Original Article

DOI: 10.7860/JCDR/2024/67264.19569

Evaluation of Faecal Calprotectin S100A8/S100A9 Levels in Patients Suffering from Irritable Bowel Syndrome: A Cross-sectional Study

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ABSTRACT

Introduction: Irritable Bowel Syndrome (IBS) is a common functional bowel disorder which remains a clinical challenge with limited therapeutic options. The diagnosis of IBS is made by the Rome IV criteria. Patients suffering from IBS often have impaired poor quality of life. Distinguishing IBS from Inflammatory Bowel Diseases (IBD) can be difficult, especially with mild disease activity. Both conditions share a symptom complex with abdominal pain and altered bowel habits but potentially have different treatments for each disorder.

Aim: To evaluate Faecal Calprotectin S100A8/S100A9 Levels (FCL) in IBS patients and compare those levels with patients suffering from the IBD group.

Materials and Methods: This was a cross-sectional study conducted for a period of one year from June 2022 to May 2023 at the Department of Pathology in collaboration with the Department of Medicine, Khaja Bandanawaz (KBN) University-Faculty of Medical Sciences, Kalaburagi Karnataka, India. The study included 130 patients aged between 18 and 65 years with chronic diarrhoea, consisting of 90 cases in the IBS group and 40 cases in the IBD group for comparison. After obtaining clinical details, FCL were analysed by Fluorescence Immunoassay (FIA). Patients were subjected to colonoscopic evaluation, and biopsies were taken for processing. FCL, colonoscopic, and histopathological findings were evaluated in IBS patients and IBD patients, respectively. Data were analysed using the Statistical Package for Social Sciences (SPSS) version 24.0 software package. Quantitative data were expressed as mean±Standard Deviation (SD). Qualitative data were expressed using U-test Fisher's-exact and Chi-square test, where a p-value <0.05 was considered statistically significant.

Results: The age range was from 18 to 65 years with a male to female ratio of 1:2 and a mean age of 42.2 years. Among the 90 IBS cases, IBS-diarrhoea predominant was the largest subgroup with 65 patients (72.2%) clinically. Seventeen IBS cases showed elevated FCL levels, with four patients having FCL levels higher than those in IBD cases in a quiescent stage. The mean±SD FCL levels in the IBS subgroup were 80.45±76.4 µg/g Out of the 17 cases with elevated FCL levels, 10 showed features of microscopic colitis {lymphocytic colitis (7), collagenous colitis (2), indeterminate (1)}, respectively. Thirty-seven IBD patients had elevated FCL levels, with a mean±SD FCL level in the IBD subgroup of 180.20 ± 386.4 µg/g, and 12 patients (30.0%) had levels higher than 500 µg/g.

Conclusion: The study concluded that FCL levels are elevated in IBS patients also. The FCL levels in the IBS-diarrhoea subgroup were elevated more than the IBD-quiescent subgroup. IBS cases with elevated FCL levels showed positive colonoscopic findings and histopathological features of microscopic colitis on biopsy. These IBS cases require anti-inflammatory treatment as they do not respond to regular anti-IBS treatment. Furthermore, these IBS patients should be followed-up as they may potentially develop into future IBD cases.

Keywords: Inflammatory bowel disease, Microscopic colitis, Rome IV criteria

INTRODUCTION

IBS is a common functional bowel disorder characterised by recurrent abdominal pain associated with a change in bowel habits (constipation, diarrhoea, or both) [1]. It is one of the most frequent reasons for patients seeking primary care through gastroenterology consultation. IBS is a multifactorial disease and remains a clinical challenge with limited therapeutic options [2]. Patients suffering from IBS often have impaired poor quality of life with increased social and economic costs due to frequent recourse to healthcare resources. The diagnosis of IBS is made as defined by the Rome IV criteria [3]. The various aetiopathogenesis includes gut-brain axis dysfunction, visceral hypersensitivity, increased intestinal hypermotility, psychological and stress disorders, and gut microbiota [4]. Many organic conditions can be falsely diagnosed as IBS, including IBD, GI malignancies, coeliac disease, etc. Rare causes including Small Intestinal Bacterial Overgrowth (SIBO), pancreatic insufficiency, and diverticular diseases can mimic IBS, but data are conflicting and uncertain with poor sensitivity of test results [5]. However, recent data in gastroenterology literature shows that IBS and IBS-like clinical features can be present in stable/subtle organic Gastro-Intestinal (GI) diseases, such as in quiescent IBD and an IBS-like scenario in post-infectious colitis and coeliac disease [6]. Recognising functional gut symptoms in these individuals is of paramount importance as attention can be focused on addressing disorders of gut-brain interaction and potential adverse effects of immune-suppressive therapy can be avoided. Also, the use of anti-inflammatory drugs in IBS is controversial [7,8].

