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ARTICLE

Impact of mechanical barrier damage and interleukin-17 on symptoms in patients with post-infectious irritable bowel syndrome

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Abstract

Aims/Background The pathogenesis of irritable bowel syndrome encompasses various factors, including abnormal gastrointestinal motility, heightened visceral sensitivity, dysfunction in the brain-gut axis, psychological influences, and disturbances in the intestinal flora. These factors manifest primarily as persistent or intermittent abdominal pain, diarrhoea, alterations in bowel habits, or changes in stool characteristics. In our investigation, we delve into the repercussions of mechanical barrier damage and immune dysfunction on symptoms among patients with post-infectious irritable bowel syndrome.

Methods This study recruited a total of 20 healthy controls and 49 patients diagnosed with irritable bowel syndrome. Among the irritable bowel syndrome patients, we categorised them into two groups based on the ROME IV diagnostic criteria: the post-infectious irritable bowel syndrome group (n=23) and the non-post-infectious irritable bowel syndrome group (n=26). To compare clinical features, we utilised the Gastrointestinal Symptom Rating Scale, Self-Rating Depression Scale, and Self-Rating Anxiety Scale. Furthermore, we employed various techniques including haematoxylin and eosin (HE) staining, electron microscopy, Enzyme-linked Immunosorbent Assay, and flow cytometry to assess changes in immune cells, immune factors, inflammatory biomarkers, and intestinal barrier function.

Results Under haematoxylin and eosin staining, post-infectious irritable bowel syndrome patients demonstrated increased neutrophils and plasma cells compared to the control group. Additionally, electron microscopy revealed ultrastructural changes such as the widening of the epithelial cell gap in the intestinal mucosa among post-infectious irritable bowel syndrome patients. Comparatively, the Gastrointestinal Symptom Rating Scale, Self-Rating Anxiety Scale, and Self-Rating Depression Scale scores were significantly elevated in the post-infectious irritable bowel syndrome group in contrast to both the control group and the non- post-infectious irritable bowel syndrome group ($p < 0.05$). Moreover, post-infectious irritable bowel syndrome patients exhibited a notably higher neutrophil-to-lymphocyte ratio compared to the control group ($p < 0.05$). Furthermore, the levels of interleukin-17 (IL-17) were elevated in post-infectious irritable bowel syndrome patients compared to the control group ($p < 0.05$). Additionally, the post-infectious irritable bowel syndrome group displayed a higher percentage of T helper 17 (Th17) cells compared to both the control and non-post-infectious irritable bowel syndrome groups ($p < 0.05$).

Conclusion Acute gastrointestinal infection can disrupt the balance of intestinal flora, leading to dysbiosis. This dysbiosis can trigger the release of pro-inflammatory factors, including interleukin-17, which contributes to the impairment of the intestinal mucosal barrier. Consequently, this sets the stage for the development of long-lasting, mild chronic intestinal inflammation, ultimately culminating in the onset of post-infectious irritable bowel syndrome. Furthermore, within the framework of the gut-brain axis interaction, anxiety and depression may exacerbate intestinal inflammation in post-infectious irritable bowel syndrome patients. This interaction can perpetuate and prolong clinical symptoms in individuals with post-infectious irritable bowel syndrome, further complicating the management of the condition.

Key words: IL-17; Mechanical barrier; Post-infectious irritable bowel syndrome; Th17

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Introduction

Irritable bowel syndrome (IBS) presents with abdominal pain, bloating, or discomfort in the abdomen, primarily associated with defecation or changes in defecation habits such as frequency and stool consistency. The functional disorders explaining these symptoms have a prevalence ranging from 1.1% to 45.0% among adults globally, with the overall prevalence of IBS in the general population of China estimated between 1.4% to 11.5% (Lovell and Ford, 2012; Villani et al, 2010; Chang et al, 2022). Irritable bowel syndrome is classified into four types based on defaecation patterns: IBS with diarrhoea (IBS-D), IBS with constipation (IBS-C), mixed type (IBS-M), and unclassified type (IBS-U) (Bonetto et al, 2021). It significantly diminishes patients' quality of life, exerting a substantial negative impact on their work, studies, daily lives, and mental wellbeing. Notably, individuals with IBS often report a lower quality of life compared to those with other chronic conditions such as diabetes and gastroesophageal reflux disease (GERD). Intestinal infection represents one of the risk factors for developing IBS. Following an acute intestinal infection, approximately 10%–11.5% of patients develop a condition known as post-infectious irritable bowel syndrome (PI-IBS) (Barbara et al, 2019; Ghoshal, 2022).

