

Evaluation of Colonic Mucosa of Non-IBS And Non-Endoscopic Large Bowel Disorders in Metabolic Syndrome

Mohamed Darwish Ahmed Abd Alla^{1*}, Ayman Mohammed Abd Alazez², Mohammed Ragab Sayed², Sara Mamdouh Youssif², Mohammed Abdel Moneam², Osama Abdallah Ismail², Ahmed Youssif Montasser³

¹Department of Hepatology, Gastroenterology, and Infectious diseases, Faculty of Medicine, Al- Azhar University, Cairo, Egypt.

²Department of Hepatology, Gastroenterology, Theodor Bilharz research institute.

³Department of pathology, Theodor Bilharz research institute.

Corresponding Author: Mohamed Darwish Ahmed Abd Alla, E-mail: darwish0716@azhar.edu.eg

ABSTRACT

Background: Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder. Metabolic syndrome (MetS) is suspected to play a pro-inflammatory role in colonic mucosal microenvironment.

Aim of the work: To evaluate the mucosal histopathological and histochemical alterations of the colonic mucosa in metabolic syndrome (MetS) patients with non-organic and non-IBS large bowel disorders.

Patients and Methods: This cross-sectional study analyzed 199 symptomatic adults with normal colonoscopy findings, classified into four groups based on Rome IV criteria and MetS guidelines: Group I (IBS without MetS, n=47), Group II (IBS with MetS, n=80), Group III (non-IBS with MetS, n=49), and Group IV (non-IBS without MetS, n=23). Biopsies obtained from the right and left colon underwent blinded histopathological analysis to grade mucosal cellular infiltrates and architectural features.

Results: In the left colon, lymphocyte cellular infiltrate (LCI) differed significantly among the groups ($p = 0.002$), with moderate-to-severe grades predominantly clustered in Group II (15.0%) compared to Group I (2.1%) and Group III (6.1%). Significant pairwise LCI differences were verified between Groups I vs. II ($p < 0.001$) and II vs. III ($p = 0.038$). In the right colon, abnormal eosinophil cellular infiltrate (ECI) was significantly more frequent in Group II than Group III (21.3% vs. 6.1%, $p = 0.049$). A verified positive correlation was identified between LCI and MetS parameters in both the left colon ($\rho = 0.261$, adjusted $p = 0.003$) and right colon ($\rho = 0.200$, adjusted $p = 0.032$).

Conclusion: Metabolic syndrome acts as a histological catalyst, inducing distinct mucosal low-grade inflammation (especially lymphocytic infiltration) in patients with IBS functional bowel symptoms.

Keywords: Irritable bowel syndrome; Metabolic syndrome; Colonic mucosa; Low-grade inflammation.

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by symptoms such as abdominal pain or discomfort, alteration of bowel habits, relief of symptoms upon defecation, aggravation because of psychological strain and absence of organic abnormalities on colorectal examination. Prevalence ranges from 10% to 15% of the general population in major developed countries [1].

No known structural or anatomic explanation accounts for the pathophysiology of IBS, although several mechanisms have been proposed, including low-grade mucosal inflammation, visceral hypersensitivity, alterations in fecal flora, and bacterial overgrowth [2].

The current gold standard for IBS diagnosis is through the Rome IV symptom-based diagnostic criteria although it doesn't have high reliability. In addition, a few organic diseases share symptoms with IBS, including Crohn's disease, ulcerative colitis, and celiac disease. Because of similarities in clinical presentation, many physicians proceed with invasive testing to rule out organic disease before confirming a diagnosis of IBS. ROME IV defined IBS as a recurrent abdominal pain on average at least 1 day/week in the last 3 months that is associated with two or more of the following criteria: a. Relation to defecation b. Association with a change in frequency of bowel evacuation c. Changes in consistency of stool. These criteria should be fulfilled for the last 3 months, with onset of symptoms for 6 months prior to the diagnosis [3].

Colonoscopy is a common test used to confirm the absence of pathology that might be responsible for a patient's intestinal symptoms, such as IBD or colon cancer. Histopathological examination of the gut mucosa is useful to exclude microscopic colitis, eosinophilic enteritis, and amyloidosis. Because microscopic colitis is diagnosed using histological criteria, a colonoscopy should be considered in cases of refractory IBS [4].

Microscopic colitis (MC) consists of 2 primary subtypes: collagenous and lymphocytic colitis. Collagenous colitis (CC) differs from lymphocytic colitis (LC) in that it presents with a subepithelial collagen band adjacent to the basal membrane, whereas the hallmark of LC is dense lymphocytic infiltration in the epithelium. The incidence of MC has been reported as 2.6 to 21.0 per 100,000 per year. It has 3 key elements: a clinical history of chronic watery diarrhea, normal or almost-normal macroscopic appearance of the colon at colonoscopy, and a distinct

histologic pattern. Up to 10%-20% of chronic diarrhea is thought to be secondary to MC. The diagnosis is made by both clinical history and endoscopic biopsies^[5].

