The Egyptian Journal of Hospital Medicine (April 2022) Vol. 87, Page 1991-1999

Role of Intestinal Ultrasound with Doppler in Assessment of Inflammatory Bowel Disease

Ahmed M. Ashmawy[1], Nour Eldeen A.M EL Hefny[1], Hayam Fathy[1], Zainab Gaber Mahran[2], Mohamed A.A. Abozaid[1], Wageeh A. Ali[3]

[1]Internal Medicine Department, Hepatogastroenterology Unit, [2] Department of Gastroenterology and Tropical Medicine, [3]Radiology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.

Corresponding author: Hayam Fathy Nasr, ORCID :0000-0001-5289-303X,

Email address: hyamfathy1@gmail.com,Telephone number:002 01062054664

ABSTRACT

Background and aim: accurate diagnosis of inflammatory bowel disease (IBD) are mandatory steps for a good outcome. Its standard method for assessment is still ileo-colonoscopy; however, many recent reports described the utility of intestinal ultrasound (IUS) and duplex US in evaluating patients with IBD. We aimed to explore the efficacy of IUS and duplex US for the accurate diagnosis and follow-up of patients with IBD.

Methods: The current study was performed for >2 years between September 2018 and September 2020. A total of 60 patients diagnosed with IBD and 51 control subjects who underwent colonoscopy for reasons other than IBD were included. The two groups underwent colonoscopy and IUS with Doppler for the diagnosis and follow-up of patients after 1 year of treatment. **Results**: The mean age of patients with IBD was 29.23 ± 5.62 years, and 56.7% of them were females. The majority of them presented with abdominal pain, diarrhea, and bleeding per rectum. Patients with IBD had significantly lower hemoglobin, iron, and ferritin levels with higher C-reactive protein and erythrocyte sedimentation rates. The IBD group had significantly higher wall thickness (WT), peak systolic velocity (PSV), and end-diastolic velocity (EDV). After 1 year, the IBD group showed a significant reduction of WT and PSV. The PSV had the best diagnostic performance for the prediction of histological and clinical responses. **Conclusion**: IUS and duplex US are considered rapid noninvasive tools for the assessment of patients with IBD. Moreover, they can be used to predict histological and clinical responses.

Keywords: Colonoscopy, End-diastolic velocity, Inflammatory bowel disease, Intestinal ultrasound, Peak systolic velocity

INTRODUCTION

Inflammatory bowel disease (IBD), such as Cohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing, and destructive inflammatory illnesses of the gastrointestinal tract that can cause organ damage and affect the quality of life. Although there is no one reference standard for diagnosing IBD, ileo-colonoscopy with histologic examination is considered the most common approach. Ileo-colonoscopy, on the other hand, is an invasive and costly technique that patients dislike[(1,2)].

Intestinal ultrasound (IUS) with Doppler on superior mesenteric artery has become more widely available, and technical developments in the ultrasound equipment, as well as enhanced operator competence, have boosted the role of IUS with Doppler in assessing gastrointestinal tract illnesses in recent years. IUS is a noninvasive, low-cost, and easily repeatable technology that has been employed in various settings [(3)].

IUS with Doppler US is included as a diagnostic modality in the European Crohn's and Colitis Organization guidelines for the management of IBD, for the initial evaluation of patients with clinically suspected IBD, for monitoring the therapeutic response, for suspicion of relapse, and for the detection of complications [(4)]. In this study, we aimed to assess the accuracy of IUS and duplex US in the diagnosis of patients with IBD.

MATERIALS AND METHODS

Ethical approval

The study protocol was approved by the Medical Ethics Committee of the Institutional Review Board of the Faculty of Medicine, Assiut University, Egypt (IRB No. 17200162). Informed written consent was obtained from all participants according to the declaration of Helsinki. The study protocol is registered at Clinicaltrails.gov ID: NCT03445624.

Study participants

A case-control study was conducted at the IBD Clinic from September 2018 to September 2020. Sixty patients with IBD based on laboratory, colonoscopy, and histopathological data were enrolled (16 patients had colonic CD and 44 patients had UC). In addition, the control group (n = 51) was included for comparative purposes. Patients in this group were subjected to colonoscopy, but for purposes other than IBD.

All patients were subjected to full history evaluation in addition to baseline laboratory parameters such as complete blood picture, liver and kidney function tests, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Based on standard guidelines, the management plan was performed.

Based on the results of colonoscopy and histopathology, participants were subgrouped into the study (those with confirmed IBD) and control groups.

