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MR enterography in small bowel diseases, adding multipoint Dixon sequence, is it worth?

Alaa Mohamed Reda^{1*} , Rasha Aly Saleh¹, Mahmoud Abdelhamid Elgawish¹ and Ahmed Elsharkawy¹

Abstract

Background MR enterography (MRE) has been increased in the last decade as a modality of choice in diagnosis and evaluation of small bowel diseases in both children and adults. Lacking ionizing radiation, non-invasiveness, excellent soft tissue resolution, adequate luminal distension of small bowel loops are the advantages of this technique. Acquisition of images was used to be: T2WI, steady-state free precession, and T1WI, fat-suppressed gadolinium contrast-enhanced sequences. Multipoint Dixon sequence was added recently to the routine MR enterography protocol. The current study aimed to evaluate the added value of multipoint Dixon sequence as a valuable modification of MR enterography protocol for better assessment of small bowel lesions as activity of Crohn's disease, small bowel polyps, fibrotic strictures, with modification of MRE protocol in the future with less time consumption and better radiological evaluation.

Methods The current research was a prospective cross-sectional study. Data were collected prospectively after getting ethical approval from the ethical committee of the faculty of medicine, in our university.

Study population The sample size was 69 adult patients with suspected small bowel lesions, within the period from January 2022 to 30th December 2022. Written informed consent was obtained from all studied cases before the study, who were enrolled in this study. This number was calculated by using MedCalc 19 program by setting alpha error significance of 0.05%, 95% confidence level, and 80% power sample.

Results This study enrolled 69 patients with recurrent abdominal pain and/or bleeding in stool. The patients' age ranged from 18 to 50 years, with a mean of 32.6 ± 8.8 . There is slightly higher female prevalence. The most common positive imaging findings in the studied patients were mucosal thickening in terms of submucosal fat deposition (36.23% of the MRE examination and 39.13% of the MRE with added Dixon sequence). Adding Dixon sequence yielded significantly higher AUC (97.2% vs. 90.5%, $p = 0.047$), higher sensitivity (100% vs. 92.16%), specificity (94.4% vs. 88.89%), accuracy (98.55% vs. 91.3%), positive predictive value (98.08% vs. 95.92%), and negative predictive value (100% vs. 80%).

Conclusions MRE is an excellent imaging modality in the assessment of small bowel diseases without the use of ionizing radiation. Developing MR-based sequences as multi-point Dixon sequence have the potential to improve the ability of MRE to image the subtle changes as Crohn's accompanying early inflammatory changes and fibrosis, as well as small intestinal polyps. Familiarity with MR enterography is essential for radiologists and gastroenterologists who participate in the clinical management of small intestinal diseases.

Keywords MR enterography, Crohn's disease, Small bowel diseases, Polyps, Dixon sequence, Inflammatory bowel diseases (IBD), MRE

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Background

Small bowel diseases including inflammatory bowel disease (IBD), as Crohn's disease (CD), intestinal tumors as lymphoma, polyps are chronic debilitating diseases that currently lack to have a single gold standard [1]. Crohn's disease is a chronic relapsing disorder that affects the gastrointestinal tract (GIT), particularly at terminal ileal region in the form of ulceration, small polyps, submucosal edema [2]. Owing to occult location of small bowel lesions from both upper and lower GI endoscopic procedures, there are delay of diagnosis, clinical treatment, and worse complications [3]. Conventional contrast-enhanced MRI can detect small intestinal lesions as tumors, submucosal fat infiltration and edema of Crohn's disease, lipoma, lymphoma. However, small lesions could be missed in conventional MRI [4].

Lack of ionizing radiation, excellent soft tissue contrast resolution, non-invasiveness, proper intestinal distension by fluid are advantages of MR enterography as a mandatory recent imaging modality in diagnosis of small bowel diseases [5]. Typical MR pulse sequences used in MR enterography include single-shot T2WI, steady-state free precession (SSFP), T1WI, dynamic post-contrast T1WI with FAT SAT, diffusion weighted image (DWI) with high b-value 1000 sec/mm^2 , addition of fat suppression using multipoint Dixon sequence [6]. Dixon is a novel MRI sequence based on chemical shift and designed to achieve more uniform fat suppression. It has been increasing popularity as it has some advantages over other fat suppression techniques, namely: more uniform fat suppression and less affected by artifacts, can be combined with a variety of sequence types (e.g., spin echo, gradient echo, and steady-state free precession sequences), can be combined with a variety of weightings (e.g. T1, T2, and proton density), provides images with and without fat suppression from a single acquisition [7]. The Dixon technique exploits the fact that water and fat molecules precess at different rates [8]. Acquiring both in-phase and opposed-phase images simultaneously allows the images to be combined in two ways which result in a total of four sequences: in-phase = (water + fat), opposed-phase = (water - fat), fat only = in-phase - opposed phase = (water + fat) - (water - fat), water only = in-phase + opposed phase = (water + fat) + (water - fat). The water only image can be used as a fat-suppressed image [9].