Calprotectin is a calcium-binding cytosolic protein present in neutrophils. Calprotectin accumulates at the inflammatory site in the GI Tract (GIT) and is excreted in faeces, which are resistant to bacterial degradation. Various studies have shown the role of elevated FCLs in assessing the extent of inflammation in IBD patients with 93% sensitivity and 96% specificity, respectively [9]. The levels also help differentiate between organic and non organic causes of intestinal diseases in symptomatic patients referred for colonoscopy [10]. Screening the FCLs is now indicated as a non invasive test to identify persistent occult inflammation among IBD patients in clinical

remission. The use of FCLs as a biomarker of intestinal inflammation represents an interesting targeted therapeutic approach not only to rule out IBD but also to identify potential IBS candidates for anti-inflammatory treatment [11].

The aim of the study was:

(a) To assess and compare FCL in IBS patients and in patients with IBD;

(b) To compare the characteristics of patients with and without high calprotectin levels;

(c) To recognise the potential overlap between IBS and organic GI diseases, in order to aid practicing clinicians and gastroenterologists and address the need for future research in this area.

MATERIALS AND METHODS

This study was a cross-sectional study conducted in the Department of Pathology in collaboration with the Department of Gastroenterology at KBN University-Faculty of Medical Sciences, Kalaburagi, Karnataka, India. Institutional Ethical Clearance (IEC) was taken for the study (IEC No: KBNU-FM/IEC/2022-23/134). The study was carried out over a period of one year from 2022 to May 2023.

Inclusion criteria: A total of 130 patients were included in the study. Patients were subgrouped into the IBS group according to the Rome IV criteria and had normal colonoscopy findings [1]. Ninety patients were classified into the IBS subgroup and 40 patients into the IBD subgroup. Clinical features of IBD patients included abdominal pain, diarrhoea, sometimes with blood, urgency to have a bowel movement, faecal incontinence, rectal bleeding, and weight loss for chronic and longer durations extending up to months. If these features were present, the patients were classified as IBD-active, and if the clinical features were subtle, they were classified as IBD-quiescent. These IBD patients were further subgrouped into active and quiescent states (based on clinical features) for comparison [9]. Patients were aged from 18 to 65 years old.

Exclusion criteria: Patients presenting with diarrhoea due to infections, drug-induced diarrhoea, and pancreatic/bile acid deficiency, as well as patients suffering from any organic GI diseases, were excluded from the study. Cases in the paediatric age group were also excluded.

Study Procedure

Clinical details, radiological investigations, and routine laboratory investigations including complete haemogram, liver and renal function tests, serum electrolytes, inflammatory markers-C-Reactive Proteins (CRP), and thyroid profile were compared. CRP was increased in IBD cases compared to IBS patients, giving a clue of the inflammatory process in the GIT of IBD patients. The above parameters were not tabulated since none of the parameters were significantly related to the IBS or IBD group except for CRP.

FCLs were analysed by FIA based on the sandwich immune detection method. The detector antibodies bind to antigens present in the faecal sample, forming antigen-antibody complexes that migrate to the nitrocellulose matrix, which is detected by a fluorescence signal. The manufacturer-quoted cut-off values for most FCL assays are similar. Most laboratories have taken 50 µg/g faeces as the recommended cut-off.

Patients were further subjected to colonoscopic evaluation, and segmental biopsies were taken from the right-sided colon (cecum, ascending colon, and transverse colon), left-sided colon (descending colon and sigmoid colon), and the rectum. The biopsies were placed in 10% formalin bottles and sent for histopathological processing. The colonic tissue was stained with routine Haematoxylin-Eosin (H&E) staining. In suspected cases of microscopic collagenous colitis, Masson trichrome staining was performed for the collagen layer. In suspected cases of microscopic lymphocytic colitis, the mean number of Intraepithelial Lymphocytes (IEL) was expressed per 100 epithelial cells. Colonoscopic findings, histopathological findings, and FCL in IBS patients were compared with those in IBD patients. The biopsies were considered normal when there were less than five IEL/100 surface epithelial cells, the collagen layer was less than 5 µm (by morphometry), and no other pathological changes in the epithelium and lamina propria were found. The biopsies were considered abnormal when there were ≥20 IEL per 100 surface epithelial cells, thickening of a subepithelial collagen layer of more than 10 µm, and pathological changes in the lamina propria. IEL between 5-19 per 100 surface epithelial cells were considered indeterminate [8].