Intestinal and duplex ultrasound

After 4-h of fasting, the procedure was performed in the supine position. To avoid the interobserver variability, the procedure was performed by a radiologist and an endoscopist, both of them were blinded with the results of

Received: 19/11/2021
Accepted: 16/01/2022

IUS and colonoscopy. IUS and the duplex US (superior mesenteric artery) were performed using (GE logiq p6 *Ultrasound machine)*.using convex (1–7 MHz) and linear (1–15 MHz) probes.

Follow-up

The study group was followed up at IBD Clinic monthly to assess therapy, response, and side effects of therapy, whereas the control group was followed up individually based on their diagnoses. The study group was subjected to IUS and duplex after 1 year.

Statistical analysis

Data were collected and analyzed using Statistical Package for the Social Sciences (version 20, IBM, and Armonk, New York). Continuous data were expressed in the form of mean ± standard deviation (SD) and range, whereas nominal data were expressed in the form of frequency (percentage).

Chi²-test was used to compare the nominal data of different groups, whereas the McNemar test was used to compare the proportion difference between groups on follow-up.

The *Mann-Whitney test* was used to compare continuous data of different groups. Wilcoxon sign test compared baseline and follow-up data in the same group. K degree to calculate degree of agreement between colonoscopy and intestinal US.

Diagnostic accuracy of ultrasound in the diagnosis of IBD and prediction of clinical and histological responses was assessed by receiver operating characteristics curve. The confidence level was kept at 95%; hence, *P*-value was considered significant if <0.05.

RESULTS

Baseline characteristics and laboratory data among the study population

There was no significant difference between the 2 groups as regard age and sex. Among the 60 patients in the study group, 44 (73.3%) had UC, and 22/44 (50%) of them were on biological therapy.

At baseline, the study group had significantly lower hemoglobin(10.08 ± 1.9 vs. 11.39 ± 1.7: P0.37), serum iron(45.67 ± 3.8 vs. 93.80 ± 4.6: P < 0.001), and ferritin levels (57.27 ± 5.9 vs. 99.49 ± 4.7: P < 0.001). Patients with IBD had significantly higher CRP level (29.87 ± 4.5 vs. 9.90 ± 1.3: P < 0.001) and ESR (36.82 ± 3.9 vs. 10.22 ± 5.5: P < 0.001). Other laboratory data are summarized in **Table 1.**

Table 1: Baseline characteristics and laboratory data among the study population

	Control group (n= 51)	Study group (n= 60)	*P* value
Age (years)	27.45 ± 5.40	29.23 ± 5.62	0.09
Range	16-36	14-45	
Sex			0.66
Male	20 (39.2%)	26 (43.3%)	
Female	31 (60.8%)	34 (56.7%)	
Presentation			< 0.001
Bleeding per-rectum	2 (3.9%)	23 (28.3%)	
Diarrhea	10(19.6%)	30 (50%)	
Abdominal pain	20 (39.2%)	50 (83.3%)	
Extra-intestinal manifestations	0	3 (5%)	
Weight loss for investigations	8 (15.7%)	0	
Constipation	7 (13.7%)	0	
Screening for CRC	4 (7.8%)	0	
Hemoglobin (g/dl)	11.39 ± 1.7	10.08 ± 1.9	0.37
MPV (fl)	8.56 ± 1.4	7.70 ± 1.5	0.002
Monocytes (10^3/ul)	0.68 ± 0.1	0.70 ± 0.1	0.29
Leucocytes (10^3/ul)	7.63 ± 0.3	7.12 ± 1.4	0.21
Platelets (10^3/ul)	351.41 ± 7.9	312.57 ± 9.5	0.34
Serum iron (mcg/dl)	93.80 ± 4.6	45.67 ± 3.8	< 0.001
Serum ferritin (ng/ml)	99.49 ± 4.7	57.27 ± 5.9	< 0.001
Serum albumin (mg/dl)	38.29 ± 2.7	35.67 ± 6.1	0.006
1st hour ESR (ml)	10.22 ± 2.5	36.82 ± 3.9	< 0.001
2nd hour ESR (ml)	13.53 ± 1.34	47.35 ± 4.3	< 0.001
CRP (mg/dl)	9.90 ± 1.3	29.87 ± 4.5	< 0.001

Data were expressed as mean (SD), range, and frequency (percentage), n: number, CRC: colorectal cancer; IDA: iron-deficiency anemia; IBD: inflammatory bowel disease; CRP: C-reactive protein; MPV: mean platelets volume; ESR: erythrocyte sedimentation rate

Colonoscopy and ultrasound findings among the study population

At baseline, pancolitis, left-sided colitis, and rectosigmoid lesion were found in 8, 26, and 26 patients, respectively; however, a marked improvement in patients was observed at post-therapy, where 68.3% of patients had normal

colonoscopy findings. At baseline, the control group had normal findings except for only two cases who had a rectosigmoid lesion (angiodysplasia), no follow-up colonoscopy was performed in this group.