The aim of this study was to evaluate the added value of multipoint Dixon sequence as a valuable modification of MR enterography protocol for better assessment of small bowel lesions as activity of Crohn's disease, small bowel polyps, fibrotic strictures, with modification of MRE protocol in the future with less time consumption (as it

replaces 4 sequences) with more uniform fat suppression and better radiological evaluation.

Methods

This prospective cross-sectional study was carried out in the period from 1st January 2022 to 30th December 2022 after local ethical committee approval in our institution. Written informed consent was obtained from all studied cases, who were enrolled in this study after explanation of the benefits of the examination. Privacy and confidentiality of all patients' data were guaranteed. All data provision was monitored and used for scientific purpose only.

A total of 69 patients were referred from the Internal Medicine Department to the Radiodiagnosis Department in our institution, with recurrent attacks of non-specific unexplained abdominal pain and/or blood in stool.

Full general and local abdominal examination was performed by a well-experienced gastroenterologist of 12 years of experience, and stool analysis and blood tests were performed.

Inclusion criteria

Patients who were referred with documented signs, symptoms, physical, and laboratory results that were suspicious for inflammatory bowel disease. Patients with recurrent unexplained nonspecific abdominal pain with/without bloody stool were included in this study, and who had negative ultrasound findings, were also enrolled in this study as suspected small bowel diseases. Pregnant females and children with suspected small intestinal lesions were included in the current study as well, owing to lack of ionizing radiation.

Exclusion criteria

Exclusion criteria include patients with abdominal pain caused by organic lesions that was already diagnosed by ultrasound or computed tomography, patients with end-stage kidney disease (GFR less than 30 ml/min), allergic to MR contrast material, as well as claustrophobic patients, and patients with MR noncompatible metallic devices, cardiac pacemakers, and cochlear implants.

Patient preparation

All cases underwent MRI at 1.5 Tesla MRI unit, GE (General Electric) machine (closed magnet). The patient removed metal pins and entered the machine in supine position.

Oral preparation before the procedure

Fasting for about 8–12 h prior to the examination was recommended; low-fiber diet was used for 3–5 days

prior to the procedure. Bowel cleansing is performed by enema, laxatives, avoidance of dairy products.

Bowel distention

Adequate small bowel distention was very essential for the assessment of bowel wall thickening as well as for mural enhancement. Polyethylene glycol 350 ml over 1300 ml water was preferred for proper bowel distension and meticulous mucosal assessment. Patients were advised to start drinking one hour prior to the examination (250 ml/10 min). No sedation is required. This was just reassurance for the patients.

MRI acquisition

All patients underwent MR enterography using a 1.5 T MR scanner: GE Signa Explorer, using phased array body coil. They were in a supine position. They were in a supine position with their feet were introduced first. 3-plane localizer: from the xiphisternum to symphysis pubis was done. Injection of I.V. anti-peristaltic agent (20 mg buscopan). Remove all metallic objects like pins, bra, credit cards, etc.

MRI sequences

- Axial and coronal: T1WI, T2-single-shot fast spin echo sequences (SSFSE) and diffusion-weighted sequences.
- I.V. Injection of 10 ml magnevist (0.1 mmol/kg BW)
- Coronal 3D T1-weighted gradient echo sequences with fat suppression.
- Post-contrast axial 3D T1-weighted gradient echo sequences
- Coronal post-contrast fat suppression by Dixon multipoint sequence
- 5 mm slice thickness and 0.5-mm interval gaps.

The data were reviewed on PACS (picture archiving and communication system). The radiological findings were correlated with clinical data and analyzed by two experienced radiologists with 10,11 years of experience in GIT imaging; the final decision was given by a third experienced consultant of GIT imaging who had 15 years of experience in advanced imaging field who gave his opinion in controversy opinions.

Each patient took a different ID number, and all patient data were blinded.

Statistical analysis

The SPSS for Windows version 28.0 software package (SPSS Inc, Chicago, IL, USA) was used for statistical data analysis. Categorical variables were described by frequency (number of patients), percentage, and proportion,

and quantitative variables were described by using mean, SD, and range. Quantitative data were compared by using the Student *t* test. *p* Value less than 0.05 represented a significant difference. ROC curve analysis was used to determine the optimum cut-off values and maximal area under curve (AUC) with evaluation of diagnostic accuracy, sensitivity, specificity, positive and negative predictive values (PPV and NPV, respectively) of standardized MR enterography and adding multi-point Dixon sequence for localization and early assessment of small bowel diseases.