Reports indicate that alterations in mucosal permeability, the gut microbiome, epithelial structure, and immune responses contribute to the pathophysiology of IBS. Additionally, a growing body of literature suggests that environmental factors such as diet, infections, and social-psychological factors play significant roles in IBS (Beatty et al, 2014; Berumen et al, 2021). Recent studies have linked psychological factors like depression and anxiety with the development of PI-IBS following infections. Post-infectious irritable bowel syndrome is further associated with low-grade inflammation and increased intestinal permeability (Card et al, 2018; Cryan et al, 2019). However, there is limited understanding of the connections among intestinal barrier function, immune responses, and psychological factors in PI-IBS. In this study, we recruited patients diagnosed with IBS and categorised them into PI-IBS and non-PI-IBS groups based on ROME IV criteria (Lacy et al, 2021). We conducted comparisons of clinical features, changes in immune cells, immune factors, inflammatory biomarkers, and intestinal barrier function to gain insight into PI-IBS.

Methods

Inclusion and exclusion criteria

We recruited 49 patients diagnosed with IBS, comprising 26 individuals with non-PI-IBS and 23 with PI-IBS, from the outpatient service of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China, between 2021 and 2022. All patients met the Rome IV diagnostic criteria for IBS. Post-infectious irritable bowel syndrome was defined as IBS onset following an episode of acute gastroenteritis with symptoms such as fever, diarrhoea, or vomiting (Beatty et al, 2014). Additionally, we enrolled 20 healthy controls from subjects undergoing regular physical examinations. Pregnant women and individuals aged over 70 years or under 18 years were excluded from the study. Patients with severe cardiovascular and cerebrovascular diseases were also excluded. Those who declined to participate were not included. All participants underwent electronic colonoscopy and biochemical tests to rule out other organic diseases. Informed consent was obtained from every participant, and the study protocol was approved by the institutional research ethics committee of Chongqing Medical University (Ethical approval numbers: 2019301).

Subjects and clinical data

In this study, we employed the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire to evaluate the gastrointestinal symptoms experienced by IBS patients. The GSRS is a symptom-associated rating scale comprising 15 items grouped into five clusters: reflux, abdominal pain, dyspepsia, diarrhoea, and constipation. Higher scores on the GSRS indicate greater severity of IBS symptoms (Svedlund et al, 1988). Additionally, all participants completed the Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS) questionnaires to assess their psychological states. The SAS and SDS questionnaires consist

of twenty items each, measuring participants' levels of anxiety and depression, respectively. Anxiety is indicated by a SAS standard score of ≥ 50, while depression is indicated by an SDS standard score of > 53 (William et al, 1965; William and Zung, 1971).

Histological assessment

Intestinal mucosal samples were collected with the informed consent of the subjects. Under colonoscopy, experienced gastroenterologists obtained intestinal mucosal specimens measuring approximately 2 mm in diameter from the caecum and rectum. These specimens were then fixed in 4% paraformaldehyde (BL539A, biosharp, Beijing, China) for 24 hours, followed by haematoxylin and eosin (HE) staining. Additionally, samples were stored in glutaraldehyde (BY2741, Boer, Chongqing, China) for transmission electron microscopy analysis.

Tissue specimens were sectioned at 5 μm, mounted onto slides, deparaffinized, and rehydrated according to standard protocols. Routine HE staining (DW2148, Dowobio, Shanghai, China) was performed to assess the presence of immune cells. Microscopic observations were conducted using a microscope (CX21, Olympus, Tokyo, Japan), and cell counts were performed. Statistical analysis of the cell counting results was carried out using GraphPad Prism (version 9.5.1, GraphPad Software, Boston, MA, USA).

Enzyme-linked immunosorbent assay

Peripheral blood samples were collected from the subjects with their informed consent. An experienced nurse collected 3 mL of peripheral blood, which was anticoagulated with Ethylene Diamine Tetraacetic Acid (EDTA). Following heparin anticoagulation, the blood was left at room temperature for 25 minutes, then centrifuged at 3500 rpm for 10 minutes. The supernatant was carefully pipetted and transferred into EP tubes.

For the assay, the samples were coated and then incubated with antibodies for interleukin-17 (IL-17) (ml058052, Mlbio, Shanghai, China), interferon-γ (IFN-γ) (ml077386, Mlbio, Shanghai, China), and interleukin-10 (IL-10) (ml064299, Mlbio, Shanghai, China). Following termination of the reaction, the assay was conducted using a Microplate Reader (VLBLATGD2, Thermo Fisher Scientific, Waltham, MA, USA).

Ultrastructural analysis

Following fixation in a phosphate buffer containing glutaraldehyde and paraformaldehyde, samples from the caecum and rectum were dehydrated and embedded in resin. These samples were then visualised using a JEOL JEM-1400PLUS electron microscope (Jeol Ltd., Tokyo, Japan). Image acquisition and analysis were performed using Digital Micrograph© software.