STATISTICAL ANALYSIS

Data were analysed using SPSS version 24.0 software package. Quantitative data were expressed as mean±SD. Qualitative data were expressed in terms of frequency and percentage and were evaluated using U-test Fisher's-exact and Chi-square tests. A p-value <0.05 was considered statistically significant.

RESULTS

The age ranged from 18 to 65 years with a male to female ratio of 1:2 and a mean age of 42.2 years. The clinical details of IBS patients are listed in [Table/Fig-1]. The diarrhoea-predominant IBS subgroup constituted the largest subgroup with 65 (72.2%) of total patients, with 22 (24.4%) patients presenting with post-infectious IBS symptoms. Sixteen (17.8%) patients also complained of significant weight loss.

Clinical features	n (%)
Diarrhoea predominant	65 (72.2)
Constipation predominant	15 (16.7)
Alternating	8 (8.9)
Undetermined	2 (2.2)
Post-infectious IBS	22 (24.4)
Worsening of symptoms by stressful events	43 (47.8)
Nocturnal pain	17 (18.9)
Weight loss	16 (17.8)

[Table/Fig-1]: Clinical profile of IBS patients.

FCLs were estimated by FIA in all IBS patients, IBD active, and IBD quiescent subgroups, respectively. The results of FCLs in various IBS subgroups are tabulated in [Table/Fig-2]. FCL levels were more elevated in the IBS-diarrhoea subgroup compared to other groups. Seventeen IBS patients had elevated FCL. The mean FCL in the IBS subgroup was 80.45±76.4 µg/g, and a subgroup of 17 patients (18.89%) had levels higher than 50 µg/g [Table/Fig-2].

	Faecal Calprotectin Levels (FCL) (µg/g)						
IBS-subgroups (90)	0-25	25-50	50-100	100-200	200-300	300-400	400-500
IBS-Diarrhoea (65)	24	31	04	02	02	01	01
IBS-constipated (15)	04	06	03	01	01		
IBS-alternating (08)	02	04	01	01			
IBS-undetermined (02)	01	01					
p-value	0.0589						

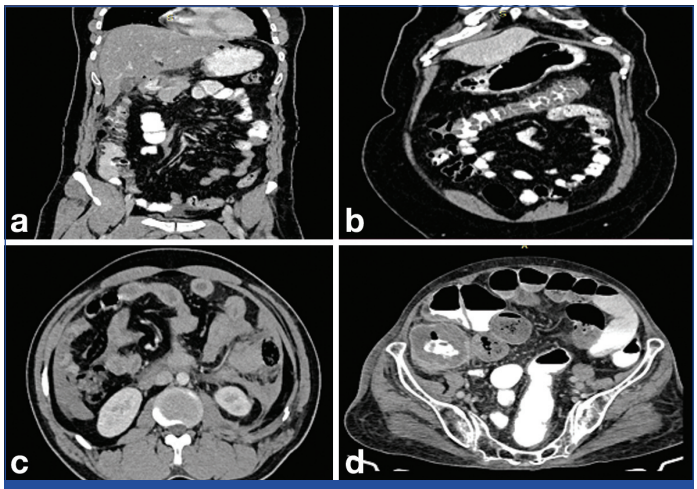
[Table/Fig-2]: Faecal Calprotectin Levels (FCL) in IBS subgroups.

Variations in FCL levels in IBD subgroups were evaluated and tabulated in [Table/Fig-3]. Thirty-seven IBD patients had elevated FCL levels. The mean FCL in the IBD subgroup was 180.20±386.4 µg/g, and a subgroup of 12 patients (30.0%) had levels higher than 500 µg/g.

