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Sigmoid wall thickness correlates with fecal calprotectin during irritable bowel syndrome exacerbation

Tarek Mazzawi[1], Sara Haj Ali[1], Kim Nylund[2,3], Odd Helge Gilja[2,3]

[1]Department of Internal Medicine, Faculty of Medicine, Al-Balqa Applied University, Salt, Jordan, [2]National Centre for Ultrasound in Gastroenterology, Haukeland University Hospital, Bergen, Norway, [3]Institute of Clinical Medicine, University of Bergen, Bergen, Norway

Abstract

Aim: Patients with diarrhea-predominant irritable bowel syndrome (IBS-D) who present with symptom exacerbation [defined by a high IBS-Symptom Severity Scale (IBS-SSS)] may have increased fecal calprotectin (FC). Intestinal ultrasound (IUS) is used to measure bowel wall thickness (BWT). The aim of our study was to measure BWT in IBS-D patients, ulcerative colitis (UC) and healthy controls (HC), and compare BWT, IBS-SSS score, and FC level. **Material and method**: Patients with IBS-D and symptom exacerbation (n=100), active UC (n=25), and HC (n=30) were included. FC was measured in all groups. IBS-D patients with an IBS-SSS score >75 and FC level >50 µg/g underwent IUS and colonoscopy. **Results**: Patients with IBS-D had a (mean±SEM) IBS-SSS score of 268±11, FC level of 260±46 µg/g, and a normal colonoscopy. IUS showed a significant difference ($p<0.0001$) between sigmoid BWT for IBS-D (3.16±0.09 mm) and HC (1.6±0.12 mm). Color Doppler signals were also absent. A significant correlation between sigmoid BWT and FC level (r=0.48, $p=0.0012$) was found in patients with IBS-D. **Conclusion**: Compared to HC, sigmoid BWT was significantly increased in IBS-D patients with symptom exacerbation. Sigmoid BWT correlates significantly with FC levels during IBS-D symptom exacerbation.

Keywords: Bowel wall thickness; Color Doppler; Intestinal ultrasound; Irritable Bowel Syndrome Symptom Severity Scale; Fecal calprotectin; Ulcerative colitis

Introduction

Irritable bowel syndrome (IBS) is a chronic disorder of the gastrointestinal (GI) tract. Its etiology is unknown [1] and symptoms range from mild to severe [2]. According to Rome IV criteria, IBS is characterized by recurrent abdominal pain at least once weekly that is related to defecation and is associated with changes in stool frequency and/or form. The criteria must occur in the prior 3 months with symptom onset at least 6 months before diagnosis [3].

The diagnosis of IBS is based on clinical assessment because there are no specific tests or biomarkers to confirm the disease. Therefore, it is considered a diagnosis of exclusion [4]. Fecal calprotectin (FC), an S-100 protein family member found in neutrophils, is detected by a non-invasive ELISA stool test, which indicates intestinal inflammation or irritation [5]. It has been used in the diagnosis and follow-up of patients with inflammatory bowel diseases (IBDs) and as a way to differentiate IBD from IBS [6]. In patients with IBS, FC levels vary from normal (<50 µg/g) to slightly elevated (50-200 µg/g) [7-9]. Higher levels of FC (>200 µg/g) typically indicate an inflammatory condition in the GI tract, and levels of

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Corresponding author: Tarek Mazzawi, MD, PhD
Department of Internal Medicine,
Faculty of Medicine,
Al-Balqa Applied University,
19117, Salt, Jordan
E-mail: tarek.mazzawi@bau.edu.jo
Phone/fax: +96253491111 / +96253532312

>500 μg/g definitely indicate an inflammatory condition [7].

Intestinal ultrasound (IUS) is a clinically significant method to assess bowel diseases [10-12]. It is often used to measure the thickness of the wall of the intestine. A cutoff value >3 mm is typically used to detect active inflammation in the bowel wall [10,12,13]. IUS enhances predictive value of endoscopic evaluation in patients with ulcerative colitis (UC) [14]. The sensitivity and specificity of IUS to assess disease activity in patients with UC can be increased when combined with the level of FC [15,16]. Both FC and IUS could be used when patients experience an exacerbation of their GI symptoms. It is important to assess the relationship between these parameters and different GI diseases such as IBS.

