 89.5%, 94.4%, and 89.5%, respectively. Furthermore, mural stratification loss was correctly identified by BUS in all 30 out of the 37 diseased segments when compared to MRE, with sensitivity, specificity, and accuracy of 100%.

Regarding mural vascularity, BUS showed relatively low to intermediate sensitivity figures for grade 1, 2, and 3 Limberg scores compared to the corresponding MRE vascular grading, with sensitivities of 46.5%, 0%, and 62.5% for grades 1, 2, and 3 Limberg scores, respectively, and specificity ranging from 81.8 to 100%. However, grade 4 Limberg score by US showed high sensitivity and specificity compared to the corresponding vascular grading by MRE (sensitivity, specificity, and accuracy = 100%, 83.3%, and 100%, respectively) (Figs. 2, 3, 4, 5, 6).

Regarding complications, all abscesses, fistulae, and their types detected by MRE were correctly identified by BUS, with 100% sensitivity, specificity, and accuracy. Furthermore, the size of localized collections or abscesses detected by BUS showed excellent correlation ($r = 0.915$, $p = 0.004$). Diagnostic performance of BUS compared to MRE is summarized in Table 4.

Discussion

In the present study, 25 patients with a total of 38 segments were evaluated using ileocolonoscopy, MRE, and BUS. Considering disease presence and localization in the current study, comparative analysis of MRE and BUS with endoscopy showed that both imaging modalities demonstrated equivalent sensitivity, specificity, and overall accuracy (97.4%, 100%, and 97.4%, respectively) to ileocolonoscopic findings. Such results align with the systematic review by Panes et al. [6], who reported a pooled sensitivity and specificity of 84–88% and 73–92% for BUS compared to endoscopy, respectively, and the METRIC study conducted by Taylor et al. [7] who reported comparable sensitivity for BUS (92%) and MRE (97%) relative to endoscopy, with marginally higher specificity values for MRE (96%) compared to BUS (84%). Additionally, a more recent meta-analysis, by Lee et al. [8], reported pooled sensitivity and