Metabolic syndrome (MetS) is a cluster of cardio-metabolic risk factors that include abdominal obesity, dyslipidemia, hypertension, and insulin resistance. It has become a worldwide epidemic in parallel with the increase in unhealthy behaviors especially in developed countries^[6].

Emerging evidence suggests that systemic conditions such as dyslipidemia may play a causative role in the initiation or exacerbation of these histological changes through multiple mechanisms, including: • Increased exposure of the colon to secondary bile acids (e.g., deoxycholic acid), which can irritate and damage the mucosal surface. • Disruption of the mucosal barrier and increased intestinal permeability, allowing bacterial antigens to penetrate and trigger chronic immune responses. • Elevated systemic inflammatory mediators (e.g., TNF- α , IL-6) associated with dyslipidemia, which may impair mucosal integrity^[7].

Aim of work

To assess the histopathologic and histochemical changes of colonic mucosa in the expected nonorganic and non-IBS colon diseases in patients with metabolic syndrome.

Patients and Methods

Study Design and Setting

This cross-sectional study was conducted at the Department of Hepatology, Gastroenterology, and Infectious Diseases, Faculty of Medicine, Al-Azhar University (Cairo, Egypt), in collaboration with the Departments of Hepatology, Gastroenterology, and Pathology at the Theodor Bilharz Research Institute.

Patient Recruitment and Selection Criteria

A total of 496 adult patients presenting with lower gastrointestinal symptoms were initially assessed. Following comprehensive clinical and endoscopic evaluations, 199 patients who exhibited normal macro structural colonoscopic findings were enrolled in the final analysis.

Inclusion Criteria: Symptomatic adult patients aged 18 years or older, undergoing either primary (naïve) or repeated screening colonoscopy, with no detectable gross structural mucosal lesions.

Exclusion Criteria: Patients aged < 18 years. Individuals with a documented history or ongoing follow-up for established structural gastrointestinal diseases (e.g., Inflammatory Bowel Disease, Colon cancer), gastrointestinal infections (including HIV, HBV, HCV, COVID-19, Salmonellosis, Shigellosis, or pathogenic E. coli), or systemic malignancies (except hepatocellular carcinoma 6 months' post-cure or extrahepatic malignancies 2 years disease-free) were excluded. Pregnant females and patients with severe, chronic debilitating disorders were also excluded.

To control for potential confounding variables that could independently induce or modulate microscopic colonic low grade inflammation, a comprehensive pharmacological and clinical screening was conducted. For essential maintenance medications common in metabolic syndrome cohorts (such as metformin and low-dose aspirin), drug history was systematically documented and analyzed as a potential confounding factor during clinical interpretation, rather than being modified, to reflect real-world clinical presentations."

Clinical and Biochemical Profiling

All participants underwent detailed medical history taking and systemic clinical examinations. Routine laboratory investigations included complete blood count (CBC), liver function tests (LFTs), and renal function tests (RFTs). To evaluate Metabolic Syndrome (MetS), targeted anthropometric and biochemical assessments were performed, including waist circumference (cm), systolic and diastolic blood pressure, fasting blood glucose (FBG), HbA1c, and a comprehensive lipid profile (total cholesterol, triglycerides, LDL, VLDL, and HDL).

Symptom-Based Patient Stratification

Enrolled subjects (n = 199) were segregated based on the validation of their symptoms against the Rome IV criteria into two primary cohorts: IBS (n = 127) and non-IBS (n = 72).

IBS Cohort: Fulfilling the rigorous definitions of Rome IV criteria.

Non-IBS Cohort: Included patients with "IBS-like" symptoms (partially fulfilling Rome IV criteria, characterized by recurrent abdominal pain with alternating bowel habits for less than the required threshold) and "IBS-free" subjects presenting with vague gastro-colonic manifestations (e.g., postprandial dyspnea, isolated abdominal distension, persistent flatulence, or borborygmi, etc).

Following screening for the presence of Metabolic Syndrome (MetS), the cohort was further stratified into four mutually exclusive groups:

Group I (IBS / no-MetS (n = 47): Fulfilling Rome IV criteria without metabolic syndrome.

Group II (IBS / MetS (n = 80)): Fulfilling Rome IV criteria with concurrent metabolic syndrome.

Group III (Non-IBS / MetS (n = 49)): Presenting with non-IBS symptoms with concurrent metabolic syndrome.

Group IV (Non-IBS / no-MetS (n = 23)): Presenting with non-IBS symptoms without metabolic syndrome.