Results

This study included 69 patients with recurrent abdominal pain and/or bleeding in stool. The patients' age ranged from 18 to 50 years, with a mean of 32.6 ± 8.8 . Slightly higher female prevalence was noted (56.5%), and the mean patients' BMI was 29.7 ± 5.4 kg/m². The patients complain duration ranged from 0.75 to 13 months with a median of 1.25. They experienced the abdominal pain daily (10.14%), 1–3 times weekly (46.38%), 1–3 times monthly (26.09%), or sporadic (17.39%) (Table 1).

MRE and MRE with added Dixon study demonstrated normal study in 27.54% and 24.64% of the patients, respectively. The most common positive imaging findings in the study patients were mucosal thickening in terms of submucosal fat deposition (36.23% of the MRE examination and 39.13% of the MRE with added Dixon examination) and submucosal edema (39.13% of the MRE examination and 43.48% of the MRE with added Dixon examination); similar results were obtained in cases of segmental small bowel dilatation (49.28%), and lymphadenopathy (23.19%) in both techniques as well. Other findings in order of frequency were perienteric edema, fibrofatty proliferation, vascular engorgement, ulcers, sinus tracts, neoplastic lesions, polyps/polypoid

Table 1 Clinical findings and data of the studied cases

		Study patients (N=69)
Age (year)	Mean \pm SD	32.6 \pm 8.8
Sex	Female	39 (56.5%)
	Male	30 (43.5%)
BMI (Kg/m ²)	Mean \pm SD	29.7 \pm 5.4
Disease duration (years)	Median (range)	1.2 (0.75–13)
Frequency	Daily	7 (10.14%)
	1–3 times weekly	32 (46.38%)
	1–3 times monthly	18 (26.09%)
	Sporadic	12 (17.39%)

N=number, %=percentage, SD=standard deviation

Table 2 Imaging findings in the studied cases using MRE versus MRE with Dixon

Imaging findings		MRE N (%)	MRE + Dixon N (%)
Mural thickening	Submucosal fat deposition	25 (36.23)	27 (39.13)
	Submucosal edema	27 (39.13)	30 (43.48)
Multiple polyps/polypoid mucosal thickening		2 (2.9)	3 (4.35)
Segmental small bowel dilatation		34 (49.28)	34 (49.28)
Lost jejunal folds		2 (2.9)	2 (2.9)
Sinus / fistulous tracts		2 (2.9)	5 (7.25)
Ulcers		7 (10.14)	9 (13.04)
Fibrofatty proliferation		12 (17.39)	15 (21.74)
Perienteric edema		13 (18.84)	14 (20.29)
lymphadenopathy		16 (23.19)	16 (23.19)
Vascular engorgement		8 (11.59)	8 (11.59)
Fat necrosis		2 (2.9)	3 (4.35)
Neoplastic lesions		3 (4.35)	4 (5.8)
Normal study		19 (27.54)	17 (24.64)

MRE: magnetic resonance enterography, N = number, % = percentage, SD = standard deviation

mucosal thickening, fat necrosis, and lost jejunal folds as described in Table 2.

According to the final diagnosis based on histopathological diagnosis (in 39 cases) and/or clinical and laboratory follow-up by the gastroenterology consultant (in 30 patients), most of the patients (35 cases; 50.72%) had Crohn's disease. Other diagnoses were small bowel neoplasms (5.8%, 2 cases of small lipomas, 1 case of adenoma, and 1 case of lymphoma), focal fat infarction (3 cases; 4.35%), celiac disease, small bowel tuberculosis, chronic appendicitis, non-specific peritoneal inflammation (2 cases each; 2.9%), and intestinal polyposis (1 case; 1.45%). No disease was encountered in 18 cases (26.09%). Two cases of false positive Crohn's disease diagnosis were found in the MRE examination, and one case was found in the MRE with Dixon examination based on false depiction of skipped mucosal ulcers. The endoscopic and laboratory investigations confirmed the disease absence. Four cases of missed diagnosis were experienced with MRE examination. Two of them were diagnosed to be early Crohn's disease: one case was found to be small mural lipoma and the remaining case was found to be peritoneal focal fatty infarction. All of them were correctly diagnosed by Dixon study as mentioned in Table 3.

The overall diagnostic performance of both studies is presented in Fig. 1 and Table 4. Adding Dixon study yielded significantly higher AUC (97.2% vs. 90.5%, $p=0.047$), higher sensitivity (100% vs. 92.16%), specificity (94.4% vs. 88.89%), accuracy (98.55% vs. 91.3%), positive predictive value (98.08% vs. 95.92%), and negative predictive value (100% vs. 80%) (Figs. 2, 3, 4).