Patients with IBD had significantly higher wall thickness (WT) (5.67 ± 1.49 vs. 2.36 ± 0.50; P < 0.001), peak systolic velocity (110.52 ± 30.59 vs. 95.09 ± 15.20; P < 0.001), and end-diastolic velocity (26.68 ± 6.0)[5]. Moreover, resistive Index (RI) was significantly higher among the study group (0.72 ± 0.06 vs. 0.64 ± 0.11; *P*< 0.001). Other data are summarized in **Table 2.**

Table 2: Baseline US and duplex data among study population

	Control group (n= 51)	Study group (n= 60)	*P* value
Wall thickness (mm)	2.36 ± 0.50	5.67 ± 1.49	< 0.001
PSV (cm/s)	95.09 ± 15.20	110.52 ± 30.59	< 0.001
EDV (cm/s)	21.62 ± 2.09	26.68 ± 6.06	< 0.001
Resistive index	0.64 ± 0.11	0.72 ± 0.06	< 0.001
Liver			0.29
Normal	41 (80.4%)	49 (81.7%)	
Enlarged	2 (3.9%)	0	
Fatty liver	8 (15.7%)	11 (19.3%)	
Other sonographic findings			
Multiple LNs		3 (5%)	
Minimal IPF		3 (5%)	
Baseline colonoscopy			< 0.001
Normal	49 (96.1%)	0	
Pancolitis	0 (0%)	8 (13.3%)	
Left-sided colitis	0 (0%)	26 (43.3%)	
Rectosigmoiditis	2 (3.9%)	26 (43.3%)	
Follow up colonoscopy			
Normal		41 (68.3%)	
Pancolitis		3 (5%)	
Left-sided colitis		6 (10%)	
Rectosigmoiditis		10 (16.7%)	

Data were expressed as mean (SD), and frequency (percentage), n: number, PSV: peak systolic velocity; EDV: end-diastolic velocity; US: ultrasound; LNs: lymph nodes; IPF: intraperitoneal free fluid

Baseline and follow-up radiological findings among the study group

Follow-up US and duplex revealed that EDV and RI showed no significant changes during follow-up; however, a significant reduction was observed in WT (3.69 ± 1.38 vs. 5.67 ± 1.49 (mm); *P* < 0.001) and PSV (100.08 ± 28.34 vs. 110.52 ± 30.59 (cm/s); *P*= 0.03) during follow-up, as shown in **Table 3.**

Table 3: Baseline and follow up US and duplex data among study population

	Baseline (n= 60)	Follow up (n= 60)	*P* value
Wall thickness (mm)	5.67 ± 1.49	3.69 ± 1.38	< 0.001
PSV (cm/s)	110.52 ± 30.59	100.08 ± 28.34	0.03
EDV (cm/s)	26.68 ± 6.06	26.03 ± 6.93	0.43
Resistive index	0.72 ± 0.06	0.68 ± 0.09	0.07
US diagnosis			< 0.001
Normal	3 (5%)	40 (66.7%)	
Pancolitis	8 (13.3%)	3 (5%)	
Left-sided colitis	25 (41.7%)	6 (10%)	
Rectosigmoiditis	24 (40%)	11 (18.3%)	

Data were expressed as mean (SD), and frequency (percentage), n: number, PSV: peak systolic velocity; EDV: end-diastolic velocity; US: ultrasound

Diagnostic accuracy of baseline US and duplex findings in the diagnosis of IBD and the prediction of clinical and histological responses

Baseline intestinal WT at a cut-off of >3 mm had the best area under the curve (0.94) for diagnosis of IBD in comparison to PSV, EDV, and RI with 88% sensitivity and 100% specificity **(Table 4).**

Table 4: Diagnostic accuracy of US and duplex findings in diagnosis of IBD

Indices	Wall thickness	PSV	EDV	RI
Sensitivity	88%	73%	60%	50%
Specificity	100%	75%	94%	84%
PPV	100%	77%	92%	79%
NPV	88%	70.4%	67%	59%
Cut off point	> 3 mm	> 102	> 24	> 0.72
AUC	0.94	0.70	0.81	0.67
P value	< 0.001	< 0.001	< 0.001	< 0.001