Colonoscopy and Tissue Sampling Protocol

Following standardized bowel preparation, colonoscopies were performed under conscious sedation utilizing midazolam, with continuous vital signs monitoring. High-definition visualization was achieved using Olympus EVIS EXERA III (CF-HQ190) and Fujifilm 600 series video colonoscopes. Complete examination up to the terminal ileum was verified in all cases to exclude macro structural pathology. A minimum of two mucosal biopsy pieces were systematically obtained from each of the anatomical sites, (2 from the terminal ileum, 2 from ascending colon) representing right colon, and (2 from sigmoid colon, 2 from rectum) representing left colon using standard biopsy forceps. Specimens were immediately fixed in 10% neutral-buffered formalin.

Histopathological and Histochemical Evaluation

Formalin-fixed, paraffin-embedded tissue blocks were sectioned at 4–5 μ m thickness. Sections were stained with Hematoxylin and Eosin (H&E) for baseline histopathological screening, and Masson's Trichrome stain was utilized for the histochemical evaluation of mucosal collagenous deposition. All slides were evaluated by an experienced pathologist blinded to the clinical groups.

A semi-quantitative scoring system was applied to assess cellular and architectural parameters based on reference normal values:

Lymphocyte Cellular Infiltrate (LCI): Quantified as 11–30/HPF or 30 < HPF.

Eosinophil Cellular Infiltrate (ECI): Quantified as 11–30/HPF or 30 < HPF.

Goblet Cell Density (MGCD): Graded by percentage reduction (%20–10 > of average).

Architectural Parameters: Evaluated for gland number/size atrophy (%90 > of average), crypt depth shortening (%90 > of average), ileal villi length atrophy (%90 > of average), dysplastic or metaplastic changes, and mucosal collagenous material (MCM) thickness (%10 < of average).

Statistical Analysis and Sample Size Calculation

Statistical analysis was conducted using SPSS version 25.0. Normality of quantitative data was verified via the Shapiro-Wilk test, and homogeneity of variance was assessed using Levene's test. Quantitative variables are expressed as Mean, Standard Deviation (SD) and analyzed using the independent Student's T-test or ANOVA where appropriate. Categorical variables are presented as frequencies and percentages (%) and analyzed using the Chi-Square test or Fisher's Exact test. Pairwise comparisons were executed using post-hoc tests with adjustment for multiple comparisons. Statistical significance was established at a threshold of ($p < 0.05$).

Sample Size Justification: Based on pilot data estimating a control group standard deviation of 0.9 and a regression error standard deviation of 1.94, a minimum sample size of 35 subjects per group was calculated to achieve 90% statistical power ($\beta = 0.10$) to reject the null hypothesis of a zero slope (assuming a true regression slope of 1.4) at a Type I error rate (α) of 0.05.

Ethical Aspects

- 1- Approving study: - this study was approved by Committee of HGID Department and Committee of Faculty of Medicine at Al-Azhar University (approval certificate Trop.Med._1Med.Research_Inf.GIT.Liv.Dis._104).
- 2- Patient Consent: - All patients that were included in the current study signed approved consent from Al-Azhar University ethical committee.

Results

1. Baseline Clinical Characteristics and Comorbidities

The distribution of comorbid conditions across the four studied groups revealed highly significant variation. As detailed in Table 1, the prevalence of Diabetes Mellitus (DM) demonstrated a prominent overall clinical difference ($p < 0.001$). Group III (Non-IBS/MetS) exhibited the highest rate of diabetic cases (46.9%), followed by Group II (IBS/MetS) at 26.3%, whereas Group I (IBS/no-MetS) showed minimal prevalence (2.1%) and Group IV (Non-IBS/no-MetS) had none. Post-hoc pairwise comparisons confirmed significant differences between Group I vs. II ($p < 0.001$), I vs. III ($p < 0.001$), II vs. III ($p = 0.016$), II vs. IV ($p = 0.003$), and III vs. IV ($p < 0.001$). Similarly, disturbed lipid profiles differed significantly across the cohorts ($p < 0.001$), predominating sharply in metabolic syndrome groups: Group II (65.0%) and Group III (57.1%). Conversely, normal lipid profiles were almost universally sustained in non-metabolic groups (Group I: 97.9%, Group IV: 100.0%). Pairwise variations were verified as highly significant across all metabolic vs. non-metabolic group combinations ($p < 0.001$). In

contrast, the occurrence of oral ulcers (OU) showed no statistical differences globally or in pairwise comparisons ($p = 0.6$).