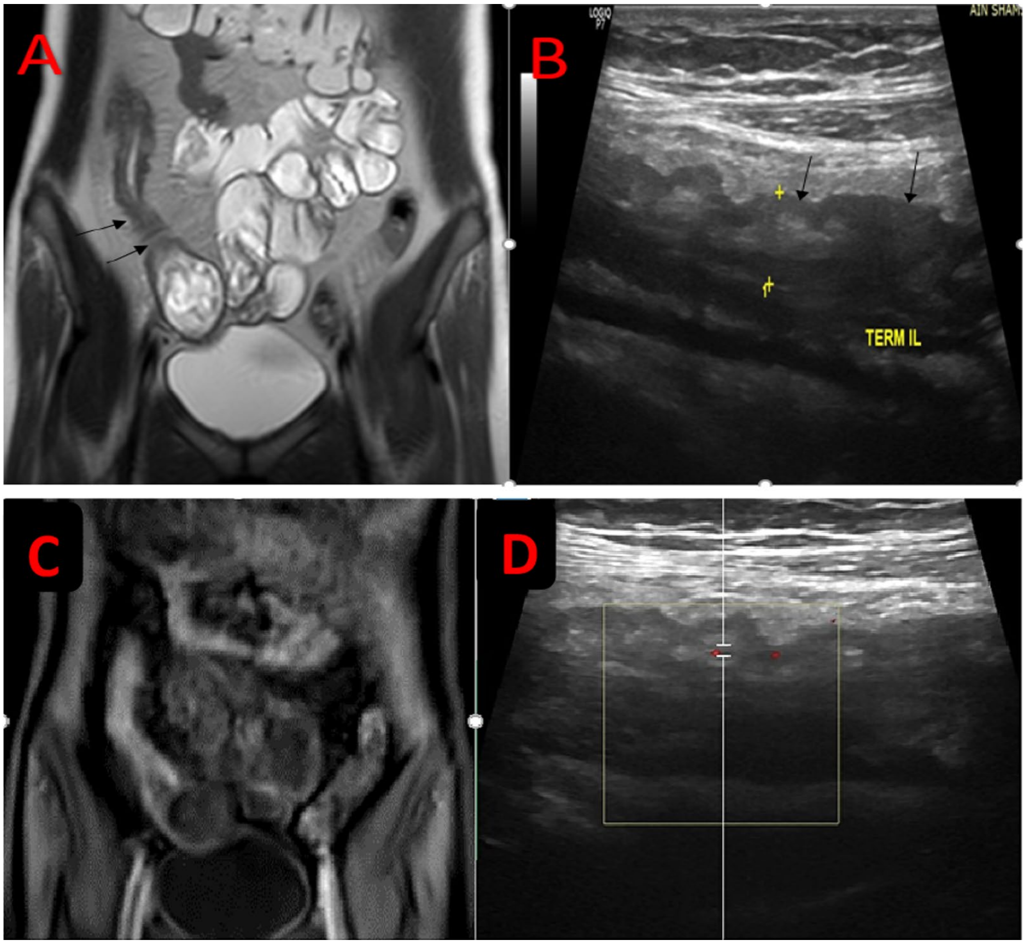

Fig. 3 A 20-year-old female patient diagnosed with ileal Crohn's disease. MRE T2 coronal image (**A**) and BUS image (**B**) show increased mural thickness of the terminal ileum with areas of mural stratification loss (black arrows). MRE T1 post-contrast image (**C**) shows mild mural enhancement of the affected terminal ileum, and BUS image with color Doppler (**D**) shows mild increased wall vascularity (Limberg 2)

specificity for BUS of 86% and 88%, respectively, compared to 88% and 87% for MRE. Despite the near-identical numbers for BUS and MRE in all referenced studies, their relatively lower sensitivity and specificity figures compared to the current study can be partially explained by using per-patient comparisons in addition to per-segment comparisons in the meta-analyses, besides our smaller sample.

As regards measured bowel wall thickness and ileal disease extent, our data showed excellent correlation, between both BUS and MRE for both parameters ($r=0.8$, $p<0.01$—$r=0.96$, $p<0.01$ for mural thickness and ileal length, respectively). This contrasts the results by Taylor et al. [7] who demonstrated lower sensitivity figures (70%) in BUS compared to MRE (80%), with a 10% difference between both modalities. This can be attributed to our approach using quantified thickness and length by both modalities and then conducting direct correlation opposed to qualitative visual assessment of diseased segments applied by the mentioned study.

For assessment of disease activity, mural characteristics, and complications, the estimation of inflammatory activity in Crohn's disease through increased mural vascularity has been established as an indicator for disease activity besides mural thickening. In our study, we employed Doppler ultrasound using Limberg scoring system to evaluate mural vascularity, which showed low sensitivity but high specificity figures for mild and moderate vascular grades (1–3) compared to their corresponding