The primary objective of this study was to measure the bowel wall thickness (BWT) of the sigmoid colon in patients with diarrhea-predominant IBS (IBS-D) and to compare it with the BWT of the sigmoid colon in patients with UC and healthy controls (HC). The secondary objective of the study was to investigate the relationship between BWT of the sigmoid colon in patients with IBS-D, the IBS-Symptom Severity Scale (IBS-SSS), and FC levels.

Materials and methods

Study design

This was a prospective, case-control study. Patients with active IBS-D as defined by the IBS-SSS who were referred to the outpatient clinic of Gastroenterology at New Salt Hospital, Al-Balqa Applied University (Al-Salt, Jordan) were included. Patients with UC who were referred due to suspected active disease (a clear pathological condition) and healthy participants (with normal health condition) at our hospital were included as control groups to compare them with IBS-D patients.

The inclusion criteria for patients with IBS-D included patients from both sexes and older than 18 years of age with an established IBS-D diagnosis and an exacerbation defined as an IBS-SSS score >75 and FC level >50 μg/g. The exclusion criteria included the following: IBD; pregnant or breastfeeding females; colorectal cancer; history of bowel resection; current nonsteroidal anti-inflammatory drug use; comorbidities that could cause inflammatory reactions; active infection, acute infectious diarrhea, incomplete ileocolonoscopy; inability to provide blood and stool samples; and mental or psychological problems that hinder the patient's ability to sign the consent form.

The inclusion criteria for the UC group were patients with an established diagnosis and active disease defined as total Mayo score >2 and FC level >50 μg/g [17]. Patients with UC who were younger than 18 years or with a previous history of operations including colectomy or colostomy or complications such as fistulation were excluded.

Thirty healthy participants with normal blood tests, an FC level <50 μg/g, and a normal screening colonoscopy were included as healthy controls (HC). They also underwent IUS.

Clinical disease activity

All the patients completed an IBS-SSS questionnaire. Scores >75 indicate mild symptoms, scores >175 indicate moderate symptoms, and scores >300 indicate severe symptoms [18]. Patients with UC were assessed using the Mayo score where scores 3-5 indicate mild disease, 6-10 indicate moderate disease, and 11-12 indicate severe disease [17].

As part of the routine investigation, blood tests (complete blood count, kidney and liver functions tests, C-reactive protein and erythrocyte sedimentation rate) and stool samples were collected for analysis of infection (stool analysis and stool culture) and for measuring FC level [Chorus Calprotectin; Diesse Diagnostica Senese S.p.A., Monteriggioni (SI) Italy]. The patients were requested to deliver freshly collected (<60 min) stool samples to the hospital's laboratory in a clean container that did not contain any urine or water, to prevent any potential contamination. The stools samples were stored in a refrigerator at 2-8 °C and processed within 48 h after collection.

Ultrasound examination and measurements

Using a high-performance color Doppler ultrasound machine (S22 Ultrasound; Sonoscape, Guangzhou, China) with a 7-12 MHz linear array probe, bowel examination started from the terminal ileum towards the rectum. Patients were positioned in a supine position following a 4 h fast. Using B mode, the total wall thickness of the ascending and sigmoid colon with distended lumen along with the different wall layers [15] were measured by the main investigator (first author) starting from and excluding the central luminal hyperechoic line until the end of the outer hypoechoic wall margin of the proper muscle layer in a segment of the bowel. A preset with frequency, focus, and gain optimized for bowel examination was used.

Color Doppler was set with an optimized wall filter, frequency, and velocity scale 5-7 cm/s for registering bowel wall vessels. The color Doppler signal was identified as either absent (0) or present (1) [19].

Colonoscopy

After recruitment and subsequent performance of the laboratory tests and ultrasound, all patients were scheduled for colonoscopy within 48 h after bowel cleansing

using the standard procedure of polyethylene glycol. Colon biopsy samples were taken from each colon segment (at least 4/segment) for microscopic assessment to rule out any other pathology.

Statistical analysis

Using a sample size calculator (www.clincalc.com) and setting the mean BWT for the UC group at 3.0±0.6 and anticipated mean for the IBS-D group at 2.5 as well as the alpha to 0.05 and beta to 0.2 with 80% power, the estimated sample size was 23 participants in each group.