Table 1: Baseline Characteristics and Comorbidities Across the Studied Groups

Study populations	Diabetes Mellitus		Oral Ulcers (OU)			Disturbed Lipid Profile			
	No DM	Current ly Diabeti c	No OU	Past History Of OU	Reccurre nt OU	Norma l	Prese nt	Pendin g	Under TTT
A. group I: IBS/no.MetS.(n=47)	46 (97.9%)	1 (2.1%)	38 (80.9%)	8 (17.0%)	1 (2.1%)	46 (97.9%)	1 (2.1%)	0 (0.0%)	0 (0.0%)
B. group II: IBS/MetS.(n=80)	59 (73.8%)	21 (26.3%)	61 (76.3%)	13 (16.3%)	6 (7.5%)	28 (35.0%)	23 (28.8%)	1 (1.3%)	28 (35.0%)
C. Group III: no.IBS/MetS.(n=49)	26 (53.1%)	23 (46.9%)	37 (75.5%)	6 (12.2%)	6 (12.2%)	21 (42.9%)	11 (22.4%)	0 (0.0%)	17 (34.7%)
D. group IV: no.IBS/no.MetS.(n=23)	23 (100.0%)	0 (0.0%)	17 (73.9%)	3 (13.0%)	3 (13.0%)	23 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
p values:									
A vs B	<0.001		0.5			<0.001			
A vs C	<0.001		0.2			<0.001			
A vs D	>0.900		0.2			>0.900			
B vs C	0.016		0.6			0.800			
B vs D	0.003		0.7			<0.001			
C vs D	<0.001		>0.9			<0.001			
Overall	<0.001		0.6			<0.001			

Abbreviations: DM, Diabetes Mellitus; TTT, treatment; IBS; irritable bowel syndrome; MetS., Metabolic syndrome. Diabetes mellitus and Disturbed lipid profile differ significantly among study groups, most prevalent in MetS. Group (II & III)

2. Clinical History and Symptom Presentation

Regarding general somatic symptoms (**Table 2**), distinct variations were observed. Headache patterns showed overall statistical significance ($p = 0.039$), clustered more frequently in non-metabolic groups (Group IV: 26.1%, Group I: 23.4%) compared to Group II (10.0%), with verified pairwise differences between Group I vs. II ($p = 0.041$) and II vs. IV ($p = 0.017$). Fatigue followed a matching trend ($p = 0.021$), appearing higher in Group IV (21.7%) and Group I (14.9%) than in Group II (3.8%).

Pharmacological profiles highlighted a significantly higher intake of aspirin/colchicine in metabolic cohorts ($p = 0.004$), peaking in Group III (24.5%) and Group II (12.5%), while completely absent in non-metabolic cohorts. Anti-diabetic medication use mirrored the DM distribution ($p < 0.001$), driven mostly by Group III (48.0%) and Group II (28.9%). No statistical variance was identified in the overall distribution of general colonic complaints ($p = 0.2$)

Table 2: Clinical History and Medication Profiles Among the studied Groups

Study populations	Headache	Fatigue	Colonic symptoms	Gastro-colonic	Aspirin / colchicine	Anti-diabetics
A. group I: IBS/no.MetS.(n=47)	11 (23.4%)	7 (14.9%)	39 (83.0%)	0 (0.0%)	0 (0.0%)	1 (2.1%)
B. group II: IBS/MetS.(n=80)	8 (10.0%)	3 (3.8%)	68 (85.0%)	3 (3.8%)	10 (12.5%)	23 (28.9%)
C. Group III: no.IBS/MetS.(n=49)	6 (12.2%)	3 (6.1%)	38 (77.6%)	4 (8.2%)	12 (24.5%)	23 (48%)

D. group IV: no.IBS/no.MetS.(n=23	6 (26.1%)	5 (21.7%)	21 (91.3%)	0 (0.0%)	0 (0%)	0 (0.0%)
p values:						
A vs B	0.041	0.038	0.8		0.019	<0.001
A vs C	0.2	0.2	0.052		<0.001	<0.001
A vs D	0.4	0.5	0.3		-	>0.9
B vs C	0.7	0.7	0.12		0.2	0.089
B vs D	0.17	0.013	0.6		0.3	0.014
C vs D	0.083	0.10	0.4		0.027	<0.001
Overall	0.039	0.021	0.2		0.004	<0.001

There is a significant difference between study groups regarding use of aspirin and anti-DM medications, Medication use is higher in MetS Group II & III

3. Gastrointestinal Presentation

Analysis of specific gastrointestinal presentations (Table 3) showed that upper GIT signs, including dyspepsia ($p = 0.7$) and GERD symptoms ($p = 0.2$), remained comparable across all groups. However, lower colonic symptom patterns showed stark differences ($p < 0.001$). Pain combined with alternating bowel habits (ABH) was heavily concentrated in the designated IBS cohorts (Group I: 85.1%, Group II: 91.3%). In contrast, non-IBS groups presented mainly with isolated structural manifestations like chronic constipation (Group III: 32.7%, Group IV: 30.4%) or chronic diarrhea (Group III: 26.5%, Group IV: 8.7%). These descriptive trends established highly significant pairwise variances between all functional (IBS) and organic (non-IBS) pairs ($p < 0.001$).