Antibodies and flow cytometry

10 mL of venous blood from subjects was drawn into EDTA vacuum tubes. Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation according to standard protocols. Peripheral blood mononuclear cells were then stained with monoclonal antibodies including Anti-CD3-FITC (555339, BD Biosciences, Franklin Lakes, NJ, USA), anti-CD4-PE (550630, BD Biosciences, Franklin Lakes, NJ, USA), and anti-IL-17A-BV421 (562933, BD Biosciences, Franklin Lakes, NJ, USA). Subsequently, data analysis was conducted using a CytoFLEX flow cytometer (B53004, Beckman Coulter, Brea, CA, USA).

Intracellular cytokine staining

1×10^6 PBMCs were resuspended in 1 mL of serum-containing RPMI-1640 medium and stimulated *in vitro* with 50 ng/mL of phorbol-12-myristate-13-acetate (PMA) (407950, Sigma, Darmstadt, Germany), 1 μg/mL of ionomycin (407950, Sigma, Darmstadt, Germany), and 1μg/mL of brefeldin A (B5936, Sigma, Darmstadt, Germany). The cells were then incubated at 37°C in a 5% CO_2 incubator for 5 hours. After incubation, the cells were harvested, fixed, and permeabilized using the Fixation/Permeabilization Diluent (554714, BD Biosciences, Franklin Lakes, NJ, USA). Next, the cells were incubated with fluorochrome-conjugated intracellular cytokine antibodies, including anti-IL-17A-PE (560487, BD Biosciences, Franklin Lakes, NJ, USA) and anti-IFN-γ-APC (562017, BD Biosciences,

Franklin Lakes, NJ, USA) for 30 minutes at room temperature. Finally, the samples were analysed by flow cytometry.

RNA extraction, reverse transcription, and real-time PCR

Total RNA was extracted from PBMCs using TRIzol reagent (15596018CN, Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Reverse transcription was performed using a T100 Thermal Cycler (BIO-RAD, Hercules, CA, USA), and real-time PCR was conducted on a C1000 Touch Thermal Cycler (BIO-RAD, Hercules, CA, USA). Data analysis was carried out using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008). The primer sequences used for real-time PCR were as follows: *IL-17:* Forward: 3'-*AGATTACTACAACCGATCCACCT*-5'; Reverse: 3'-*GGGGACAGAGTTCATGTGGTA*-5'; Retinoic acid-associated orphan receptor gamma-T (*RORγt*): Forward: 3'-*AGATACCCTCACCTACACCTTG*-5'; Reverse: 3'-*CCGCTCAGGGCTGTATTCAA*-5'; β-actin: Forward: 3'-*GTTGTCGACGACGAGCG*-5'; Reverse: 3'-*GCACAGAGCCTCGCCTT*-5' (Primers from TSINGKE, Beijing, China).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (version 9.5.1, GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics 22 (IBM, Armonk, NY, USA). For normally distributed data such as SAS and SDS scores, differences were analysed using One-Way analysis of variance (One-Way ANOVA) followed by post-hoc (Bonferroni) tests. The GSRS scores, which exhibited non-normal distributions, were assessed using the Mann-Whitney U test and the Kruskal-Wallis test. The percentage of neutrophils, lymphocytes, monocytes, and Systemic Immune Inflammatory Index (SII) were analysed using *t* tests. A *p* value of less than 0.05 was considered statistically significant.

Results

Clinical characteristics

The study comprised a total of 20 controls, 26 non-PI-IBS patients, and 23 PI-IBS patients. Table 1 presents the clinical characteristics of the participants in this investigation. No significant difference was observed in the ages of the subjects among the three groups ($p > 0.05$). However, SAS and SDS ratings were notably higher in IBS patients compared to controls, and PI-IBS patients exhibited higher scores than non-PI-IBS patients ($p < 0.05$).

Abdominal discomfort and abnormal stool patterns are common symptoms of IBS (Lacy et al, 2021). Table 2 presents the questionnaire data for the IBS subsets. Gastrointestinal Symptom Rating Scale scores indicated a higher level of gastrointestinal symptoms in

Table 1. Clinical data of subjects (mean ± SD)

	control	non-PI-IBS	PI-IBS	F	*p* value
Case (n)	20	26	23	–	–
Age	43.10 ± 13.23	43.19 ± 8.56	43.35 ± 12.49	0.003	0.997
SAS scores	36.75 ± 5.87	44.27 ± 5.62	49.78 ± 7.59	22.195	< 0.001***#
SAS scores > 50 (n, %)	1 (5.00%)	6 (23.08%)	13 (56.52%)	–	–
SDS scores	36.30 ± 5.49	44.27 ± 8.79	50.74 ± 10.66	13.398	< 0.001**#
SDS scores > 53 (n, %)	0 (0.00%)	5 (19.23%)	11 (47.83%)	–	–