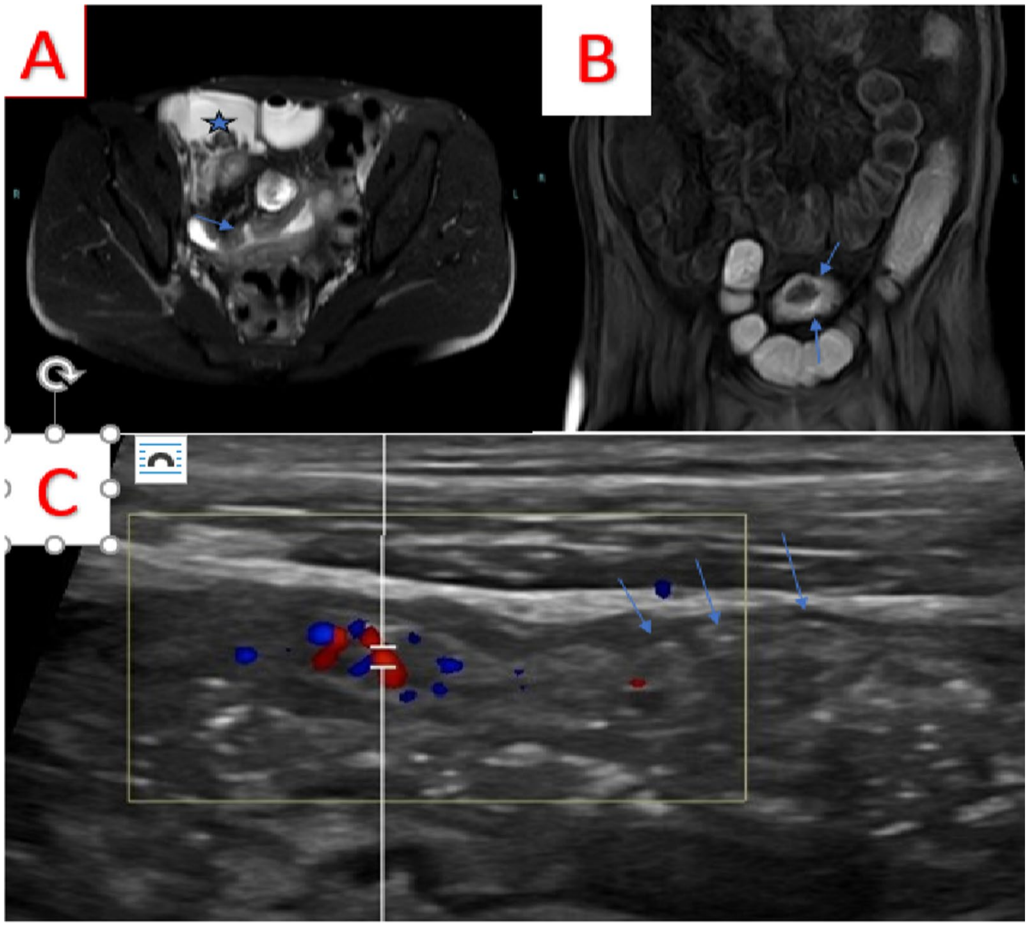

Fig. 4 A 29-year-old male, diagnosed with ileal Crohn's disease, presented with abdominal pain and constipation, **A** MRE axial T2 image shows mild free fluid (star), circumferential mural thickening of the distal ileum, and areas of mural stratification loss, **B** MRE coronal T1 post-contrast image shows marked mural enhancement of the affected segment. **C** BUS with color Doppler of the distal ileum shows increased mural thickness with increased wall vascularity (Limberg 3)

degrees of enhancement on MRE. However, marked hypervascularity (Limberg grade 4) showed high sensitivity, specificity, and overall accuracy (100%, 83.3%, and 88.9%, respectively), compared to its corresponding contrast enhancement degree on MRE.

Our results agree with Allocca et al. [9] and Moraes et al. [10], who showed high specificity (92–100%) for bowel vascularity compared to contrast-enhanced MRI. However, our varying results for sensitivity (46–62% for mild—moderate, and 100% for marked vascularity) compared to 81.8–87% in their study could be attributed to our implementation of Limberg Doppler grading, comparing it to the corresponding MRI enhancement, while they resorted to estimation of positive Doppler vascular signal correlating it to presence of MR mural enhancement.

Besides mural vascularity, another indicator of presence inflammatory activity in CD is the loss of mural stratification. This was established in a recent study by Bhatnagar et al. [5] who compared mural stratification loss on BUS and pathological evaluation of surgical specimens, showing highly significant correlation between loss of mural stratification and degree of inflammation using the histological acute inflammation scoring (AIS), highlighting its validity an indicator of inflammatory activity. On that basis, regarding mural stratification loss in our study, BUS showed high specificity, sensitivity, and overall accuracy for detection of mural stratification loss (100% for all) and presence of perimural fluid (89.5%, 94.4%, and 89.5%, respectively) compared to MRE.