PSV: peak systolic velocity; EDV: end-diastolic velocity; US: ultrasound; RI: resistive index, PPV: positive predictive values; NPV: negative predictive value; AUC: Area under the ROC curve

Follow-up PSV at a cut-off of <135 cm/s had the best area under the curve (0.94) for the prediction of clinical response in comparison to WT, EDV, and RI with 98% sensitivity and 91% specificity **(Table 5).**

Table 5: Accuracy of follow up US and duplex in prediction of clinical response

Indices	Wall thickness	PSV	EDV	RI
Sensitivity	89%	98%	90%	89.8%
Specificity	55%	91%	90.9%	90.9%
PPV	89.4%	98%	98%	97.8%
NPV	50%	91%	66.7%	66.7%
Cut off point	< 3	< 135 cm/s	< 29 cm/s	< 0.75
AUC	0.71	0.94	0.84	0.90
P value	< 0.001	< 0.001	< 0.001	< 0.001

PSV: peak systolic velocity; EDV: end-diastolic velocity; US: ultrasound; RI: resistive index; PPV: positive predictive values; NPV: negative predictive value; AUC: Area under the ROC curve

Follow-up PSV at a cut-off of <132 cm/s had the best area under the curve (0.94) for the prediction of histological responses in comparison to WT, EDV, and RI with 98% sensitivity and 92% specificity **(Table 6).**

Table 6: Accuracy of follow up US and duplex in prediction of histological response

Indices	Wall thickness	PSV	EDV	RI
Sensitivity	87%	98%	92%	91.7%
Specificity	50%	92%	92%	91.7%
PPV	87%	98%	99%	97.8%
NPV	50%	92%	73.3%	73.3%
Cut off point	< 4 mm	< 132 cm/s	< 29 cm/s	< 0.75
AUC	0.68	0.95	0.86	0.90
P value	< 0.001	< 0.001	< 0.001	< 0.001

P-value was significant if <0.05. PSV: peak systolic velocity; EDV: end-diastolic velocity; US: ultrasound; RI: resistive index PPV: positive predictive values; NPV: negative predictive value; AUC: Area under the ROC curve

US diagnosis of patients with IBD showed a strong degree of agreement with colonoscopy at baseline *(K* degree = 0.79) and follow-up (K degree = 0.92). Illustrated cases are shown in **Figures 1 and 2.**

Figure 1: A 21-year-old female patient presented with abdominal pain and diarrhea for 1 month.
A) Colonoscopic appearance of the descending colon cobblestone.
B, C) Intestinal US: showing colonic wall thickness of 8 ml with hypervascularity.
D) Duplex US (superior mesenteric artery): peak systolic velocity was 135 and resistive index was .8.