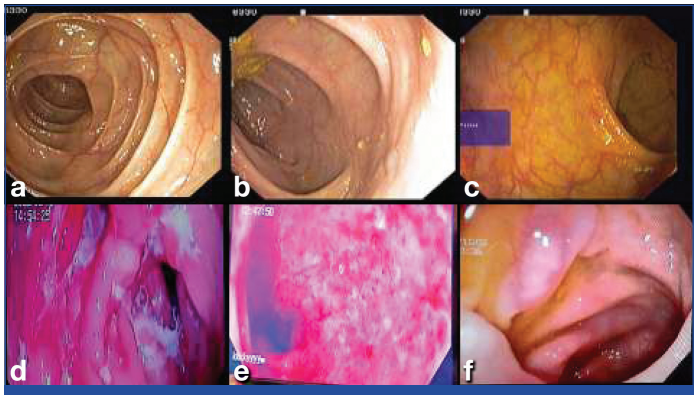
IBD-subgroups (n=40)	Faecal Calprotectin Levels (FCL) (µg/g)									
	0-25	25-50	51-100	100-200	200-300	300-400	400-500	500-1000	1000-1500	>1500
IBD-UC-quiescent (16)		01	02	06	05	01	01			
IBD-UC-active (09)						01	01	03	02	02
IBD-Crohns-Quiescent (09)		02	02	01	02	01				
IBD-Crohns-Active (06)							01	02	02	01
p-value	0.039									

[Table/Fig-3]: Faecal Calprotectin Levels (FCL) in IBD subgroups.
UC: Ulcerative colitis

Radiological details were compared in [Table/Fig-4], and patients were subjected to colonoscopic evaluation, and segmental biopsies were taken from the right-sided colon, left-sided colon, and the rectum. Out of 130 cases, a colonoscopy procedure was performed on 121 patients, while nine patients in the clinically diagnosed IBS subgroup did not agree to undergo colonoscopy. Comparative differences in colonoscopic findings in IBS and IBD subgroups are shown in [Table/Fig-5]. The colonoscopic findings in IBS patients ranged from near-normal to mild erythematous changes, while in IBD patients, positive colonoscopic findings included erythematous changes, multiple ulcerated mucosa, blood oozing from friable mucosa to complete stricture in the terminal ileum. The colonoscopic



[Table/Fig-4]: MRI contrast images: a) Longitudinal view showing normal bowel thickness in IBS case; b) Longitudinal view showing moderate increased bowel thickness in IBD case; c) Cross-sectional view showing mild enhanced echogenecity in IBS case; d) Cross-sectional view showing altered enhanced echogenecity in IBD case.



[Table/Fig-5]: Colonoscopic findings: a-c) Showing colonoscopic findings in IBS patients with near normal colonic images in comparison with; d-f) Images of Inflammatory Bowel Disease (IBD), deformed ileum-terminal ileal stricture? TB/IBD; d) rectum-erythamatous areas, fraible mucosa, spontaneous oozing S/O ulcerative colitis; e) erthamatous areas with apthae- descending colon (f).

findings suggest that IBS is a functional disorder compared to IBD, which is an inflammatory disorder. Histopathological analysis of 121 biopsies was done. The histopathological findings were compared in both IBS and IBD subgroups and tabulated in [Table/Fig-6]. There was a statistically significant difference (p-value <0.05) between microscopic colitis compared to the IBD subgroup regarding the inflammatory marker FCL.

Histopathological findings showed that out of 17 IBS patients with elevated FCL levels, 10 patients showed features of Microscopic colitis {lymphocytic colitis (07) [Table/Fig-7], collagenous colitis (02) [Table/Fig-8], indeterminate (01)} on histopathology, respectively, compared to the IBD subgroup [Table/Fig-9]. There was a statistically significant difference (p-value <0.038) between microscopic colitis compared to the IBD subgroup regarding the inflammatory marker FCL. Regarding FCL, the mean FCL in normal biopsies was 2.2±0.98176 mg/L, while it was 6.2±1.4 mg/L in Microscopic colitis patients.