Table 3 Final diagnosis of the studied patients

Final diagnosis: N (%)		MRE	MRE + Dixon
Crohn's disease: 35 (50.72%)	TP	33	35
	FP	2	1
	FN	2	0
Celiac disease: 2 (2.9%)	TP	2	2
	FP	0	0
	FN	0	0
Small bowel tuberculosis: 2 (2.9%)	TP	2	2
	FP	0	0
	FN	0	0
Focal fat infarction: 3 (4.35%)	TP	2	3
	FP	0	0
	FN	1	0
Chronic appendicitis: 2 (2.9%)	TP	2	2
	FP	0	0
	FN	0	0
Intestinal polyposis: 1 (1.45%)	TP	1	1
	FP	0	0
	FN	0	0
Small bowel neoplasm: 4 (5.8%)	TP	3	4
	FP	0	0
	FN	1	0
Non-specific peritoneal inflammation: 2 (2.9%)	TP	2	2
	FP	0	0
	FN	0	0
No disease: 18 (26.09%)	TN	15	17
	Total: 69 (100%)	TP	47
	FP	2	1
	FN	4	0
	TN	16	17

N = number, % = percentage, SD = standard deviation, MRE: magnetic resonance enterography, TP: true positive, FP: false positive, FN: false negative, TN: true negative

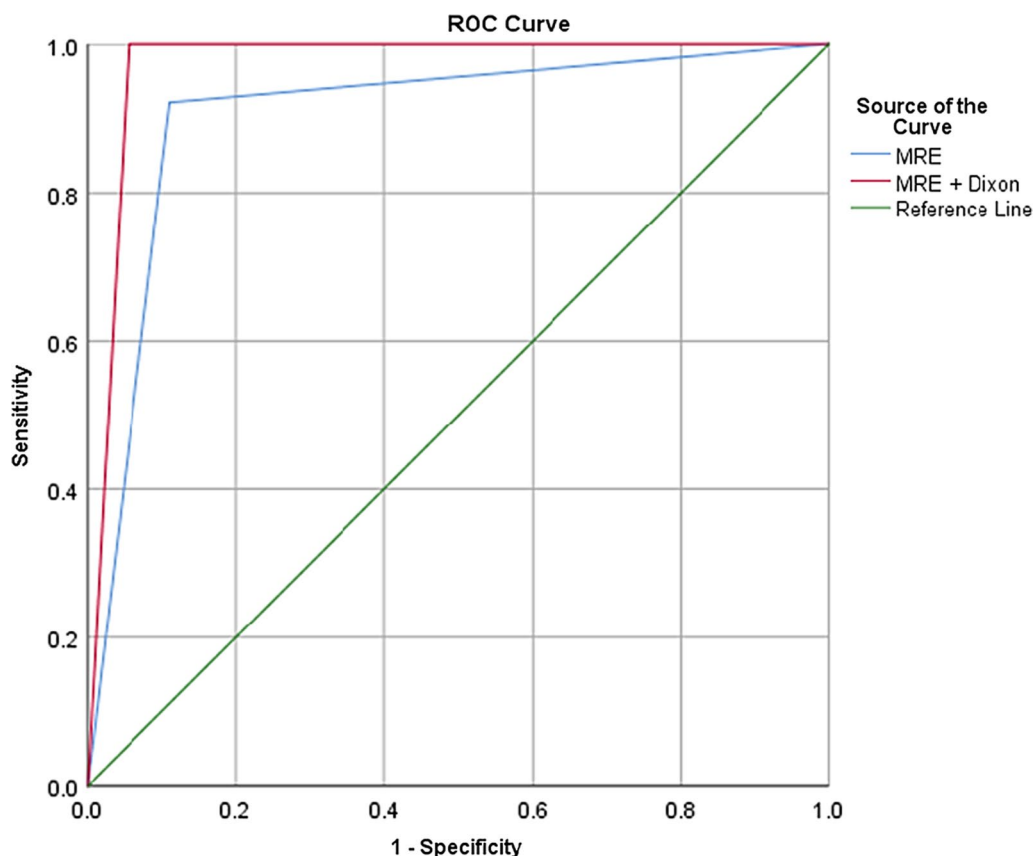


Fig. 1 ROC (receiver operating characteristic curve) shows the diagnostic performance and added value of MRE versus MRE with added Dixon sequence

Table 4 Diagnostic performance and added value of MRE versus MRE with added multi-point Dixon sequence

	MRE	MRE + Dixon
Area under curve (AUC)	90.5%	97.2%
95% confidence interval (CI)	81–100%	91–100%
Sensitivity	92.16%	100%
Specificity	88.89%	94.4%
Accuracy	91.3%	98.55%
Positive predictive value	95.92%	98.08%
Negative predictive value	80%	100%
AUC difference	6.7%	
<i>p</i>	0.047*	

N = number, % = percentage, MRE: magnetic resonance enterography, AUC: area under curve, CI: confidence interval

* means statistically significant difference (less than 0.05)

Discussion

The lack of ionizing radiation, the enhanced tissue contrast that can be obtained by using a variety of pulse sequences,

and the capability to perform real-time functional imaging are just a few of the characteristics of magnetic resonance (MR) imaging that make it well suited for imaging the small bowel [1]. When the distension of the loop is suboptimal, MR enterography (MRE), which offers numerous time points during which the small bowel is seen, can be very useful in diagnosing small-bowel disorders, which was a superadded value for diagnostic accuracy of MRE in early diagnosis and assessment of small bowel lesions [2].