Figure 1 A

Figure 1 B

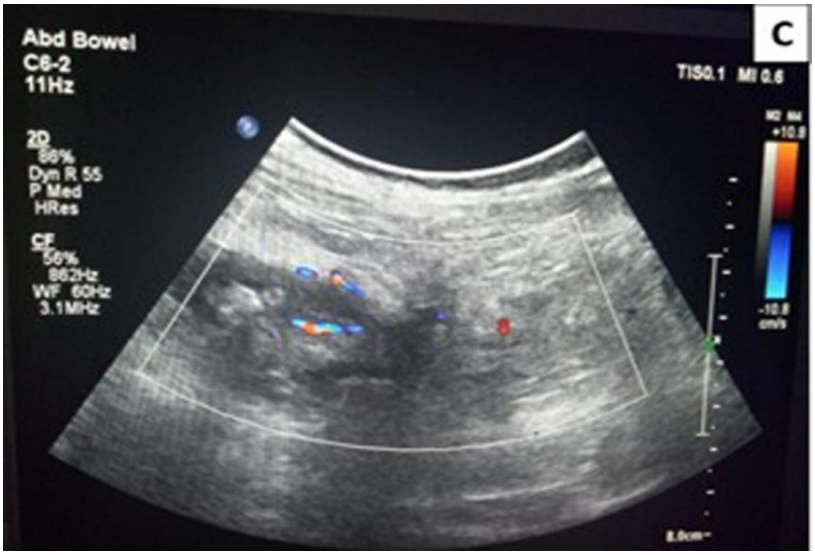

Figure 1 C

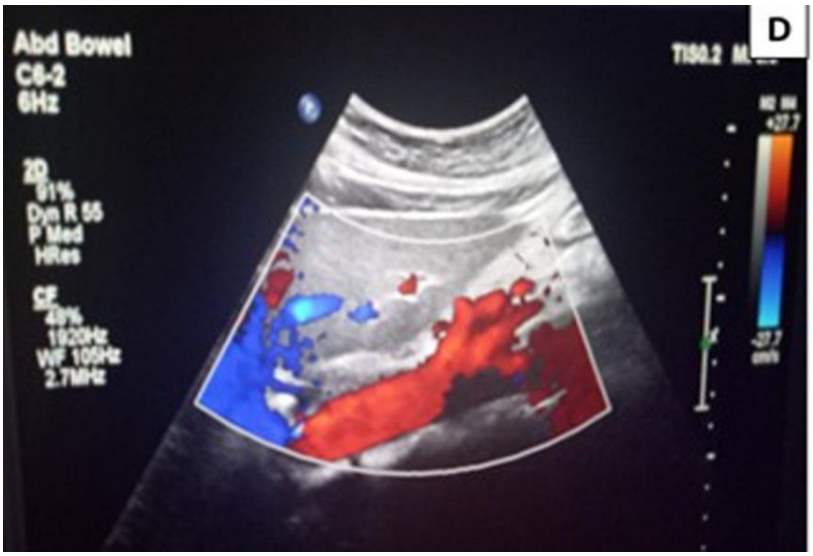

Figure 1 D

Figure 2: A 33-year-old male patient presented with bleeding per rectum diagnosed as ulcerative colitis on conventional treatment.
A) Baseline colonoscopy was severe hyperemic edematous mucosa with small pseudopolyp.
B) Baseline intestinal ultrasound shows intestinal wall thickness was 5 mm.
C) Follow-up colonoscopy with complete healing of the lesions.
D) Follow-up intestinal ultrasound shows decreased intestinal wall thickness (3 mm).