*The data are presented as mean ± standard deviation (mean ± SD). One-Way ANOVA followed by Bonferroni post-hoc tests were conducted for the analysis of age, SAS and SDS among the control, non-PI-IBS, and PI-IBS groups. Statistical significance was denoted as **p < 0.01, ***p < 0.001, compared to the control group; and #p < 0.05, compared to the non-PI-IBS group. SAS: Self-Rating Anxiety Scale. SDS: Self-Rating Depression Scale. non-PI-IBS: non-post-infectious irritable bowel syndrome. PI-IBS: post-infectious irritable bowel syndrome. One-Way ANOVA: One-Way analysis of variance. SD: standard deviation.*

Table 2. Gastrointestinal Symptom Rating Scale scores compared between non-post-infectious irritable bowel syndrome group and post-infectious irritable bowel syndrome group

Symptoms	non-PI-IBS M (p25–p75)	PI-IBS M (p25–p75)	Z	*p* value
Case (n)	26	23	–	–
Reflux	1 (0–2)	2 (1–3)	–2.274	0.023*
Abdominal pain	1 (0–1)	1 (0–1)	–0.253	0.778
Dyspepsia	2 (1–3)	3 (2–5)	–2.677	0.007**
Diarrhoea	3 (2–4)	3 (2–5)	–1.975	0.033*
Constipation	1 (0–2)	1 (0–2)	–0.380	0.704
Total scores	7 (6–9)	11 (8–13)	–3.013	0.001***

*M: median; The Mann-Whitney U test was utilised for statistical analysis of GSRS data. In comparison to the non-PI-IBS group, the total GSRS scores, reflux scores, dyspepsia scores, and diarrhoea scores were higher in the PI-IBS group ($p < 0.05$). Statistical significance is denoted as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. GSRS: Gastrointestinal Symptom Rating Scale. non-PI-IBS: non-post-infectious irritable bowel syndrome. PI-IBS: post-infectious irritable bowel syndrome.*

the PI-IBS group compared to the non-PI-IBS group ($p < 0.05$). Specifically, the levels of reflux, dyspepsia, and diarrhoea were significantly elevated in the PI-IBS group compared to the non-PI-IBS group ($p < 0.05$). However, there were no significant differences in the scores for abdominal pain and constipation between the two groups ($p > 0.05$).

Neutrophil to lymphocyte ratio (NLR) and SII are peripheral-blood-derived inflammatory indicators used to assess the condition and prognosis in the diagnosis and treatment of inflammatory diseases. Neutrophil-to-lymphocyte ratio scores demonstrated a higher level of inflammation in the PI-IBS group compared to the control group ($p < 0.05$) (Table 3, Figure 1).

Microstructural and ultrastructural changes of intestinal mucosa

During colonoscopy, patients with PI-IBS, non-PI-IBS, and those in the control group all exhibited smooth intestinal mucosa. However, slight inflammatory manifestations were observed in the PI-IBS group, while no obvious inflammatory features such as congestion, oedema, erosion, or ulcers were seen in the non-PI-IBS and control group (Figure 1A).

Compared to the non-PI-IBS group, the PI-IBS group demonstrated a significantly higher number of plasma cells ($p < 0.001$) and an increased neutrophil count ($p < 0.001$). Additionally, both the non-PI-IBS and PI-IBS groups exhibited more eosinophils compared to the control group (Figure 1B).

Observation of the ultrastructure of intestinal mucosal tissue under a transmission electron microscope revealed several differences (Figures 1C–E). In the PI-IBS group, enlargement of the endoplasmic reticulum and mitochondria in intestinal mucosal epithelial cells was noted. Conversely, in the non-PI-IBS group, enlargement of the gap between intestinal mucosal epithelial cells was observed, along with shedding of microvilli and widening of intercellular tight junctions. Furthermore, intestinal mucosal epithelial cell necrosis and formation of myeloid structures were observed (Figures 1C–E).

Expression of interleukin-17A, interferon-γ and interleukin-10

The serum level of IL-17A in patients with PI-IBS was significantly higher than that of controls ($p < 0.05$). Although the level of IL-17A in the PI-IBS group was higher compared to the non-PI-IBS group, this difference did not reach statistical significance. However, both PI-IBS and non-PI-IBS patients exhibited a dramatic decrease in IL-10 levels compared to controls ($p < 0.01$). There was no statistically significant variation in the levels of IFN-γ across the three experimental groups (Figure 2).