Table 3. Comparison of GIT specific clinical manifestations in all study groups

Study populations	C.P. + diarrhea	C.P. + Const.	C.P.+ ABH	Chr. Diarrhea	Chr. Constipation	Dyspepsia	GERD symptoms
A. group I: IBS/no.MetS.(n=47)	1 (2.1%)	6 (12.8%)	40 (85.1%)	0 (0.0%)	0 (0.0%)	5 (10.6%)	14 (29.8%)
B. group II: IBS/MetS.(n=80)	3 (3.8%)	2 (2.5%)	73 (91.3%)	1 (1.3%)	1 (1.3%)	13 (16.3%)	13 (16.3%)
C. Group III: no.IBS/MetS.(n=49)	0 (0.0%)	0 (0.0%)	3 (6.1%)	13 (26.5%)	16 (32.7%)	7 (14.3%)	6 (12.2%)
D. group IV: no.IBS/no.MetS.(n=23	1 (4.3%)	1 (4.3%)	1 (4.3%)	2 (8.7%)	7 (30.4%)	1 (4.3)	4 (17.4%)
p values:							
A vs B	0.12					0.7	0.072
A vs C	<0.001					0.7	0.034
A vs D	<0.001					0.4	0.4
B vs C	<0.001					0.9	0.5
B vs D	<0.001					0.3	>0.9
C vs D	0.3					0.6	0.7
Overall	<0.001					0.7	0.2

Abbreviations: C.P.: colonic pain; ABH: alternating bowel habits. Colonic symptom patterns differ significantly between groups. Pain with alternating bowel habits predominates in IBS groups. With no significant difference regarding upper GIT symptoms.

4. Metabolic and Biochemical Markers

As displayed in **Table 4**, glycemic control markers (HbA1c) presented explicit group deviations ($p < 0.001$), peaking in Group III ($6.6 \pm 1.6\%$) and Group II ($6.1 \pm 1.1\%$), which directly matches their metabolic grouping. Fasting blood glucose (FBG) exhibited a borderline overall trend ($p = 0.064$), though significant differences were found in pairwise assessments for Group I vs. II ($p = 0.020$) and Group I vs. III ($p = 0.030$). Lipid profiles consistently verify metabolic derangement. Total cholesterol, triglycerides, LDL, and VLDL reached maximum values in Group II, closely followed by Group III, showcasing highly significant overall variations ($p < 0.001$). Conversely, cardioprotective HDL fractions were significantly higher in the non-metabolic groups (Group IV: 56.2 ± 4.9)

Table 4. Comparison of Laboratory Findings Among the studied groups

Study populations	HBA1c Mean±SD	FBG Mean±SD	Cholesterol Mean±SD	Triglycerides Mean±SD	LDL Mean±SD	VLDL mean±SD	HDL mean±SD
A. group I: IBS/no.MetS.(n=47)	5.4±0.6	98.7±11.2	183.5±19.3	123.4±24.5	84.7±18.6	22.5±6.3	56.0±5.5
B. group II: IBS/MetS.(n=80)	6.1±1.1	111.8±27.5	222.6±36.1	147.6±26.9	112.5±27.6	32.7±8.8	50.9±8.1
C. Group III: no.IBS/MetS.(n=49)	6.6±1.6	120.0±36.1	203.0±35.7	144.4±28.2	105.8±22.0	28.5±10.3	53.2±2.0
D. group IV: no.IBS/no.MetS.(n=23)	5.5±0.6	102.4±9.5	178.5±22.6	131.3±21.6	82.6±13.3	21.2±4.8	56.2±4.9
P values:							
A vs B	<0.001	0.02	<0.001	<0.001	<0.001	<0.001	<0.001
A vs C	<0.001	0.030	<0.001	<0.001	<0.001	<0.001	0.017
A vs D	0.3	0.068	0.3	0.11	>0.9	0.7	0.6
B vs C	0.3	0.5	0.007	0.5	0.2	0.034	0.090
B vs D	0.052	0.7	<0.001	0.004	<0.001	<0.001	<0.001
C vs D	0.021	0.5	0.002	0.037	<0.001	0.002	0.028
Overall	<0.001	0.064	<0.001	<0.001	<0.001	<0.001	<0.001

The baseline mean triglyceride levels in the metabolic syndrome groups (Groups II and III) fall below the traditional diagnostic cutoff (150 mg/dL) because a substantial proportion of these enrolled patients were already receiving active lipid-lowering therapies (e.g., statins or fibrates) at the time of recruitment, which pharmacologically controlled their lipid profiles and subsequently lowered the overall statistical means. There is a significant difference between study groups regarding HBA1C and lipid profile (TG, cholesterol, LDL, VLDL, HDL).