DISCUSSION

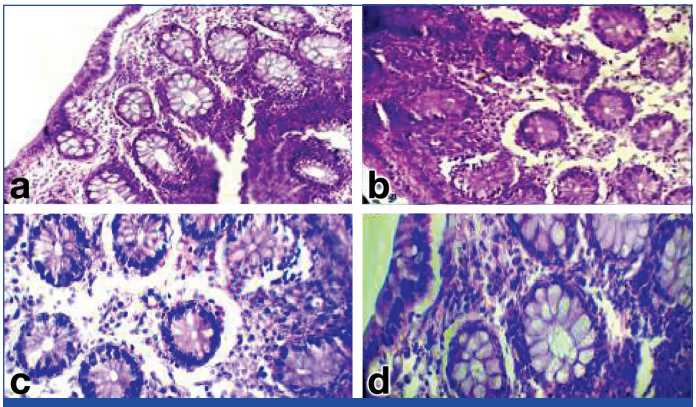
IBS is a bowel disorder associated with chronic abdominal pain, with patients presenting with multiple episodes of diarrhoea, crampy abdominal pain to severe constipation, in the absence of any organic cause [1]. It affects patients' quality of life with physical, psychological, social, and economic non productivity. The incidence of IBS is estimated to be 11.2%, but the figures may be higher due to heterogeneity in published studies [2]. In view of the recent and growing interest in the gut microbiome and GI diseases, including IBS, data from Asian research (where infections and infestations are common) may offer specific epidemiological insight. Several population-based studies show the prevalence of IBS in India varies from 4.2-7.5%, suggesting that IBS might be related to economic development associated with lifestyle changes, fast living, and psychological stress associated with reduced T regulatory cell response [3,4].

Both IBS and IBD are chronic disorders that are challenging to diagnose in the initial phase as clinical signs and symptoms overlap with on and off signs and symptoms in due course [9,10]. Hence, FCL plays an important role in differentiating both. In the majority of IBD cases, there are high FCL levels, whereas IBS patients typically exhibit nil to mildly elevated FCL levels [11]. Further evaluation through colonoscopic findings helps in confirmation. IBD patients present with multiple apthous ulcers in the gastrointestinal tract, while IBS patients show almost normal colonoscopic findings, as discussed in the results of this study. Since IBS is a disorder of gut-brain interaction, the treatment alters in comparison to IBD, which is caused by an altered immune system, and anti-inflammatory drugs are effective in treating it. This study takes a step further by examining patients who present with combined features of both IBS and IBD in

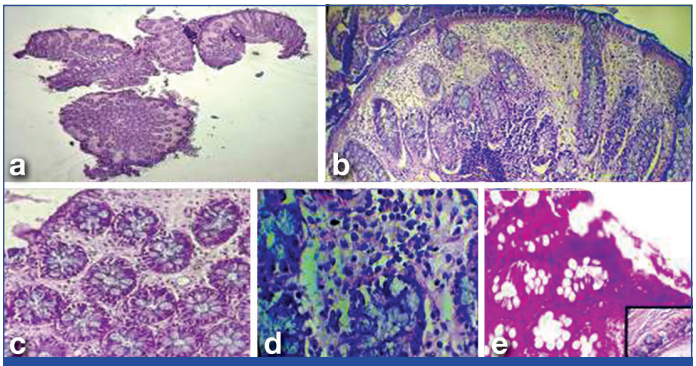
No. of patients (121)	Normal	Minimal change colitis	Chronic colitis (non specific)	Microscopic colitis			Chronic colitis due to IBD
IBS- subgroup*				Lymphocytic colitis	Collagenous colitis	Indeterminate (MCI)	
IBS-diarrhoea (59)	36	05	09	06	02	01	00
IBS-constipated (12)	07	01	03	01	00	00	00
IBS-alternating (08)	06	00	02	00	00	00	00

IBS-undetermined (02)	02	00	00	00	00	00	00
p-value	**0.0374***						
IBD-subgroup							
IBD-UC-quiescent (16)	04	02	04	00	00	01	05
IBD-UC-active (09)	00	00	02	00	00	00	07
IBD-Crohns-quiescent (09)	04	00	02	00	00	00	03
IBD-Crohns-active (06)	00	00	02	00	00	00	04
p-value	**0.027**						

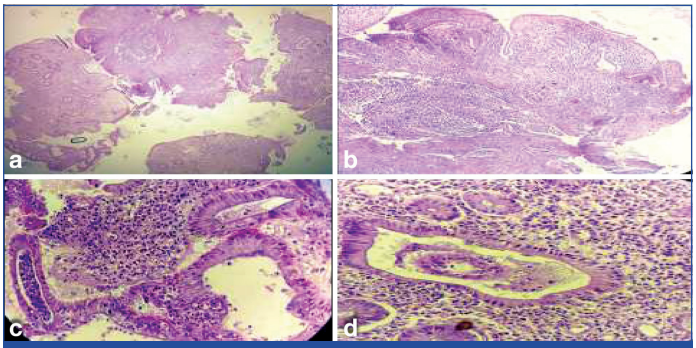
[Table/Fig-6]: Comparative analysis of histopathological findings in IBS and IBD sub-group.
*9 patients in clinically diagnosed IBS subgroup didn't agreed for colonoscopy



[Table/Fig-7]: Lymphocytic colitis: a) Intact crypt architecture (H&E-10x); b) Increased surface Intraepithelial Lymphocytes (H&E-10x). c,d) Mixed lamina propria inflammatory infiltrate (H&E-40x).