Table 3. Comparison of control and PI-IBS study group

	control	PI-IBS	Z/*t* value	*p* value
Case (n)	20	23	–	–
	M (p25–p75)	M (p25–p75)	–	–
Leukocytes	5.865 (4.820–6.890)	5.560 (4.810–6.720)	–0.471	0.644
Lymphocytes	1.900 (1.700–2.205)	1.420 (1.2800–2.090)	–2.725	0.006**
Neutrophils	3.165 (2.573–4.218)	3.530 (3.000–4.080)	–0.801	0.429
Percentage of eosinophils (%)	0.900 (0.500–2.250)	1.300 (0.450–2.125)	–0.327	0.765
Percentage of basophils (%)	0.400 (0.100–0.850)	0.450 (0.300–0.500)	–0.542	0.621
NLR	1.770 (1.448–1.995)	2.490 (1.700–2.940)	–3.015	0.002**
PLR	109.8 (84.27–126.1)	120.8 (101.9–157.8)	–1.422	0.158
	$\bar{x}$±s	$\bar{x}$±s		
Percentage of neutrophils (%)	54.23 ± 9.470	67.12 ± 9.078	4.552	< 0.001**
Percentage of lymphocytes (%)	36.01 ± 9.168	26.31 ± 6.594	4.020	< 0.001**
Percentage of monocytes (%)	6.66 ± 2.29	6.63 ± 1.43	0.052	0.959
SII	368.70 ± 162.20	489.50 ± 239.8	1.904	0.064

*Normal data are presented as mean ± standard deviation ($\bar{x}$ ± s), while non-normal data are presented as M (p25–p75). The Mann-Whitney U test was used for statistical analysis of leukocytes, lymphocytes, neutrophils, NLR, PLR, and the percentage of eosinophils and basophils. Unpaired t tests were utilised for SII and the percentage of neutrophils, lymphocytes, and monocytes. Compared to the control group, lymphocytes, NLR, and SII were higher in the PI-IBS group (**p < 0.01). NLR: neutrophil to lymphocyte ratio, PLR: Platelet to lymphocyte ratio, SII: Systemic Immune Inflammatory Index, PI-IBS: post-infectious irritable bowel syndrome.*

Flow cytometry analysis

Interleukin-17A+/interferon-γ+ cells in circulation

Post-infectious irritable bowel syndrome patients exhibited a significantly higher percentage of IL-17A+ cells compared to controls and non-PI-IBS patients ($p < 0.01$). However, no significant differences in the percentage of IL-17A+ cells were observed when comparing non-PI-IBS patients to controls. Additionally, there was no discernible variation in the percentage of IFN-γ+ cells across the three groups ($p > 0.05$) (Figure 3A).

T helper 17 cells in circulation

Due to the increased IL-17 levels, we further evaluated T helper 17 (Th17) cells. When comparing PI-IBS patients to controls, the percentage of Th17 cells was significantly higher ($p < 0.001$) (Figure 3B). Furthermore, it was observed that the proportion of Th17 cells in PI-IBS patients was substantially higher ($p < 0.001$) than in non-PI-IBS patients (Figure 3B). Compared with the control group and the non-PI-IBS group, the percentage of CD4 T cells was increased in the PI-IBS group ($p < 0.05$) (Figure 3C). Additionally, the percentage of non-T cells was decreased in the PI-IBS group compared with the control group ($p < 0.05$) (Figure 3D). Moreover, the expressions of IL-17 ($p < 0.01$) and RORγt ($p < 0.05$) were increased in the PI-IBS group compared with the control group (Figures 3E,F).

Discussion

Irritable bowel syndrome is a prevalent disorder of the brain-gut connection, affecting up to 4.8% of the global population. It not only imposes a significant burden on public health resources but also diminishes the quality of life for affected individuals. Post-infectious irritable bowel syndrome is characterised by two or more episodes of nausea, vomiting, fever, diarrhoea, or positive stool culture in patients who have previously experienced an intestinal infection (Buckley et al, 2014; Defrees and Bailey, 2017; Pellissier and Bonaz, 2017).