Statistical analysis was performed using the GraphPad Prism 10.4.1 program (GraphPad Software, Inc., San Diego, CA, United States). The Mann-Whitney and Kruskal-Wallis with Dunn's post-tests were used to compare the different groups. Correlation between the variables was assessed using the Pearson rank test (*r*). Data were summarized as mean ± the standard error of the mean. p values <0.05 were considered statistically significant.

Consent

All participants provided written informed consent to participate in the study. The study was performed in accordance with the Declaration of Helsinki [20] and approved by the Institutional Review Board committee at Al-Balqa Applied University (No. 1441/1/3/26).

Results

Study participants

A total of 100 patients with IBS-D according to the Rome IV criteria, 25 patients with UC, and 30 HCs were included. Blood test (hemoglobin, WBC, C-reactive protein, erythrocyte sedimentation rate, and FC) results for all the participants are shown in Table I. Stool analysis and culture for infectious pathogens and parasites were negative for all the participants.

Clinical disease activity

The severity of IBS symptoms in patients with IBS-D was graded using the IBS-SSS questionnaire. They were divided into mild, moderate and severe. The mean score was 268±11 for patients with IBS-D. The levels of FC for patients with IBS-D, patients with UC, and HCs are shown in Table I. They were significantly different between the groups [p=0.0003 (IBS-D *vs* UC); p<0.0001 (IBS-D *vs* HC); p<0.0001 (UC *vs* HC)].

Ultrasound examination and measurements

IUS in patients with IBS-D, patients with UC, and HCs showed that the mean total BWT of the ascending colon to be 1.08±0.04 mm, 1.96±0.20 mm, and 1.20±0.10 mm, respectively. These values were significantly different when comparing UC *vs* IBS-D and UC *vs*

Table I. Demographic characteristics of the study participants

Characteristic	IBS-D *n* = 100	UC *n* = 25	Healthy controls *n* = 30
Sex, M/F	51/49	14/11	12/18
Age in years, mean ± SEM	39.9 ± 1.4	34.0 ± 3.0	38.2 ± 2.2
Laboratory tests, mean ± SEM			
Hemoglobin in g/dL	14.40 ± 0.16	13.80 ± 0.37	14.20 ± 0.20
WBC as × 10^9/µL	7.10 ± 0.17	8.00 ± 0.34	7.70 ± 0.30
CRP in mg/L	2.2 ± 0.2	9.4 ± 2.6	3.3 ± 0.4
ESR in mm/h	9.5 ± 0.7	18.4 ± 2.2	7.2 ± 0.4
FC in µg/g	251 ± 19	1097 ± 385	30 ± 2
Disease activity[1]			
Mild	4	4	-
Moderate	61	20	-
Severe	35	1	-
Medications			
5-ASA	0	24	0
Budesonide	0	3	0
Azathioprine	0	6	0
Biologic	0	1	0

[1]Disease activity in diarrhea-predominant irritable bowel syndrome (IBS-D) was defined by the Irritable Bowel Syndrome-Symptom Severity Scale where a score >75 indicates mild disease, a score >175 indicates moderate disease, and a score >300 indicates severe disease. Disease activity in ulcerative colitis (UC) was defined as the total Mayo score where a score of 3-5 indicates mild disease, 6-10 indicates moderate disease, and 11-12 indicates severe disease. 5-ASA: 5-Aminosalicylic acid; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; F: Female; FC: Fecal calprotectin; M: Male; SEM: Standard error of the mean.

HC (p<0.0001 and p=0.0045, respectively) but were not different when comparing IBS-D *vs* HC (p>0.9).

The total BWT (fig 1) and the layers of the sigmoid colon in patients with IBS-D, patients with UC, and HC differed significantly as shown in Table II. The relative wall thickness for each sigmoid layer [defined by dividing the thickness of each layer (mucosa, submucosa and proper muscle) over the total BWT] for patients with IBS-D was 0.20±0.01 mm, 0.50±0.01 mm, and 0.40±0.01 mm, respectively, and for UC patients was 0.20±0.01 mm, 0.50±0.02 mm, and 0.30±0.08 mm, respectively. When comparing the relative thickness of each wall layer in patients with IBS-D and patients with UC, the only significant difference was in the proper muscle layer (p=0.039).