5. Histopathological and Architectural Features Right Colon Assessment

As detailed in Table 5, right colon mucosal cellularity demonstrated a borderline overall trend for Lymphocyte Cellular Infiltrate (LCI) ($p = 0.053$ = however, post-hoc analysis identified a significant pairwise elevation of high-grade LCI (30<HPF) in Group I (12.8%) and mild-to-moderate LCI (11–30/HPF) in Group II (48.8%) compared to each other ($P = 0.007$). Eosinophil Cellular Infiltrate (ECI) showed no global differences ($p = 0.2$), but a significant pairwise variation occurred between Groups II and I ($P = 0.045$) with abnormal infiltrate being more frequent in Group II (18.8% mild, 2.5% severe) than Group III (6.1% mild, 0% severe). Goblet cell density remained statistically uniform ($p = 0.3$)

Architectural parameters Table 6 (revealed that ileal villi atrophy, glandular structural alterations, and mucosal collagenous material were balanced across groups ($p = 0.05$) Crypt depth shortening showed a localized pairwise difference between Group II and IV ($p = 0.045$) with moderate structural crypt shortening observed exclusively in Group IV (19.0%)

Table 5: Right Colon Mucosal Cellular Infiltration Patterns

Study populations	Lymphocyte cellular infiltrate		Eosinophil cellular infiltrate		Goblet cell density
	11-30/HPF	>30/HPF	11-30/HPF	>30/HPF	10-20% <average
A. group I: IBS/no.MetS.(n=47)	12 (25.5%)	6 (12.8%)	8 (17.0%)	0 (0.0%)	5 (10.6%)
B. group II: IBS/MetS.(n=80)	39 (48.8%)	15 (18.8%)	15 (18.8%)	2 (2.5%)	7 (8.8%)
C. Group III: no.IBS/MetS.(n=49)	21 (42.9%)	5 (10.2%)	3 (6.1%)	0 (0.0%)	2 (4.1%)
D. group IV: no.IBS/no.MetS.(n=23)	9 (39.1%)	1 (4.3%)	5 (21.7%)	0 (0.0%)	0 (0.0%)

p values:			
A vs B	0.007	0.8	0.8
A vs C	0.2	0.12	0.3
A vs D	0.4	-	0.2
B vs C	0.3	0.049	0.5
B vs D	0.13	0.9	0.3
C vs D	0.7	0.10	>0.9
Overall	0.053	0.2	0.3

Lymphocyte cellular infiltrate on right colon shows a borderline overall difference with higher infiltrate in Group II.

Table 6. Right colon mucosal architectural study

Study populations	Ileal Villi atrophy (<90%of average)	Glands atrophy (<90%of average)	Crypt depth shortening (<90%of average)	Collagenous material (>10%of average)
A. group I: IBS/no.MetS.(n=47)	6 (15.0%)	5 (10.6%)	10 (21.3%)	0 (0.0%)
B. group II: IBS/MetS.(n=80)	5 (7.0%)	11 (13.8%)	16 (20.0%)	1 (1.3%)
C. Group III: no.IBS/MetS.(n=49)	5 (11.9%)	6 (13.8%)	11 (22.4%)	0 (0.0%)
D. group IV: no.IBS/no.MetS.(n=23)	0 (0.0%)	3 (13%)	4 (19%)	0 (0.0%)
P values:				
A vs B	0.2	0.4	0.2	>0.9
A vs C	0.7	0.7	0.8	>0.9
A vs D	0.2	0.8	0.6	>0.9
B vs C	0.4	0.8	0.5	-NA
B vs D	0.7	>0.9	0.047	-NA
C vs D	0.3	>0.9	0.2	-NA
Overall	0.3	0.8	0.2	>0.9

Moderate crypt shortening is noticed exclusively in Group IV.

6. Histopathological and Architectural Features left Colon Assessment

In the left colon (Table 7), LCI varied significantly across groups ($p = 0.002$). Advanced structural grades of infiltration were clustered in Group II (mild LCI: 57.5%, moderate-to-severe LCI: 15.0%) compared to Group I (mild: 38.3%, moderate: 2.1%), Group III (mild: 44.9%, moderate: 6.1%), and Group IV (mild: 30.4%, moderate: 4.3%). Post-hoc analyses confirmed highly significant pairwise variations for Group I vs. II ($p < 0.001$), II vs. III ($p = 0.038$), and II vs. IV ($p = 0.004$). ECI ($p = 0.5$) and goblet cell density ($p = 0.8$) showed no structural or statistical variances.