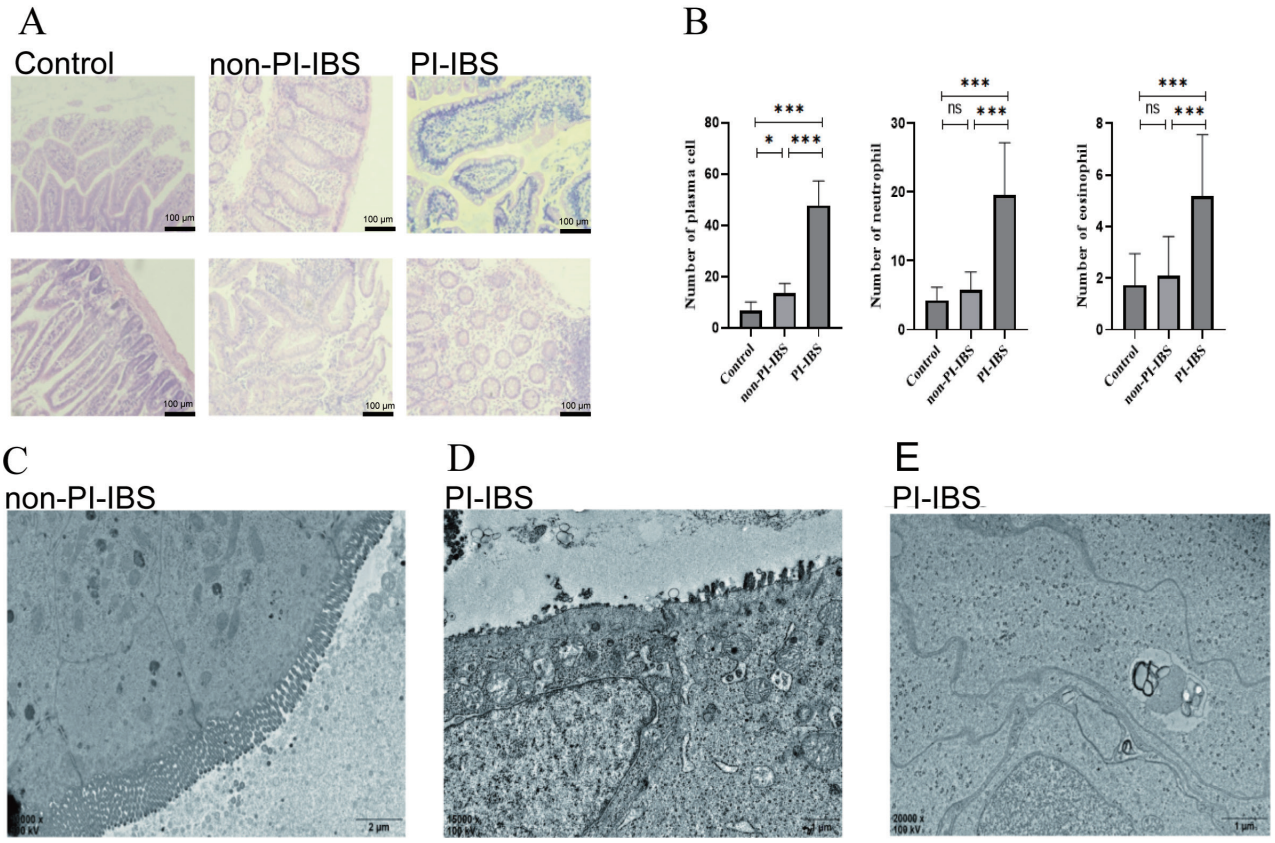

Figure 1. Microstructural and ultrastructural changes of the intestinal mucosa. (A) HE staining of intestinal mucosa tissue (200×). Scale bar=100 μm. (B) Cell counting by HE staining. One-way ANOVA and Bonferroni post-hoc tests were performed for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not significant. (C, D, E) Transmission electron microscope (TEM) of intestinal mucosa. non-PI-IBS (10,000×), scale bar=2 μm; PI-IBS (15,000×), scale bar=1 μm; PI-IBS (20,000×), scale bar=1 μm. control group (n=10), non-PI-IBS group (n=10), PI-IBS group (n=10). non-PI-IBS: non-post-infectious irritable bowel syndrome. PI-IBS: post-infectious irritable bowel syndrome. HE: haematoxylin and eosin. One-Way ANOVA: One-Way analysis of variance.

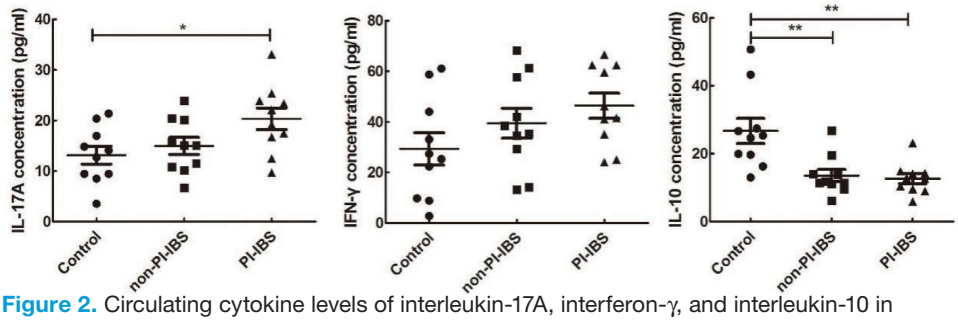

Figure 2. Circulating cytokine levels of interleukin-17A, interferon-γ, and interleukin-10 in subsets of irritable bowel syndrome and controls. One-Way ANOVA and Bonferroni post-hoc tests. Error bars represent the mean and standard error. *$p < 0.05$, **$p < 0.01$. control group (n=10), non-PI-IBS group (n=10), PI-IBS group (n=10). non-PI-IBS: non-post-infectious irritable bowel syndrome. PI-IBS: post-infectious irritable bowel syndrome. IL-17A: interleukin-17A. IFN-γ: interferon-γ. IL-10: interleukin-10. One-Way ANOVA: One-Way analysis of variance.