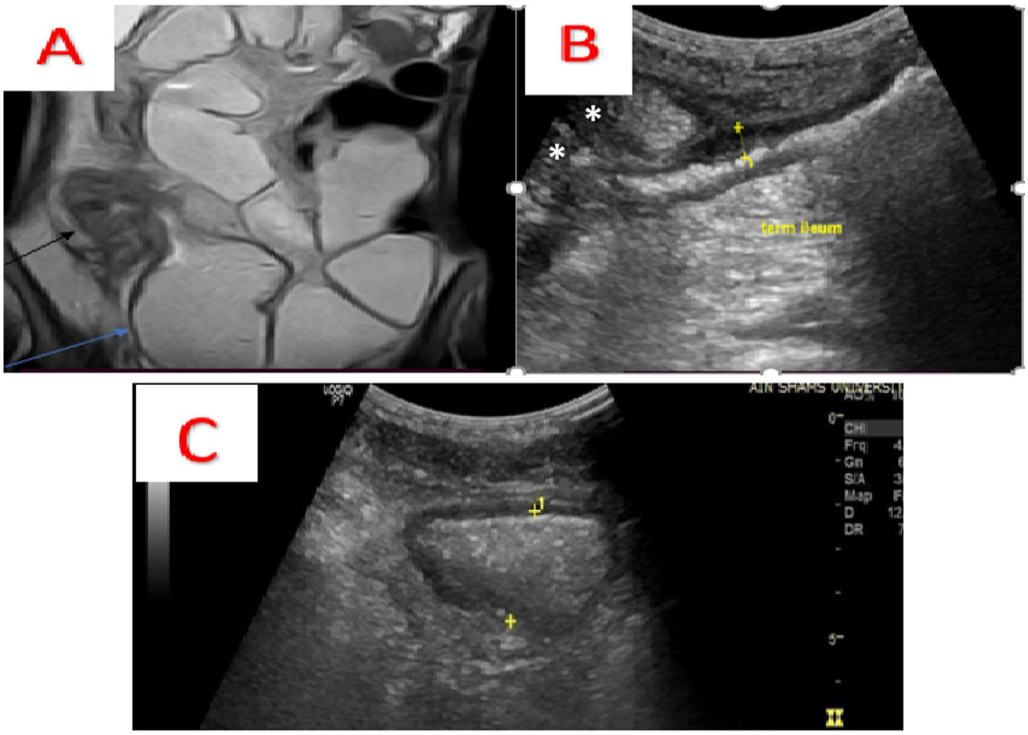

Fig. 5 A 19-year-old male patient known with ileal Crohn's disease presented with right iliac pain, constipation, and abdominal distension. **A** MRE T2 coronal image shows increased mural thickness of the terminal ileum with loss of stratification (black arrow) complicated by ileal stenosis and pre-stenotic dilatation (blue arrow). **B** BUS shows increased mural thickness of the terminal ileum with loss of stratification(*). **C** BUS image shows pre-stenotic dilatation

This was explored by Yuksel et al. [11] who showed a relatively higher sensitivity of MRE versus BUS (34.5%, 16%, respectively) compared to pathological results for estimation of mural stratification loss but lower specificity (89%, 98%, respectively), with the same overall diagnostic accuracy for both modalities (71.4% for both); however, the discrepancy between their results and the current study could be attributed to the fact that they included patients with remission (inactive) status and active disease comparing BUS and MRE to disease activity according to pathology as reference, whereas our study included only patients with active disease comparing BUS to MRE as reference.

Finally, regarding assessment of CD complications, we found equivalent results for both BUS and MRE for detection of abscesses, and fistulae with 100% sensitivity, specificity, and accuracy of BUS compared to MRE as reference; moreover, BUS showed excellent correlation with MRE regarding the size of the abscess ($r=0.915$, $p=0.004$). Our findings agree with the study by Allocca et al. [9] and Imsirovic et al. [12], who showed 100% sensitivity and 96% specificity for BUS compared against MRE, with no significant difference between both modalities regarding abscess detection.

However, the results of the current study are limited by the small sample size and also by lack of patients with non-active disease (CD in remission), hence, future research utilizing larger sample sizes and patients in remission to further validate such findings.

Conclusion

In this preliminary study, we found that BUS demonstrated comparable results to MRE in the identification, localization, and assessment of findings related to disease activity and complications. The results suggest that BUS may serve as a viable alternative to MRE in the diagnosis and management of Crohn's disease. The importance of these findings lies in the potential for BUS to provide a non-invasive, radiation free, and more cost-effective diagnostic tool for patients with Crohn's disease,