Furthermore, all architectural parameters in the left colon (Table 8), including glandular atrophy ($p = 0.3$), crypt depth shortening ($p = 0.4$), metaplastic variations ($p = 0.2$), and collagenous material deposition ($p > 0.05$), remained balanced and statistically non-significant across the groups.

Table 7: Left Colon Mucosal Cellular Infiltration Patterns

Study populations	Lymphocyte cellular infiltrate		Eosinophil cellular infiltrate		Goblet cell density 10-20% <average
	11-30/HPF	>30/HPF	11-30/HPF	>30/HPF	
A. group I: IBS/no.MetS.(n=47)	18 (38.3%)	1 (2.1%)	5 (10.6%)	0 (0%)	1 (2.1%)
B. group II: IBS/MetS.(n=80)	46 (57.5%)	12 (15.0%)	8 (10.0%)	0 (0%)	5 (6.3%)
C. Group III: no.IBS/MetS.(n=49)	22 (44.9%)	3 (6.1%)	3 (6.1%)	0 (0%)	3 (6.1%)

D. group IV: no.IBS/no.MetS.(n=23)	7 (30.4%)	1 (4.3%)	4 (17.4%)	0 (0%)	1 (4.3%)
P values:					
A vs B	<0.001		>0.9		0.4
A vs C	0.5		0.5		0.6
A vs D	0.6		0.5		>0.9
B vs C	0.038		0.5		>0.9
B vs D	0.004		0.5		>0.9
C vs D	0.5		0.2		>0.9
Overall	0.002		0.5		0.8

Lymphocyte cellular infiltrate on left colon differs significantly between groups with higher grades of infiltrate in Group II.

Table 8. Left colon mucosal architectural study between study groups

Study populations	Glands atrophy (<90%of average)	Crypt depth shortening (<90%of average)	Collagenous material (>10%of average)	Metaplastic changes
A. group I: IBS/no.MetS.(n=47)	3 (6.4%)	5 (10.6%)	0 (0.0%)	0 (0.0%)
B. group II: IBS/MetS.(n=80)	12 (15.2%)	16 (20.2%)	0 (0.0%)	0 (0.0%)
C. Group III: no.IBS/MetS.(n=49)	5 (10.2%)	8 (16.3%)	0 (0.0%)	2 (4.1%)
D. group IV: no.IBS/no.MetS.(n=23)	0 (0.0%)	3 (13%)	0 (0.0%)	0 (0.0%)
P values:				
A vs B	0.3	0.3	-NA	-NA
A vs C	0.7	0.4	-NA	0.5
A vs D	0.5	>0.9	-NA	0.14
B vs C	0.8	0.9	-NA	>0.9
B vs D	0.10	0.2	-NA	-NA
C vs D	0.2	0.3	-NA	-NA
Overall	0.3	0.4	-NA	0.2

No significant differences between groups regarding glands, crypt depth, metaplastic changes, and mucosal collagenous material deposition on left colon

DISCUSSION

The current study demonstrates a crucial histopathological and metabolic link, revealing that metabolic syndrome (MetS) acts as a distinct systemic driver of low-grade colonic mucosal inflammation. While Irritable Bowel Syndrome (IBS) has been traditionally classified as a functional disorder lacking structural alterations, our findings indicate that the presence of concurrent MetS introduces an organic inflammatory component, characterized primarily by intraepithelial and mucosal lymphocytic and eosinophilic infiltration ^[1, 2].

A central contribution of this study is the application of established diagnostic frameworks to distinguish functional from organic manifestations. While baseline patient stratification was strictly performed using the established Rome IV criteria, it's insightful to contextualize these clinical outcomes within the newly introduced Rome V diagnostic process (2026). This transition in the literature does not affect our study cohorts, which remain validated under Rome IV; rather, the reintroduction of the broader term "discomfort" alongside recurrent pain under Rome V highlights the subtle clinical overlap between systemic metabolic changes and functional bowel symptoms ^[3].

Our cohort underscores this challenge; patients in the non-IBS groups presented primarily with isolated, chronic diarrhea or constipation without meeting the mandatory pain thresholds of Rome criteria. As supported by Ford et al. (2020), isolated chronic alterations in bowel habits should prompt proactive investigations for underlying microscopic colitis, bile acid malabsorption, or low-grade organic pathologies rather than default functional labeling ^[8,9].