To explore the potential pathophysiological mechanisms of PI-IBS, patients with IBS were categorised based on their history of intestinal infection in this study. The study aimed to compare the differences in clinical symptoms, anxiety and depression scores, cytokines, and inflammatory indices among PI-IBS, non-PI-IBS, and control group.

In our investigation, we observed an increase in intestinal mucosal neutrophils and plasma cells stained with HE in IBS patients. Using electron microscopy, we further studied

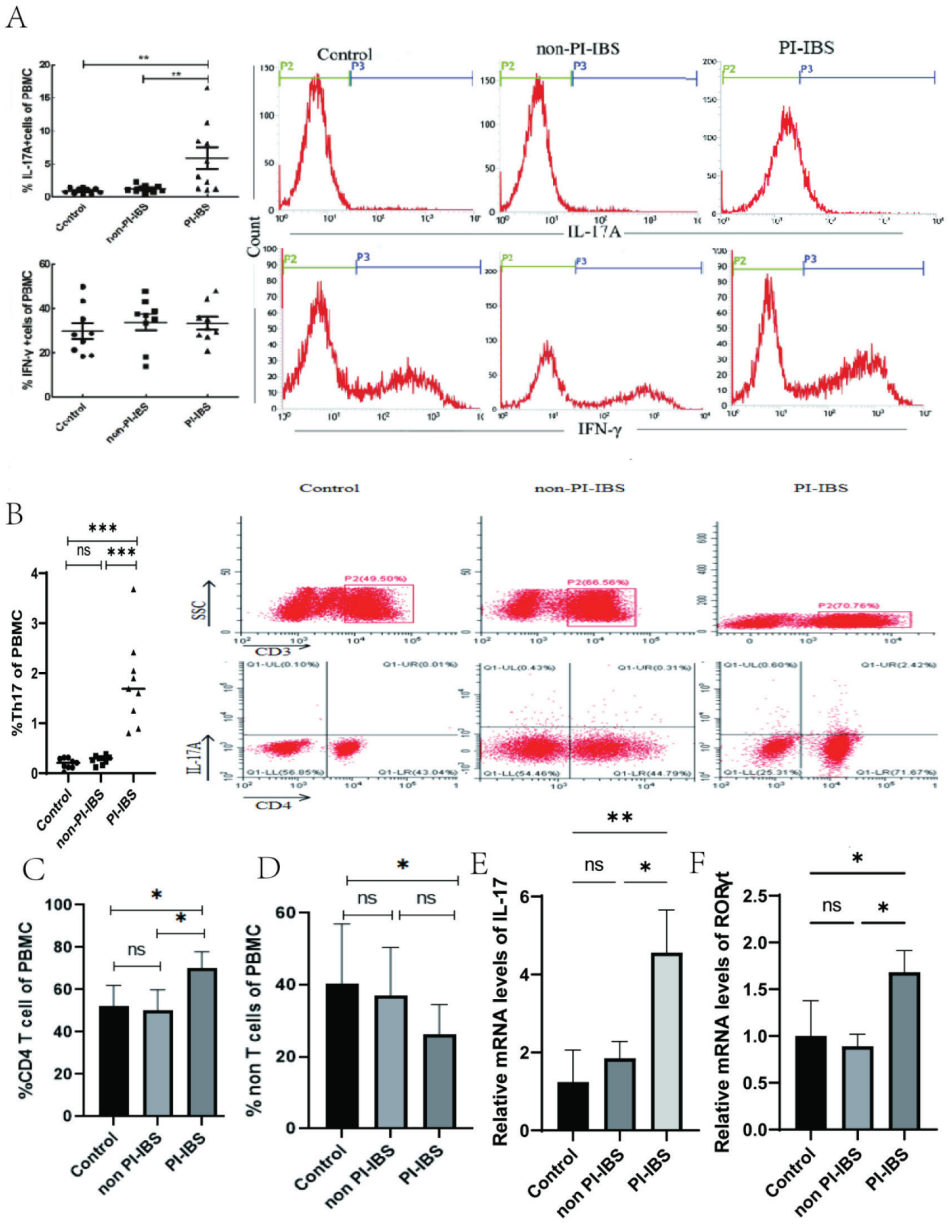

Figure 3. IL-17A +/IFN-γ+ cells and Th17 cells in circulation. (A) Circulating percentage of IL-17A+/IFN-γ+ cells in subsets of IBS and controls. Kruskal-Wallis test and Mann-Whitney U test were used for analysis. Bars show mean and standard error. (B) Circulating percentage of Th17 in subsets of IBS patients and controls. The scatter diagram displays the percentage of Th17 cells in peripheral blood among the three groups. Th17 cells were defined as CD3+CD4+IL17A+. CD4 T cells were defined as CD3+CD4+. Non-T cells were defined as CD3-. (C) The percentage of CD4+T cells. (D) The percentage of non-T cells. (E) Relative mRNA levels of IL-17. (F) Relative mRNA levels of RORγt. Kruskal-Wallis tests were performed for statistical analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not significant. control group (n=9), non-PI-IBS group (n=9), PI-IBS group (n=9). non-PI-IBS: non-post-infectious irritable bowel syndrome. PI-IBS: post-infectious irritable bowel syndrome. IL-17A: interleukin-17A. IFN-γ: interferon-γ. Th17: T helper 17. PBMC: Peripheral blood mononuclear cell. RORγt: Retinoic acid-associated orphan receptor gamma-T.