Dixon sequence was first introduced in 1984 as a "simple proton spectroscopic imaging" technique to distinguish between fat and water signal using voxel intensity variations between in- and opposed-phase MR images [3]. This method has developed and been widely used by major MRI vendors during the past forty years [4].

Recent research has shown that the Dixon approach can be used to examine the fat fraction, analyze joint cartilage, study bone marrow, assess sacroiliitis, and reduce magnetic susceptibility artefacts when metallic implants are present [10].

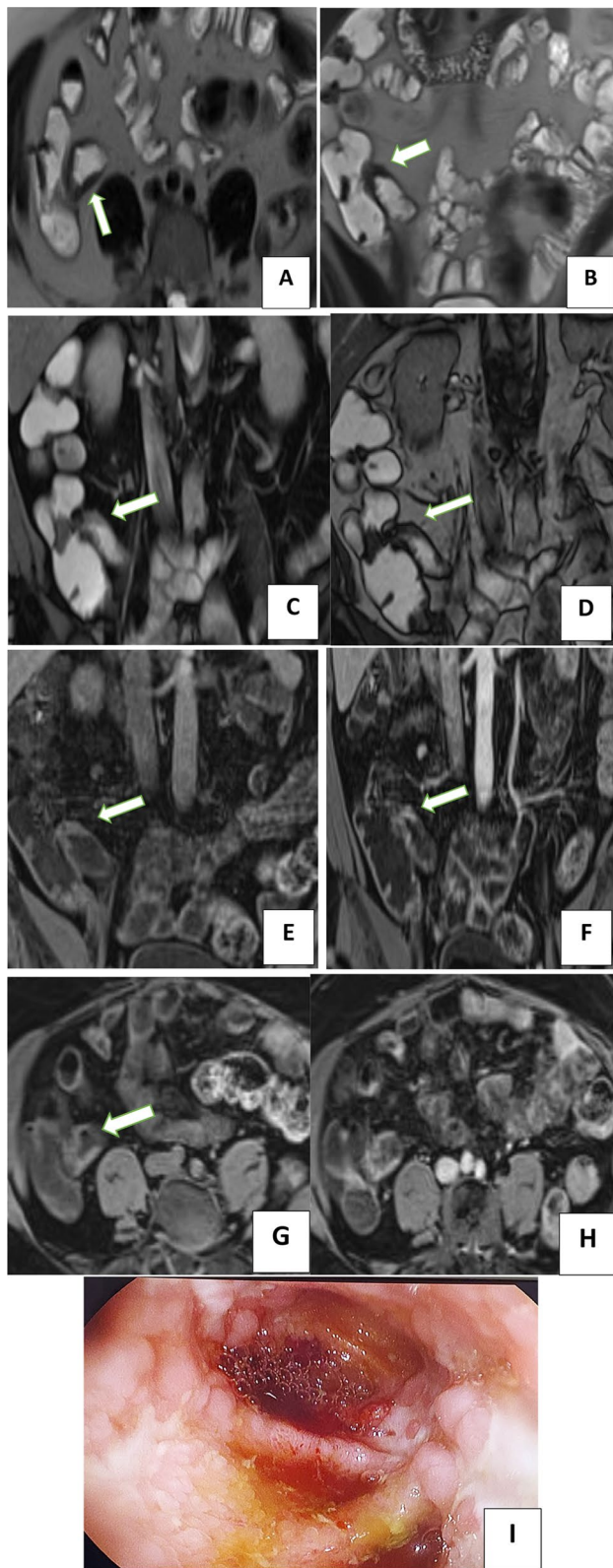


Fig. 2 (Case 1): A 39-year-old male patient, presented with persistent diarrhea and weight loss, ultrasound performed was free, MRE was performed. Axial and coronal T2 Haste (half-Fourier acquisition single-shot turbo spin-echo) images **A, B** show circumferential mural wall thickening at the distal ileum with stricture formation at the ileo-caecal junction (white arrows), coronal T2 Haste (half-Fourier acquisition single-shot turbo spin-echo) FAT SAT image (arrow) **C**, coronal TRUFI: true fast imaging with steady-state free precession with FS **D**, coronal T1 vibe Dixon pre-contrast and post-contrast images with FS **E, F** show enhancement of the distal ileum with stricture formation as well as mid-ileal loops with enhanced thickened walls (skip lesions), few enhancing reactive mesenteric lymphnodes are seen, axial T1 vibe Dixon pre-contrast and post-contrast images **G, H** show thickening of distal and mid ileal loops. Ileo-colonoscopy image **I** showed severe hyperemia, inflammation, thickening, wall thickening and irregularities with multiple ulcerations of terminal ileum. Laboratory investigations & endoscopy (ileo-colonoscopy) were done by experienced gastroenterologist and confirmed the radiological diagnosis of Crohn's disease

To the best of our knowledge, no studies have evaluated the accuracy of the Dixon approach for identifying small intestinal lesions. In order to assess the accuracy of MRE with adding the Dixon approach for the diagnosis and characterization of small bowel disorders, we therefore carried out this prospective study.