The biochemical and lipid profiles obtained in our metabolic groups (Groups II and III) strongly reinforce the systemic impact of metabolic dysfunction on intestinal health. Significant elevations in triglycerides, total cholesterol, LDL, and HbA1c fractions directly matched the metabolic syndrome cohorts. This microenvironment provides a plausible mechanistic pathway for mucosal alterations. High circulatory lipids and hyperglycemic states are known to induce oxidative stress and break down the integrity of the intestinal epithelial barrier, promoting the well-documented "leaky gut" phenomenon. According to Guo et al. (2019), this compromised mucosal barrier allows the translocation of luminal bacterial endotoxins, which subsequently activates local immune cells and triggers visceral hypersensitivity ^[10]. Furthermore, hyperglycemia and insulin resistance contribute to systemic low-grade inflammation and autonomic neuropathy, which can impair colonic motility, delay transit time, or induce osmotic changes that mimic or worsen IBS symptoms ^[11].

Histopathologically, the overall preservation of standard mucosal architecture—such as crypt depth, gland size, and goblet cell density across all groups—confirms the classical definition of IBS and MetS as disorders that lack gross erosive structural destruction, clearly separating them from classic inflammatory bowel diseases (IBD). However, the significant increase in mucosal and intraepithelial lymphocytic infiltration (LCI), predominantly clustered in Group II (IBS+MetS) in both the right and left colon, points toward a state of metabolic endotoxemia. As proposed by Boutari and Mantzoros (2023), metabolic syndrome triggers chronic recruitment of T-lymphocytes into the lamina propria via circulating adipokines and pro-inflammatory cytokines. The diffuse presence of LCI across multiple colonic segments verified in our study supports the hypothesis that systemic metabolic mediators exert a global, rather than localized, immunological stress on the gastrointestinal tract ^[12]. In addition to LCI, the significant pairwise elevation of eosinophil cellular infiltrate (ECI) observed in Group II (IBS+MetS) compared to Group III highlights a complex synergistic effect when metabolic derangements are superimposed on functional bowel disorders. This finding aligns with Mensen et al. (2020), showing that metabolic distress signals, including specialized endotoxins, can selectively recruit eosinophils to the gastrointestinal mucosa, altering cellular signaling and contributing to symptom exacerbation ^[13]. Conversely, the minimal detection of subepithelial collagenous band deposition in our cohorts aligns with the recognized epidemiological rarity of fully developed collagenous colitis in patients presenting without established non-functional risks ^[14].

An intriguing finding in our clinical history analysis was the significantly higher prevalence of anti-diabetic medications (e.g., metformin) and aspirin intake among the non-IBS metabolic cohort (Group III). Emerging evidence suggests that common anti-diabetic therapies can significantly modulate gut microbiota composition and alter mucosal signaling pathways. Metformin, which peaked in usage within our non-IBS metabolic cohort (Group III), has been shown to profoundly reshape the intestinal microbiota by promoting the growth of short-chain fatty acid (SCFA)-producing bacteria, such as *Akkermansia muciniphila*, which strengthens the mucosal barrier. Furthermore, as observed by Tseng (2021), metformin activates cellular adenosine monophosphate-activated protein kinase (AMPK), a critical pathway that exerts down-stream anti-inflammatory actions that can reduce or shift mucosal lymphocytic infiltration patterns [15]. Concurrently, low-dose aspirin also (highly prevalent in Group III) exerts a dual effect on mucosal histology; it can stabilize the colonic epithelial barrier and reduce systemic endotoxemia via cyclooxygenase-1 (COX-1) inhibition, preventing microvascular endothelial stress within the lamina propria. As emphasized by Boyle et al. (2022), the specific colonic complaints and distinct histological presentations seen in these patients are likely a combined product of the underlying metabolic disease and its pharmacological management ^[16].

Study Limitations:

while our target sample size calculation recommended a minimum of 35 subjects per group to optimize statistical power, Group IV (Non-IBS/no-MetS) yielded only 23 subjects due to the clinical rarity of symptomatic individuals completely free of both functional bowel criteria and metabolic alterations. This smaller sample size in one cohort may reduce the statistical power of certain pairwise post-hoc analyses involving Group IV. However, this risk was mitigated by implementing robust, conservative post-hoc adjustments specifically designed to account for unequal cohort sizes and variances, thereby preserving the validity of our significant global findings.

CONCLUSION

This study highlights a distinctive histopathological spectrum in patients with large bowel symptoms, revealing a clear association between metabolic syndrome and the presence of colonic mucosal inflammation in the form of intracellular lymphocyte infiltration especially when associated with ROME IV-defined functional bowel symptoms. The presence of metabolic syndrome should be viewed as a 'Histological Catalyst' that introduces an

organic inflammatory component (LCI) to a traditionally functional disorder. Therefore, treating the metabolic profile may be as crucial as managing gut symptoms in this patient population.

Conflict of interest: Authors have nothing to disclose.

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Availability of data and materials: All data generated or analyzed during this study are included in this published article.

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