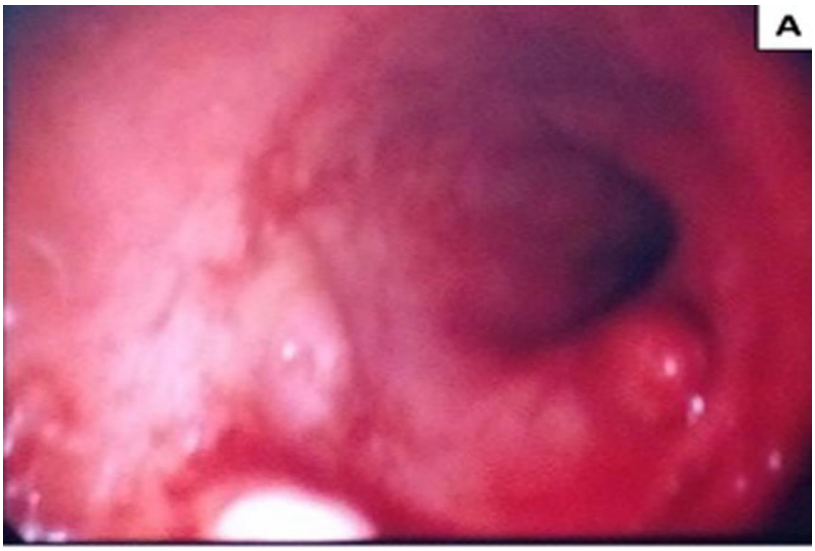

Figure 2 A

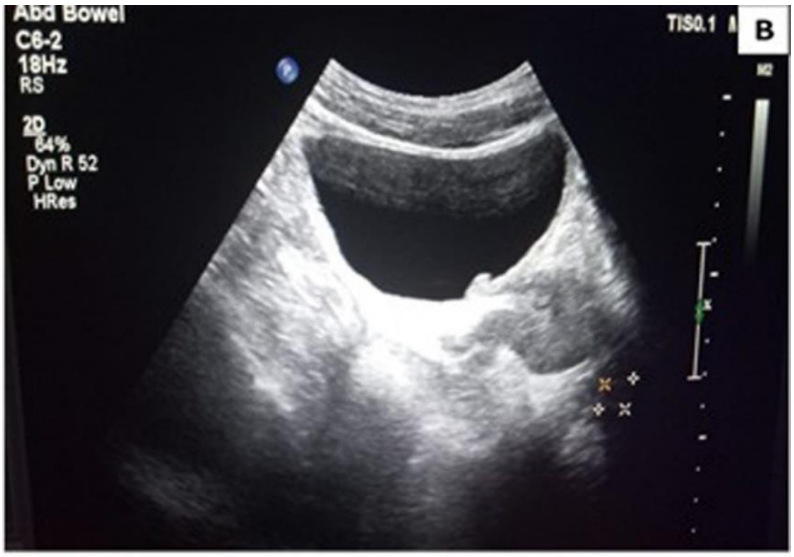

Figure 2B

Figure 2C

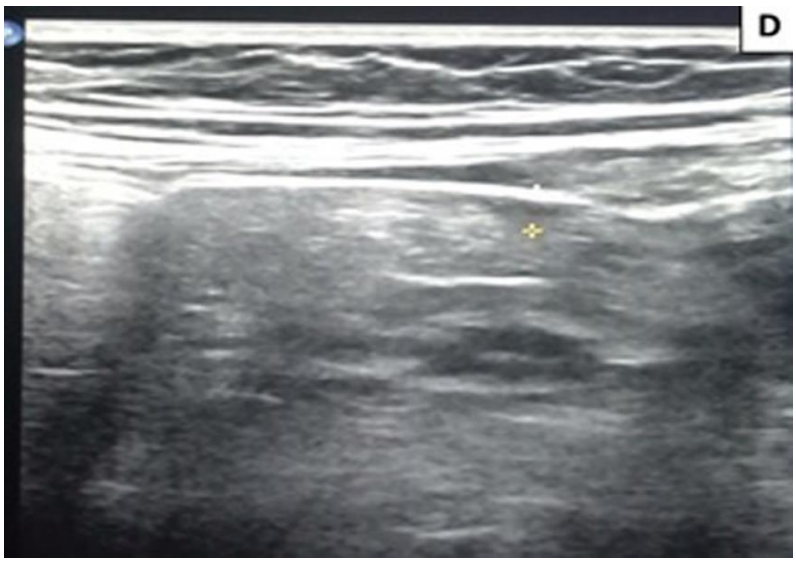

Figure 2D

DISCUSSION

Assessment of intestinal inflammatory lesions through imaging techniques is essential for the management of patients with IBD. Characterization of disease location and extension at the time of diagnosis are required to establish a proper management plan. Imaging techniques are also the accepted reference for the detection of complications, including strictures and penetrating lesions such as fistulas and abscesses[5]. Recently, there are great advances in the utility of IUS and yet there is paucity in its use in patients with IBD[6].

Sixty patients with confirmed IBD based on clinical, laboratory, radiological, and histopathological data, in addition to another 51 participants, were enrolled. The mean age of those patients with IBD was 29.23 ± 5.62 years, and 56.7% of them were females. This result was consistent with other previous studies with regard to female predominance in IBD[7]. However, several epidemiologic studies from Asian countries revealed a male predominance of IBD[8]. Based on geographic distribution, sex predominance was reported in the case of CD where female predominance was observed in Western countries, but the opposite was found in Asian patients [7].

Extra-intestinal manifestations were present in only three patients with CD. In line with the current study, previously reported studies revealed that the UC group typically has rectal bleeding, diarrhea, and tenesmus, whereas the CD group typically presents with diarrhea, abdominal pain, and weight loss[9].. The frequency of extra-intestinal manifestations in this study was relatively low (5%), and this could be explained by the fairly small sample size. This percentage was consistent with the reported percentage of such manifestations (6-47%)[10,11].

Baseline laboratory data in this study revealed that patients with IBD had significantly lower hemoglobin, serum iron, and ferritin levels, indicating that patients with IBD usually suffered from iron-deficiency anemia. This result agreed with many previously reported studies that found the prevalence of anemia in IBD was between 31% and 68%[12,13].. Therefore, it is recommended that anemia management should be considered for every patient with a hemoglobin level lower than the normal limits and iron substitution is recommended for every patient with anemia who has iron deficiency. However, treating only the underlying disease cannot sufficiently correct the hemoglobin levels[14].

The serum albumin levels were found to be lower in patients with IBD. This could be explained by low intake, reduced absorption, and/or low synthesis by the liver as previously reported[15].

Among the studied patients with IBD, 11(19.3%) had fatty liver, a finding consistent with that of the previously reported studies that revealed the prevalence of fatty liver and metabolic associated non-alcoholic fatty liver disease (NAFLD) among patients with IBD was between 8% and 40%. This is secondary to the absence of a standard diagnostic method for fatty liver and NAFLD[16.17].Due to intestinal disease-related disorders that share physiopathologic aspects with NAFLD, such as persistent relapsing inflammation and immunological activation, potentially hepatotoxic medications, surgery, and parenteral feeding, patients with IBD may be at a higher risk of NAFLD. Furthermore, changes in the gut microbiota have been associated with disease severity in both IBD and NAFLD, suggesting a possible pathogenic relationship between the two disorders[18].