In the current study, sixty-nine consecutive patients with clinically suspected small bowel diseases were assessed.

The exact visualization of numerous mural and perienteric imaging characteristics is required for the imaging assessment of small bowel disease on MRE. Overall, MRE was found to be highly sensitive and specific in detecting small bowel disease in this study. This is congruent with the studies of Miao et al. [10], Koh et al. [11], and Kavaliauskiene et al. [12], and the systematic review by Horsthuis et al. [13] who found that MRE correctly diagnosed a high percentage of patients with small bowel disease. In addition, the study of Masselli et al. [14] reported MRE performance parameters rates in the diagnosis of small bowel diseases that was close to what was found in this study. They reported accuracy, sensitivity, specificity, positive predictive values, and negative predictive value were 95.3%, 88.9%, 99%, 98%, and 94.1%, respectively.

In order to increase tissue contrast and make it easier to spot lesions, the technique of fat suppression is frequently utilized during abdominal examinations [15]. Fat has a high signal intensity in the majority of MRI sequences. Therefore, it is crucial to use fat suppression in order to improve the visibility of edematous areas.

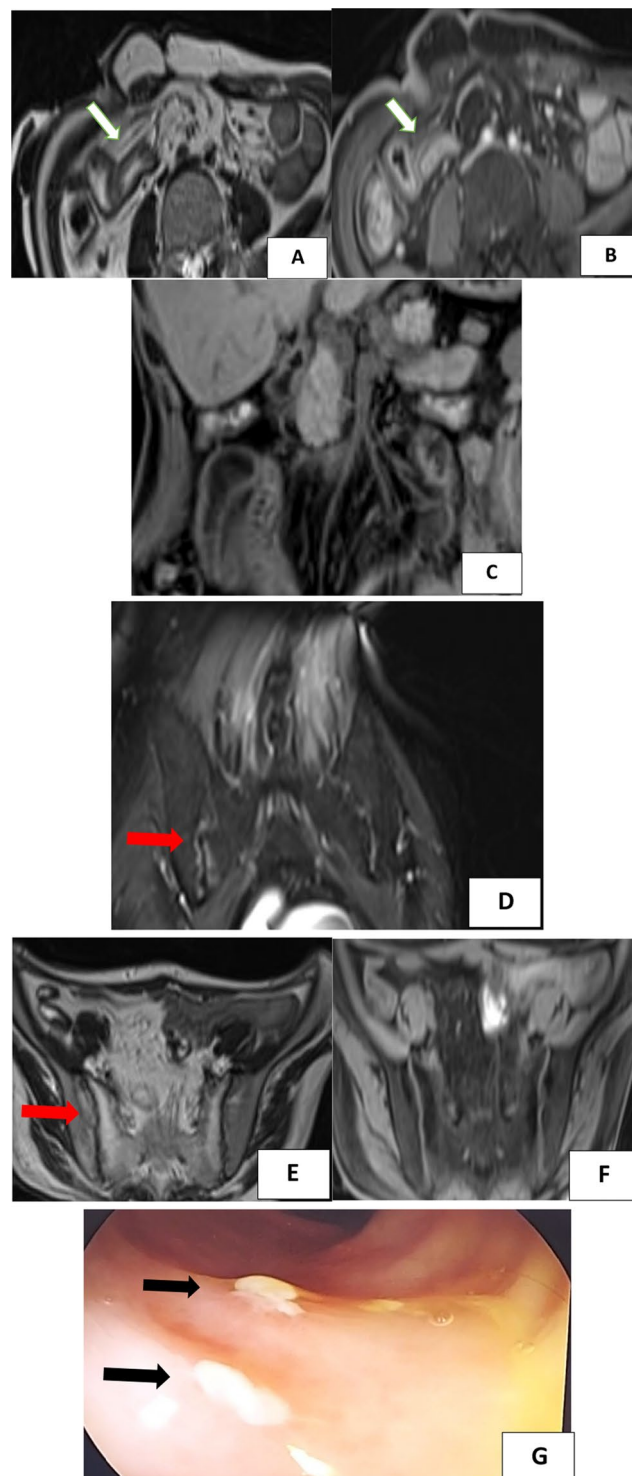


Fig. 3 (Case 2) A 33-year-old male patient, presented with status follow-up of a known case of Crohn's disease post-right ileostomy operation, ultrasound was performed with no conclusive data, MRE was performed. Axial T2WI and axial T1 Dixon post-contrast with FS images **A, B**, coronal T1 Dixon with FS **C**, show circumferential mural wall thickening at the distal ileum with stricture formation and minimal fibrotic streaks at the operative bed, enhancement of mural wall thickening, clear surrounding fat planes (white arrows). No perilesional collection. Coronal T2 Dixon with FS with contrast (water only) **D**, axial T2WI, axial T2 Dixon with FS (water only) **E, F** show bilateral sacro-iliitis in the form of subchondral bone marrow fatty changes display high T2WI signal intensity and suppressed its signal in Dixon with FS (red arrows), enhancement in post-contrast of subchondral bone marrow areas of altered signal intensity. Endoscopy image **G** showed few ulcerations of terminal ileum (black arrows). Denoting recurrence of the disease