[Table/Fig-8]: Collagenous colitis: a) Preserved architecture (H&E-4x); b) Thickened subepithelial collagen (H&E-10x); c) Lamina propria expansion (H&E-10x); d) Intraepithelial Lymphocytosis (IEL) (H&E)-40x); e) Trichrome stain positive (H&E-40x).



[Table/Fig-9]: Inflammatory Bowel Disease (IBD)- ulcerative colitis: a) Low power view of a biopsy inflammation and architectural distortion involving all biopsy fragments (H&E-4x); b) Active chronic colitis, including crypt irregularity, basal lymphoplasma cytosis (H&E-10x); c) Active chronic colitis with cryptitis (H&E-40x); d) Severe active chronic colitis with multifocal surface ulceration (H&E-40x).

the initial phase, assisting gastroenterologists in treatment decisions by comparing radiological findings, colonoscopic findings, FCLs, and histopathological features [12,13].

In this study, the diarrhoea-predominant IBS subgroup constituted the largest subgroup, with 72.2% of the total patients, and 24.4% of patients present with post-infectious IBS symptoms. Studies done by Abd El-Fattah Badran MA et al., showed that the major clinical manifestations were abdominal distention (55%), nocturnal diarrhoea (15%), and weight loss (15%), respectively [14].

Calprotectin is a calcium-binding protein that makes up 60% of the cytosolic protein content of neutrophils. In IBD, a clear relationship has been demonstrated between the magnitude of calprotectin elevation and the extent of intestinal inflammation [4]. An FCL of more than 50 mg/g of faeces indicates organic intestinal disease with 84.4% sensitivity and 94.5% specificity, respectively. Elevated FCL levels can be used as a potential biochemical biomarker of intestinal inflammation, aiding in a targeted therapeutic approach for IBD patients and potential IBS cases. Screening calprotectin levels is now indicated as a non invasive test to identify persistent occult inflammation among IBD patients in clinical remission [5,6].

In this study, calprotectin levels were quantified in IBS patients and attempted to associate these levels with histological inflammation to identify a subgroup of IBS patients for whom anti-inflammatory intestinal treatment could be discussed. In this series of 90 IBS cases, 17 patients had elevated FCL levels. FCL levels were more elevated in the IBS-diarrhoea subgroup compared to other groups. The mean FCL in the IBS subgroup was 80.45±176.4 µg/g. FCL levels in IBS patients were also higher than in a few patients with quiescent IBD (four cases).

This subgroup of IBS patients with elevated calprotectin was observed in a younger age group compared to other studies, which indicated a positive correlation between increasing age and abnormal calprotectin levels [1,7].

A study done by Kane JS et al., on 151 cases of IBS revealed that 78 (51.7%) individuals were diagnosed with CC, 59 (39.1%) with LC, and 14 (9.3%) with MC-NOS, respectively [15].

Colonoscopy results in all IBS patients showed normal colonic mucosa and vasculature without ulcers, masses, or diverticula. A study by Hilmi I et al., on 120 cases of IBS in Asian patients demonstrated normal colonoscopic findings in the majority of cases. In the IBS-D group, the macroscopic colonoscopy findings were as follows: normal findings in 58 cases (78.4%), diverticula disease in 5 cases (6.8%), diminutive polyps in 9 cases (12.2%), and haemorrhoids in 2 cases (2.7%). No subjects under the age of 40 had any significant findings. Colonoscopy findings in the control group were: normal findings in 27 cases (58.7%), adenomas in 15 cases (32.6%) (including one large rectal polyp of 1 cm, the others <1 cm), diverticula disease in 3 cases (6.5%), and haemorrhoids in 1 case (2.2%) [16].