The current study found a considerable degree of agreement between colonoscopy and IUS with regard to the diagnosis of IBD either at baseline or during follow-up. Moreover, patients with IBD had significantly higher bowel WT (5.67 ± 1.49 vs. 2.36 ± 0.50 (mm); $P < 0.001$) in comparison to the control group. It was found that a cut-off of >3 mm, baseline intestinal WT had 88% sensitivity and 100% specificity in the diagnosis of IBD with the area under the curve of 0.94.

Similar results were found in a subsequent systematic review, which revealed 88% sensitivity and 97% specificity. They described the diagnostic accuracy of IUS in the diagnosis of CD and the evaluation of disease activity[19]. Previously, WT of 3 and 4 mm had 88% and 75% sensitivity, respectively, in patients with CD. Therefore, it is better to use 4 mm as a cut-off to avoid false positives[20].

We found that patients with IBD had significantly higher baseline PSV, EDV, and RI. At the cut-off of >24 cm/s, baseline end-diastolic velocity had 60% sensitivity and 94% specificity in the diagnosis of IBD with an area under the curve of 0.81. At the cut-off of >0.72, the baseline resistive index had 50% sensitivity and 84% specificity in the diagnosis of IBD with an area under the curve of 0.67.

Increased vascularity has been observed to be associated with disease activity. Color Doppler imaging is a useful tool for detecting vascular signals from blood vessels in the gut wall as a qualitative indicator of current inflammation. IBD is known to cause neovascularization in the affected areas[21,22].

The current study revealed that EDV and RI showed no significant changes during follow-up; however, a significant reduction was observed in WT and PSV during follow-up. For the prediction of clinical and histological response, follow-up PSV had the best diagnostic performance comparable to other parameters. At the cut-off of 3–5 mm of WT, IUS had a sensitivity of 77%–81% and specificity of 86–95% in the detection of postoperative recurrence in CD[23], whereas, at 7 mm, it could assess the need for surgery in CD with 88% sensitivity and 78% specificity[24]. In a recent study, duplex performance was found to have good sensitivity, positive predictive value, and accuracy levels (>80%) in

individuals with clinically active CD, when WT and hyperemia were considered[25]. Patients with clinical remission were found to have full WT than those who failed to achieve clinical remission[26].

CONCLUSION

IUS in addition to duplex findings is found to be effective noninvasive rapid tools for the assessment of patients with IBD with good accuracy in predicting the activity, location of the lesion, and histological and clinical responses.