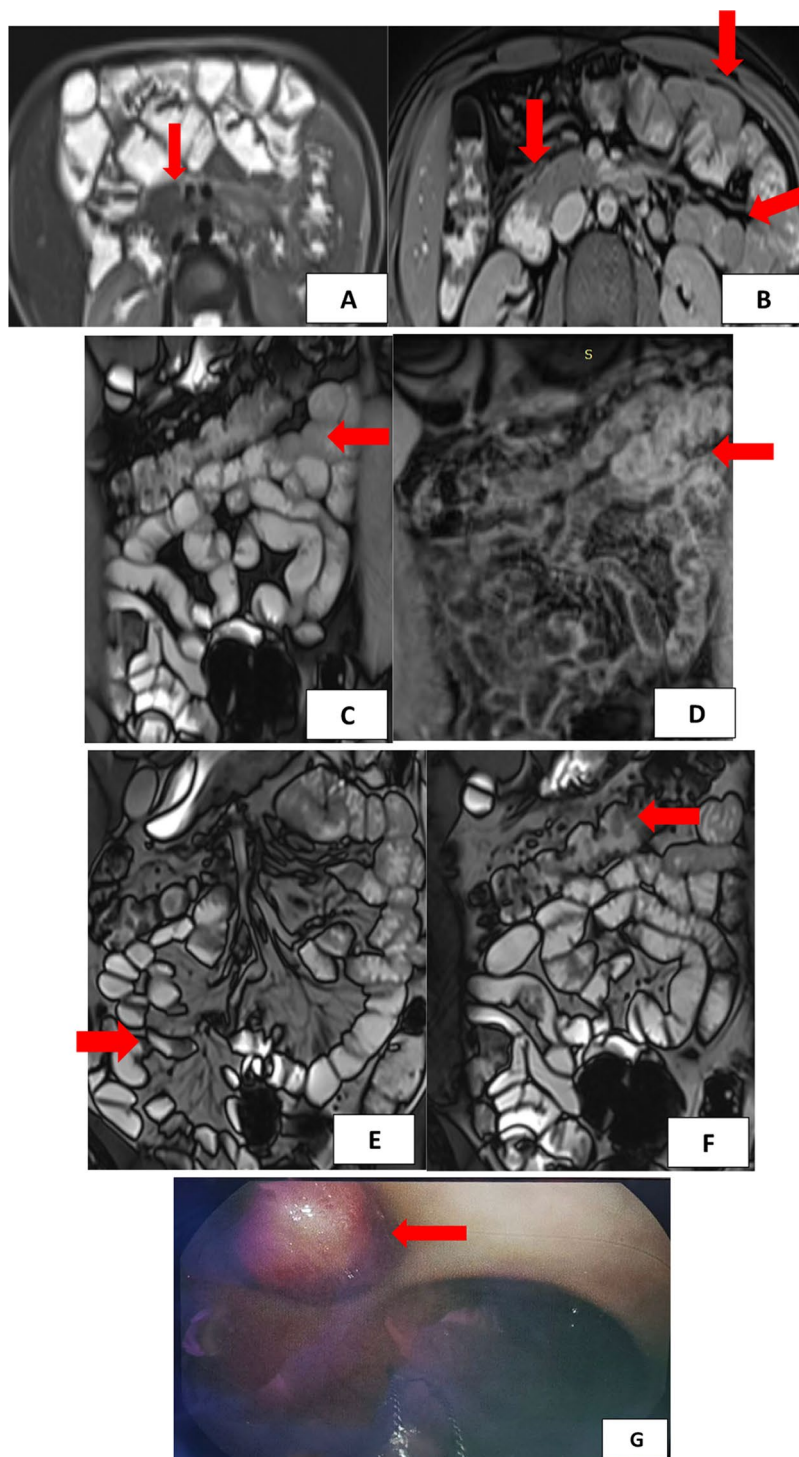


Fig. 4 (Case 3) A 29-year-old female patient, presented with recurrent attacks of abdominal pain and bloody stool, MRE was performed. Axial T2 Haste, axial T2 Trufi with FS **A, B**, revealed that the second, third parts of the duodenum and jejunal loops show multiple sessile polyps with intermediate signal intensity in T2 sequence (red arrows). Coronal T2 Haste with FS **C**, show multiple sessile polyps in jejunal loops as well as transverse colon, also multiple small nodules are seen in ileal loops (red arrows). Coronal T1 viba Dixon with FS post-contrast **D**, T2 Dixon with FS (water only) **E, F** show multiple homogeneously enhancing sessile polyps in jejunal and colonic loops and multiple ileal nodules (red arrows). Colonoscopy was performed **G** image and confirmed the diagnosis of multiple adenomatous polyposis (red arrow)

However, because it is difficult to achieve magnetic field homogeneity at the abdomen, it is difficult to get high-quality MRI images of the abdomen with consistent fat suppression [16].

According to the current study, the Dixon approach greatly improved diagnostic performance and accuracy over MRE alone in the identification of small bowel disorders.

Our results support previous findings that the Dixon technique-related water- and fat-only sequences can enhance tissue characterization and lesion conspicuity. Additionally, the Dixon method's water-only sequences have less sensitivity to magnetic field inhomogeneity and provide fat suppression that is superior to that of other frequency-selective techniques [17].

In Crohn's disease, several pathological changes are presented with fat and edema changes, such as submucosal fat deposition and fibrofatty proliferation in chronic cases, and edematous changes of active disease [11]. The superiority of adding Dixon in this study was manifested in correct diagnosis of Crohn's disease. This is supported by the stated accuracy of fat-only Dixon imaging in distinguishing edema from fat, which is of value in an MR enterography protocol for diagnosis of Crohn's disease. Also, depiction of fine details such as tiny sinuses or fistulae, shallow ulcers, and minimal nodularity requires precise distinguishing of various fat and fluid signals. This was shown in the current work where some imaging findings were depicted when adding the Dixon method.

A case of early peritoneal fat infarction was missed in MRE and diagnosed in the added Dixon study. In agreement with this study finding, fat necrosis, which occurs in response to any injury, has been reported to be accurately diagnosed by fat-only Dixon [4].

The remaining case missed in MRE was that of small submucosal lipoma, which was detected in the Dixon study due to higher potentiality of fat depiction in the fat-only Dixon study with confirmation in the fat suppression images [5].