Furthermore, 68 patients with IBS-D (68%) had a BWT of the sigmoid colon <4 mm, and 23 patients with IBS-D patients (23%) had a BWT <3 mm. In contrast 11 (44%) patients with UC had a BWT <4 mm, and only 4 patients (16%) had a BWT <3 mm. The cut-off values for BWT of sigmoid colon for patients with IBS-D and UC were 2 and 3 mm, respectively, and the sensitivity, specificity, likelihood ratio and area under the curve values for IBS-D patients were 93%, 86.67%, 6.975 and 0.92, respectively and for UC patients were 100%, 72%, 3.571 and 0.96, respectively.

Correlations

A significant correlation was found between the IBS-SSS score and FC level (r=0.73, p<0.0001) for patients with IBS-D. The total score for IBS-SSS correlated significantly with the total BWT of the sigmoid colon (r=0.39, p=0.0003) and the submucosal layer of the sigmoid colon (r=0.38, p=0.0005) but not with the ascending colon (r=0.11, p=0.3). In patients with IBS-D there was also a significant correlation between FC level and total BWT for the sigmoid colon (r=0.49, p<0.0001) (fig 2) but not for the ascending colon (r=0.08, p=0.4). Furthermore, the FC level correlated significantly with the BWT of all the layers of the sigmoid colon for patients with IBS-D as shown in Table III. In patients with UC, a significant correlation was only found between the FC level and the submucosal layer (r=0.46, p=0.02).

Color Doppler signal

None of the patients with IBS-D or HCs had visible vessels in the sigmoid colon using color Doppler. All of the UC patients had visible vessels (fig 3).

Colonoscopy

Colonoscopy for patients with IBS-D showed macroscopically normal colon mucosa and vascular markings without ulcerations. The histology of colon biopsies showed that the lamina propria was edematous and congested with mild increase in chronic inflammatory cells infiltrate, but no evidence of microscopic colitis, IBD or any other specific pathology.

Colonoscopy for patients with UC showed that 13 had proctosigmoiditis, 4 had left-sided UC, and 8 had total colitis. The severity of the disease was graded using the Mayo score (Table I).

Table II. Bowel wall thickness of the layers of the sigmoid colon in patients with irritable bowel syndrome and ulcerative colitis and healthy controls

Bowel wall layer	IBS-D	UC	HC	*P*[1]	*P*[2]	*P*[3]
Total	3.16 ± 0.09	4.20 ± 0.30	1.60 ± 0.12	0.030	< 0.0001	< 0.0001
Mucosa	0.50 ± 0.02	0.80 ± 0.08	0.30 ± 0.02	0.007	< 0.0001	< 0.0001
Submucosa	1.50 ± 0.06	2.1 ± 0.16	0.70 ± 0.06	0.017	< 0.0001	< 0.0001
Proper muscle	1.15 ± 0.04	1.30 ± 0.12	0.60 ± 0.05	0.900	< 0.0001	< 0.0001

Values are in mm and presented as mean ± standard error of the mean. Analysis was performed by the Kruskal-Wallis test with Dunn's post-test. [1]P value between the diarrhea-predominant irritable bowel syndrome (IBS-D) group and the ulcerative colitis (UC) group; [2]P value between the IBS-D group and the healthy control (HC) group; [3]P value between the UC group and the HC group.

Table III. Bowel wall thickness measurement of the sigmoid colon layers in patients with irritable bowel syndrome and their correlations to the Irritable Bowel Syndrome-Symptom Severity Scale and fecal calprotectin

Colon wall layers in mm				Investigation	*r, P* value[1]	*r, P* value[2]	*r, P* value[3]	*r, P* value[4]
Total	Mucosa	Submucosa	Proper muscle					
3.16 ± 0.09	0.50 ± 0.02	1.50 ± 0.06	1.15 ± 0.04	IBS-SSS 269 ± 11	0.39, 0.0003	0.07, 0.5	0.38, 0.0004	0.19, 0.2
				FC (µg/g) 251 ± 19	0.49, < 0.0001	0.32, 0.001	0.42, < 0.0001	0.24, 0.01

Values are presented as mean ± standard error of the mean. Spearman correlation test (r) between the investigation and the [1]total layer, [2]mucosa layer, [3]submucosa layer, and [4]proper muscle layer. P value < 0.05 was considered significant. FC: Fecal calprotectin; IBS-SSS: Irritable Bowel Syndrome-Symptom Severity Scale.