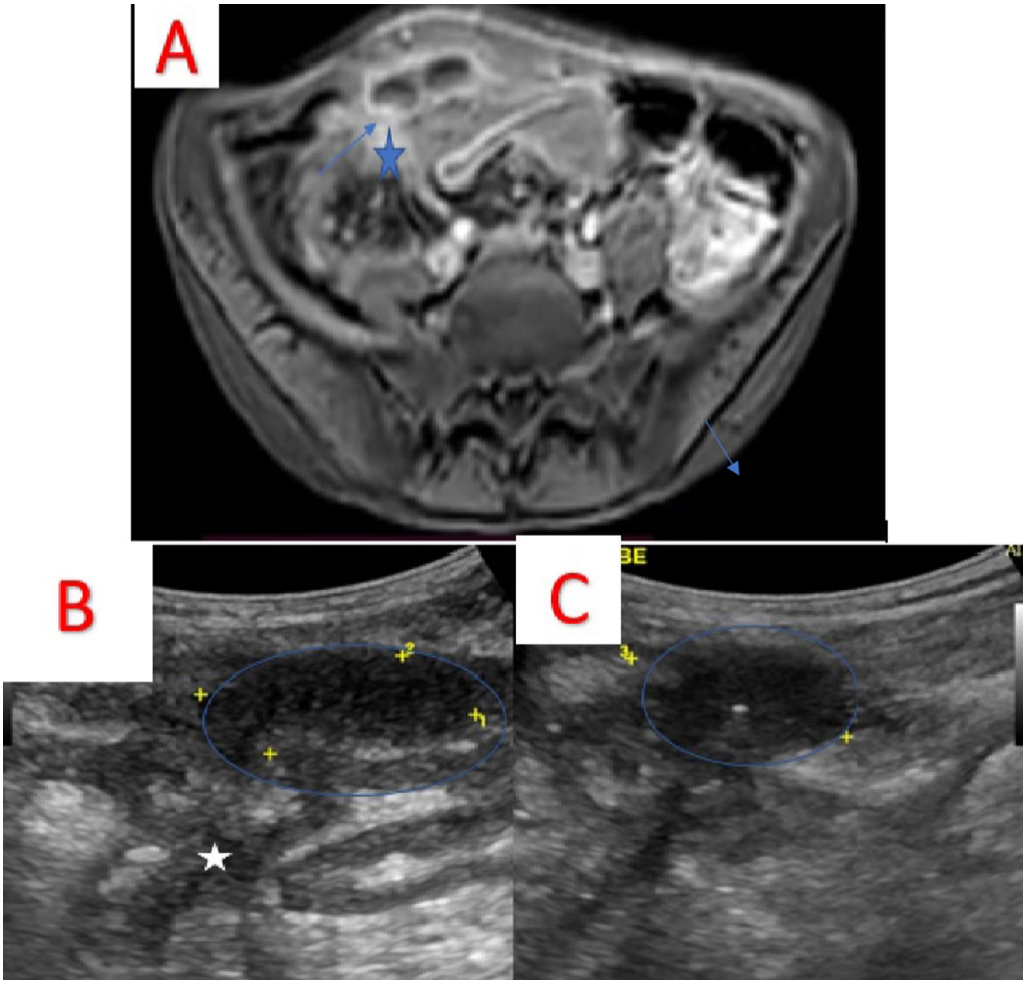

Fig. 6 A 31-year-old male patient with ileocolonic Crohn's disease, **A** MRE axial post-contrast T1 image shows localized abscess with marginal enhancement near anterior abdominal wall (arrow) with underlying complex entero-enteric fistula (star). **B** and **C** BUS images show localized fluid collection with internal echoes (circle) and air foci inside (arrow) near anterior abdominal wall related to the diseased ileal segment (star) denoting abscess formation

Table 4 Diagnostic performance indices of BUS compared to MRE

BUS parameter	Sensitivity (%)	Specificity (%)	Accuracy (%)
Perimural fluid	89.5	94.4	89.5
Mural stratification loss	100	100	100
Mural vascularity			
Limberg 1	46.5	100	80.5
Limberg 2	0	81.8	75
Limberg 3	62.5	89.3	83.3
Limberg 4	100	83.3	88.9
Fistula	100	100	100
Abscess	100	100	100

Abbreviations

MRE Magnetic resonance enterography
BUS Bowel ultrasound
IBD Inflammatory bowel disease
CD Crohn's disease
AIS Histological acute inflammatory score

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Author contributions

NM, MA contributed to the idea and data collection. NM, EA, MA contributed to organization of work. SG, KS contributed to writing and revision. KH contributed to the medical evaluation and providing endoscopic data. EA contributed to the statistical analysis and author correspondence. All authors have read and approved the manuscript.

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Availability of data and materials
Available on request with the corresponding author.

Declarations

Ethics approval and consent to participate
Ethics approvals were acquired from our institute ethical committee (Faculty of Medicine—Ain Shams University) under the code FMASU MD 191/2022.

Consent for publication
A written consent to publish this information was obtained from study participants.

Competing interests
The authors declare that they have no competing interests.

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