REFERENCES

1. **Furfaro F, Dal Buono A, Allocca M *et al.* (2021):**Bowel Ultrasound in Inflammatory Bowel Disease: How Far in the Grayscale? *Life,* 11(7):649.
2. **Fraquelli M, Castiglione F, Calabrese E, Maconi G (2020):** Impact of intestinal ultrasound on the management of patients with inflammatory bowel disease: how to apply scientific evidence to clinical practice. *Digestive and Liver Disease,* 52(1):9-18.
3. **Maconi G, Nylund K, Ripolles T *et al.* (2018)** :EFSUMB recommendations and clinical guidelines for intestinal ultrasound (GIUS) in inflammatory bowel diseases. *Ultraschall in der Medizin-European Journal of Ultrasound*,39(03):304-317.
4. **Carter D, Eliakim R *et al*.(2017) :** Feasibility of bedside bowel ultrasound performed by a gastroenterologist for detection and follow-up of inflammatory bowel disease. *Isr Med Assoc J.*,19(3):139-142.
5. **Panés J, Bouzas R, Chaparro M *et al*. (2011)**: Systematic review: the use of ultrasonography, computed tomography and magnetic resonance imaging for the diagnosis, assessment of activity and abdominal complications of Crohn's disease. *Alimentary Pharmacology & Therapeutics*,34(2):125-145.
6. **Maaser C, Petersen F, Helwig U *et al.* (2020):** Intestinal ultrasound for monitoring therapeutic response in patients with ulcerative colitis: results from the TRUST&UC study. *Gut*,69(9):1629-1636.
7. **Greuter T, Manser C, Pittet V, Vavricka SR, Biedermann L *et al*. (2020)** :Gender differences in inflammatory bowel disease. *Digestion,*101(1):98-104.
8. **Park S, Kim Y-J, Rhee K *et al.* (2019):** A 30-year trend analysis in the epidemiology of inflammatory bowel disease in the Songpa-Kangdong District of Seoul, Korea in 1986–2015. *Journal of Crohn's and Colitis*,13(11):1410-1417.
9. **Thoreson R, Cullen J *et al.* (2007)** :Pathophysiology of inflammatory bowel disease: an overview. *Surgical Clinics of North America*,87(3):575-585.
10. **Greuter T, Vavricka S *et al*. (2019)**: Extraintestinal manifestations in inflammatory bowel disease - epidemiology, genetics, and pathogenesis. *Expert review of gastroenterology & hepatology,* 13(4):307-317.
11. **Jang H, Kang B, Choe B *et al.* (2019)**: The difference in extraintestinal manifestations of inflammatory bowel disease for children and adults. *Translational pediatrics,* 8(1):4-15.
12. **Dumitrescu G, Dranga M, Pintilie I, Nedelciuc O, Mihai C, Prelipcean C *et al*. (2012**): The prevalence of anaemia in patients with inflammatory bowel diseases in North-Eastern Romania. *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi,* 116(4):968-974.
13. **Antunes C, Hallack Neto A, Nascimento C *et al*. (2015**): Anemia in inflammatory bowel disease outpatients: prevalence, risk factors, and etiology. *BioMed research international*,2015:728925.
14. **Lupu A, Diculescu M, Diaconescu R *et al*. (2015):** Prevalence of anemia and iron deficiency in Romanian patients with inflammatory bowel disease: a prospective multicenter study. *Journal of gastrointestinal and liver diseases,* 24(1):15-20.
15. **Mohammadi E, Qujeq D, Taheri H, Hajian-Tilaki K *et al*. (2017**): Evaluation of serum trace element levels and superoxide dismutase activity in patients with inflammatory bowel disease: translating basic research into clinical application. *Biological trace element research*,177(2):235-240.
16. **Sourianarayanane A, Garg G, Smith T *et al*. (2013)** :Risk factors of non-alcoholic fatty liver disease in patients with inflammatory bowel disease. *Journal of Crohn's & colitis*,7(8):e279-285.
17. **Bessissow T, Le NH, Rollet K *et al.* (2016**) Incidence and predictors of nonalcoholic fatty liver disease by serum biomarkers in patients with inflammatory bowel disease. *Inflammatory bowel diseases*,22(8):1937-1944.
18. **Saroli Palumbo C, Restellini S, Chao C-Y *et al.* (2019):** Screening for nonalcoholic fatty liver disease in inflammatory bowel diseases: a cohort study using transient elastography. *Inflammatory bowel diseases*,25(1):124-133.
19. **Dong J, Wang H, Zhao J *et al*. (2014)**: Ultrasound as a diagnostic tool in detecting active Crohn's disease: a meta-analysis of prospective studies. *European radiology,* 24(1):26-33.
20. **Fraquelli M, Colli A, Casazza G *et al*. (2005)** :Role of US in detection of Crohn disease: meta-analysis. *Radiology*,236(1):95-101.
21. **Nylund K, Hausken T, Gilja OH *et al*. (2010):** Ultrasound and inflammatory bowel disease. *Ultrasound quarterly* , 26(1):3-15.
22. **Lu C, Merrill C, Medellin A, Novak K *et al*. (2019)**: Bowel ultrasound state of the art: Grayscale and Doppler ultrasound, contrast enhancement, and elastography in Crohn disease,38(2):271-288.
23. **Andreoli A, Cerro P, Falasco G *et al*. (1998)**: Role of ultrasonography in the diagnosis of postsurgical recurrence of Crohn's disease. *The American journal of gastroenterology*,93(7):1117-1121.
24. **Castiglione F, De Sio I, Cozzolino A *et al*. (2004):** Bowel wall thickness at abdominal ultrasound and the one-year-risk of surgery in patients with Crohn's disease. *American Journal of Gastroenterology*,99(10):1977-1983.
25. **da Silva Moraes A, de Freitas Moraes G, De Araujo A *et al*. (2019)**: Abdominal ultrasonography with color Doppler analysis in the assessment of ileal Crohn's disease: comparison with magnetic resonance enterography. *Intestinal research*,17(2):227.
26. **Maconi G, Sampietro G, Cristaldi M *et al*. (2001):** Preoperative characteristics and postoperative behavior of bowel wall on risk of recurrence after conservative surgery in Crohn's disease: a prospective study. *Annals of surgery*,233(3):345.