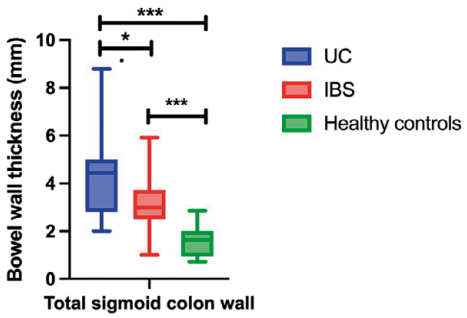

Fig 1. Bowel wall thickness in the sigmoid colon. Bowel wall thickness was measured by intestinal ultrasound in patients with diarrhea-predominant irritable bowel syndrome (IBS), ulcerative colitis (UC), and healthy controls. *p<0.05, **p<0.01, and ***p<0.001.

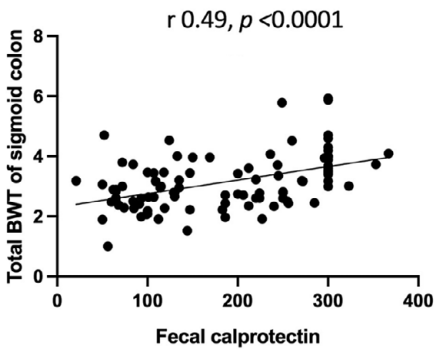

Fig 2. Correlation between fecal calprotectin levels and total bowel wall thickness of the sigmoid colon in patients with diarrhea-predominant irritable bowel syndrome. BWT: Bowel wall thickness.

Discussion

The current study investigated the BWT of the sigmoid colon in patients with IBS-D and correlated the BWT to the IBS-SSS score and FC level. We also compared the BWT of the sigmoid colon in patients with IBS-D with patients with UC. Our observations suggested that patients with IBS-D who present with elevated IBS-SSS scores and increased FC levels may also have ultrasonographic findings in the form of increased BWT without any color Doppler signals.

The measurement of the normal BWT of the colon differs between a distended colon lumen with fecal content (<2 mm) and a collapsed colon lumen without fecal content (4 mm) [21]. The measurement of BWT in the current study was conducted with distended lumen and showed that patients with IBS-D with an exacerbation of symptoms had increased wall thickness in all the layers of the sigmoid colon. The most used cutoff for BWT for determining active UC is 3 mm [22]. Patients with UC with an endoscopic Mayo 1 score could have a BWT less than 3 mm [15]. Therefore, an overlap could occur with patients with IBS-D in the current study. However, the absence of the color Doppler signal may assist in differentiating between IBS-D and IBD.

In a previous study, female patients with IBS had a muscularis thickness ranging between 1.4-10.0 mm with a mean of 3.6±1.7 mm measured by transvaginal ultrasound [23]. The same study showed that a cutoff of 3 mm for identifying IBS patients had a sensitivity of 70%, specificity of 95%, and positive and negative predictive values of 73% and 95%, respectively. This observation indicates that 3 mm is an adequate cutoff value to identify patients with IBS. However, the ultrasound equipment used in that study was not ideal for measuring the bowel wall because the images show very little detail of the bowel wall [23]. Pessarelli et al [24] showed that the total BWT of patients with IBS with cutoff values of 3 mm and 4 mm had an overall accuracy of 91% and 87%, respectively, which means that these cutoff values are able to discriminate between IBS and IBD (Crohn's disease) using IUS.