Microscopic examination of biopsies from multiple sites of the endoscopically normal colonic mucosa revealed that 51 IBS patients had normal microscopic features, while 30 IBS patients exhibited various microscopic features. This included 23 IBS diarrhoea-predominant patients, with five patients showing minimal colitis, nine with chronic non specific colitis, and nine with microscopic colitis,

comprising six cases of lymphocytic colitis, two cases of collagenous colitis, and one case of microscopic colitis indeterminate. Similarly, in the IBS-constipated subgroup, one case each of minimal colitis and lymphocytic colitis, and three cases of chronic non specific colitis were reported. Chronic non specific colitis was also seen in two cases of the IBS-alternating subgroup. In total, 10% of IBS subgroup patients showed features of microscopic colitis. A study by Abd El-Fattah Badran MA et al., revealed that 20% of cases exhibited a histologic picture consistent with microscopic colitis, with 90% of them being lymphocytic colitis and 10% being collagenous colitis [14]. A similar study by Carmona-Sánchez R et al., showed a prevalence of microscopic colitis in IBS-D patients at 18%, while the study by Kamp EJ et al., demonstrated a prevalence of microscopic colitis in 23.3% of patients [17,18].

In the comparison group of IBD cases comprising 40 patients, 15 active IBD patients exhibited classical microscopic features of IBD with elevated faecal calprotectin levels. Among the 25 quiescent IBD cases, two cases showed normal faecal calprotectin levels with minimal colitis on histopathology, and ten cases showed minimal elevation in faecal calprotectin levels with microscopy showing chronic non specific colitis.

This study revealed that the IBS-diarrhoea predominant subgroup with maximum cases with female predominance, which was comparable to previous studies. One of the reason for this high prevalence could be post-infectious IBS, even after prophylactic antibiotic treatment in suspected cases. Other factors contributing to this over-representation in our referral centre include discomfort and pain associated with diarrhoea, impacting the quality of life of patients. Faecal calprotectin levels were estimated using only one sample in the analysis. A similar study conducted by Tibble JA et al., showed significant correlation in 22 patients between the results of a single stool analysis and the 4-day faecal calprotectin expression [19].

Elevated calprotectin levels, apart from IBD and IBS patients, are also observed in conditions such as Small Intestinal Bacterial Overgrowth (SIBO), systemic sclerosis, hypersensitive patients, and patients on FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols). This aspect could not be confirmed in present study due to limited resources, a shorter duration of studies, patients' refusal of repeat colonoscopy during follow-up, superficial biopsies limited to mucosa and submucosa without involvement of deeper muscle layers to study the role of mast cells, and loss of patient follow-up over longer durations [12]. Additionally, further evaluation is needed for patients with elevated faecal calprotectin in microscopic lymphocytic colitis. Recent studies have shown the role of TNF-alpha as an inflammatory marker that increases colonic paracellular permeability, leading to mild to moderate elevation in faecal calprotectin levels in quiescent IBD patients with IBS subtype. It would be interesting to investigate whether elevated faecal calprotectin levels can be normalised through dietary interventions such as low FODMAPs and/or a gluten-free diet [13,14].

Limitation(s)

Limitations of the study include its one-year duration, small sample size, limited resources, patients' refusal of repeat colonoscopy during follow-up, and the need for long-term follow-up and more wider sample size to gain more insights into the inflammatory role in IBS cases.

CONCLUSION(S)

Faecal calprotectin testing is a simple method for detecting inflammatory processes in the colon and is elevated in IBD cases. Early cases of IBD may be misdiagnosed as IBS and may not respond to regular anti-IBS treatments. This study demonstrates that faecal calprotectin levels are elevated mainly in the IBS-diarrhoea predominant subgroup of IBS patients. Regular evaluation of faecal calprotectin levels can assist clinicians in guiding follow-up care for IBS cases, conducting colonoscopic evaluations, and determining treatment options.

REFERENCES

[1] Lacy BE, Mearin F, Chang L, Chey WD, Lembo AJ, Simren M, et al. Bowel disorders. Gastroenterology. 2016;S0016-5085(16)00222-5.

[2] Rahman MM, Mahadeva S, Ghoshal UC. Epidemiological and clinical perspectives on irritable bowel syndrome in India, Bangladesh and Malaysia: A review. World J Gastroenterol. 2017;23(37):6788-801.

[3] Melchior C, Aziz M, Aubry T, Gourcerol G, Quillard M, Zalar A, et al. Does calprotectin level identify a subgroup among patients suffering from irritable bowel syndrome? Results of a prospective study. United European Gastroenterol J. 2017;5(2):261-69.

[4] Mihaly E, Patai Á, Tulassay Z. Controversials of microscopic colitis. Front Med (Lausanne). 2021;8:717438. Doi: 10.3389/fmed.2021.717438.