This study emphasizes the promising performance of adding Dixon to the MRE studies, with higher spatial resolution of the obtained images, allowing better identification of the anatomical structures. The acquisition time is variable, ranging from 16 s to 5 min in the variable available MRI scanners [17], which is likely a reasonable cost compared to the added benefits of the examination.

Limitations of the study

This work was limited by the small sample size. However, our study according to our knowledge is the first to test the performance of combination of

multipoint Dixon sequence to conventional MRE as a fat suppression sequence. Further large-scale studies are recommended.

Conclusions

Adding Dixon technique to MRE examination yielded better and accurate diagnosis of small bowel diseases, with less time consumption. This was particularly eminent in fat-associated pathological processes. The present study supports the value of the new non-invasive MRI sequence in the elaboration of hidden small intestinal pathology.

Recommendations

- Continued data collection in a larger sample, longer duration with the ability of multi-points time of assessment is highly recommended to validate these observations.
- Multi-centric research studies in different MRI machines of higher Tesla are recommended in the future research field.

Abbreviations

MRI	Magnetic resonance imaging
T1WI	T1-weighted imaging
T2WI	T2-weighted imaging
MRE	MR enterography
T2WI	T2-weighted imaging
FAT SAT	Fat saturation
IBD	Inflammatory bowel diseases

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Author contributions

All authors have read and approved the manuscript and ensure that this is the case. AMR suggested the research idea, ensured the original figures and data in the work, minimized the obstacles to the team of work, correlated the study concept and design, and had the major role in analysis, RAS supervised the study with significant contribution to design the methodology, manuscript revision, and preparation, and MAE correlated the clinical data of patient and matched it with the radiological findings, drafted, and revised the work. AEL collected data in all stages of manuscript and performed data analysis. All authors read and approved the final manuscript.

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Availability of data and materials

The authors confirm that all data supporting the finding of the study are available within the article, and the raw data supporting the findings were generated and available at the corresponding author on request.

Declarations

Ethics approval and consent to participate

Informed written consents were taken from the patients and healthy volunteers; the study was approved by ethical committee of Tanta University hospital, faculty of medicine (36264PR21-1-23).

Consent for publication

All participants included in the research gave written consent to publish the data included in the study. Authors agreed to publish the paper.

Competing of interests

The authors declare that they have no competing of interests.

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