The current study showed that the submucosal layer is the thickest layer in the patients with UC. Our findings are consistent with the finding of a previous publication [25]. The thickened bowel wall layers in our study may

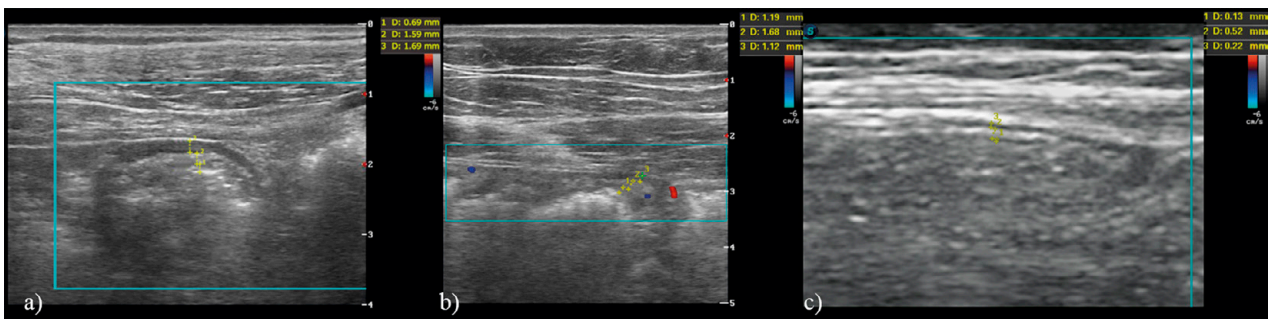

Fig 3. Ultrasonography with color Doppler signal of the bowel wall thickness: a). a patient with diarrhea-predominant irritable bowel syndrome during symptom exacerbation; b) a patient with ulcerative colitis; c) a healthy control. 1: thickness of mucosa layer (mm); 2: thickness of submucosa layer (mm); 3: thickness of proper muscle layer (mm).

be due to edematous changes due to low-grade inflammation or spasticity or a combination of both. Other studies have shown that a thick muscular layer is associated with colon spasticity and/or hypertrophy [26-29]. Another study showed that patients with IBS have increased basal colonic tone and pressure that are due to dysfunctional myenteric plexus neuronal signals [30] leading to chronic hypertonicity that could explain measurable hypertrophic wall thickness by ultrasonography [23].

Previous studies involving patients with IBS showed lower FC levels compared with our study. However, some of these studies were limited by inadequate sample sizes and did not assess IBS symptom severity using standardized symptom questionnaires. [31-35]. Another study by Melchior et al [36], showed that their subgroups of IBS patients had both high and normal FC levels and there was no significant difference in IBS-SSS scores in both subgroups. In that study, FC levels reached higher levels than 200 μg/g, especially in hypersensitive patients. A metanalysis showed that neither high nor low FC levels could rule out IBS. An FC level of 280 μg/g had an 18.8% peak predictive probability for IBS, while the same level gave a 17.0% probability for IBD. Therefore, the level of FC has a high negative predictive value but low positive predictive value [37].

Previous studies have shown that combining FC level with IUS to assess disease activity in patients with UC increases IUS sensitivity and specificity [15,16]. Our study showed that FC level correlates with the BWT of sigmoid colon, which is consistent with previous publications on UC [38,39]. This also supports the concept that patients with IBS may have low-grade inflammation that causes edema [37,40] as it is shown histologically in previous publications [41,42]. Melchior et al, also noted that samples from the colon of almost half of their IBS patients showed histologically signs of low-grade inflammation with mononuclear and mixed infiltrates [36], which is consistent with our results. However, they were not statistically different between IBS patients with normal vs. high FC levels [36].

The strength of this study is that all the participants delivered laboratory tests and underwent IUS, and colonoscopy with tissue biopsies within 48 h. This is important to rule out other organic pathologies as suggested by previous studies [23,43,44]. One limitation of the current study was the low number of patients with UC, due to the low prevalence of IBD in the Middle East region. Furthermore, the main investigator was not blinded to the diagnoses of the different groups and inter- and intra-observer variability was not performed. However, measuring the bowel wall thickness using intestinal ultrasound is reproducible, reliable and validated [45-46]. This underscores the need for future studies that address these limitations.

Conclusion

Patients with IBS-D and a symptom exacerbation presenting with a high IBS-SSS score and slightly increased FC level may present with BWT > 2 mm in the sigmoid colon on IUS. Compared to healthy controls, BWT of the sigmoid colon is significantly increased in IBS-D patients. The use of IUS with color doppler, may be beneficial in clinical practice to help in differentiating between exacerbating symptoms in conditions such as those occurring in IBS-D and UC patients. This study is the first to find a significant association between the FC level and BWT using IUS in patients with IBS-D.

Conflict of interest: none

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