[5] Ford AC, Sperber AD, Corsetti M, Camilleri M. Irritable bowel syndrome. Lancet. 2020;396(10263):1675-88. Doi: 10.1016/S0140-6736(20)31548-8.

[6] Bohr J, Wickbom A, Hegedus A, Nyhlin N, Hultgren Hörnquist E, Tysk C. Diagnosis and management of microscopic colitis: Current perspectives. Clin Exp Gastroenterol. 2014;7:273-84. Doi: 10.2147/CEG.S63905.

[7] Schmulson M, Corazziari E, Ghoshal UC, Myung SJ, Gerson CD, Quigley E, et al. A four-country comparison of healthcare systems, implementation of diagnostic criteria, and treatment availability for functional gastrointestinal disorders: A report of the Rome Foundation Working Team on cross cultural, multinational research. Neurogastroenterol Motil. 2014;26(10):1368-85.

[8] Liu Y, Chen M. Insights into the underlying mechanisms and clinical management of microscopic colitis in relation to other gastrointestinal disorders. Gastroenterol Rep (Oxf). 2022;10:goac011. Doi: 10.1093/gastro/goac011. eCollection 2022. PMID: 35401986.

[9] Aziz I, Törnblom H, Palsson OS, Whitehead WE, Simrén M. How the change in IBS criteria from Rome III to Rome IV impacts on clinical characteristics and key pathophysiological factors. Am J Gastroenterol. 2018;113(7):1017-25.

[10] Miehlke S, Guagnozzi D, Zabana Y, Tontini GE, Kanstrup Fiehn AM, Wildt S, et al. European guidelines on microscopic colitis: United European gastroenterology and european microscopic colitis group statements and recommendations. United European Gastroenterol J. 2020;20:2050640620951905.

[11] Münch A, Sanders SD, Molloy-Bland M, Hungin APS. Undiagnosed microscopic colitis: A hidden cause of chronic diarrhoea and a frequently missed treatment opportunity. Frontline Gastroenterol. 2020;11(3):228-34.

[12] Townsend T, Campbell F, O'Toole P, Probert C. Microscopic colitis: Diagnosis and management. Frontline Gastroenterol. 2019;10(4):388-93.

[13] Mars RAT, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, et al. Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. Cell. 2020;182(6):1460-73.e1417. Doi: 10.1016/j.cell.2020.08.007.

[14] Abd El-Fattah Badran MA, Rozik MS, Hammam OA, Mohamed MG. Prevalence of microscopic colitis in patients fulfilling Rome IV criteria of irritable bowel syndrome diarrheal type. Al-Azhar Med J (Medicine). 2021;50(1):707-18. Doi: 10.12816/amj.2021.139865 Available from: https://amj.journals.ekb.eg/article_139865.html.

[15] Kane JS, Irvine AJ, Derwa Y. High prevalence of irritable bowel syndrome-type symptoms in microscopiccolitis: Implications for treatment. Ther Adv Gastroenterol. 2018;11:1756284818783600.

[16] Hilmi I, Hartono JL, Pailoor J, Mahadeva S, Goh KL. Low prevalence of 'classical' microscopic colitis but evidence of microscopic Inflammation in asian irritable bowel syndrome patients with diarrhoea. BMC Gastroenterol. 2013;13:80. Available from: http://www.biomedcentral.com/1471-230X/13/80.

[17] Carmona-Sánchez R, Carrera-Álvare Z MA, Pérez-Aguilar RM. Prevalence of microscopic colitis in patients with irritable bowel syndrome with diarrhea predominance. Rev Gastroenterol Mex. 2011;76(1):39-45.

[18] Kamp EJ, Kane JS, Ford AC. Irritable bowel syndrome and microscopic colitis: A systematic review and meta-analysis. Clin Gastroenterol Hepatol. 2016;14(5):659-68.

[19] Tibble JA, Sigthorsson G, Bridger S, Fagerhol MK, Bjarnason I. Surrogate markers of intestinal inflammation are predictive of relapse in patients with inflammatory bowel disease. Gastroenterology. 2000;119(1):15-22. Doi: 10.1053/gast.2000.8523.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Aug 30, 2023
- Manual Googling: Feb 12, 2024
- iThenticate Software: Mar 20, 2024 (13%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Aug 28, 2023**
Date of Peer Review: **Dec 01, 2023**
Date of Acceptance: **Mar 23, 2024**
Date of Publishing: **Jun 01, 2024**