ultrastructural alterations, such as the expansion of the epithelial cell gap in the intestinal mucosa. Additionally, IBS patients exhibited significantly higher gastrointestinal symptom scores, SAS, and SDS scores compared to the control group.

Moreover, patients with PI-IBS demonstrated significantly higher NLR values than those in the control group. In PI-IBS, there was also a higher level of IL-17 compared to the control group, along with a somewhat higher expression of IFN-γ and lower levels of

IL-10. Furthermore, PI-IBS patients exhibited a larger percentage of Th17 cells compared to the control group. Overall, the observed alterations in these detection signs were more pronounced in PI-IBS compared to both IBS and the control group.

Observing the ultrastructure of intestinal tissue under transmission electron microscopy, we observed widened intercellular spaces in the IBS group. Additionally, intestinal mucosal specimens from PI-IBS patients displayed not only widening of the epithelial cell space but also dilated endoplasmic reticulum and mitochondrial swelling. Furthermore, we noted microvilli exfoliation, necrosis of some intestinal epithelial cells, and formation of myeloid structures. Comparatively, the PI-IBS group exhibited a significantly higher number of neutrophils and plasma cells compared to the control group.

It has been suggested in previous studies that there is impaired mucosal integrity and cell infiltration of the lamina propria in IBS (Zhao et al, 2019; Dyadyk et al, 2021). However, our study found that compared to IBS alone, the ultrastructural and microscopic changes in PI-IBS were more pronounced. The increase in plasma cells in PI-IBS may influence the local humoral response of the intestinal mucosa and promote intestinal peristalsis (Vicario et al, 2015). Moreover, the recruitment of neutrophils in the intestine of the PI-IBS group may lead to the destruction of the intestinal mucosal barrier (Denson et al, 2018). Cells, cell junctions, and gap junction proteins collectively form a mechanical barrier (Groschwitz and Hogan, 2009). Our study identified ultrastructural and microscopic changes in intestinal mucosal tissue alongside high GSRS scores in PI-IBS patients. We speculate that the damage to the intestinal mucosal mechanical barrier in PI-IBS patients contributes to gastrointestinal symptoms, with the imbalance of intestinal flora possibly being the underlying cause of mechanical barrier damage.

Platelet to lymphocyte ratio, NLR, and SII are recognised as inexpensive, widely applicable, and reliable indicators of inflammatory processes. Studies by Güçlü and Ağan (2017) and Güven et al (2022) have previously described elevated NLR, Platelet to lymphocyte ratio (PLR), and SII in IBS patients. Our analysis revealed that NLR was higher in the PI-IBS group compared to the control group, although there was no statistically significant difference in PLR. The presence of mucosal inflammatory cells in PI-IBS patients may account for the observed increase in NLR values.

Furthermore, we observed an elevation in the SII, indicating additional local intestinal mucosal barrier inflammation in PI-IBS patients. The SII index is believed to possess greater predictive power than NLR and PLR, with elevated SII typically indicating a stronger inflammatory response. In light of our findings on mucosal barrier impairment, it suggests that PI-IBS patients may experience low-grade inflammation both systemically and within the local mucosa.

Immunological damage in PI-IBS is indicated by changes in the systemic immunological inflammation index. Disruption of the mucosal barrier leads to variations in cytokine levels. Th1 cells typically secrete pro-inflammatory cytokines such as IL-17 and IFN-γ, whereas Th2 cells produce anti-inflammatory cytokines like IL-10. This study observed that compared to the control group, PI-IBS patients exhibited increased IL-17 expression and decreased IL-10 levels. These findings align with the results reported by Sundin et al (2015) and our previous animal experimental study (Yang et al, 2015). The increase in Th1 cytokines and decrease in Th2 cytokines suggest a shift from Th2 to Th1 immune cells in PI-IBS patients. It is believed that immune imbalance factors play a role in the development of PI-IBS. Disruption of the intestinal mucosal barrier leads to Th1-dominated mucosal immune activation and visceral hypersensitivity (Hasler et al, 2022; Ruan et al, 2023). Acute gastrointestinal infections compromise the intestinal epithelial barrier and antigen presentation, activating inflammatory cells to produce proinflammatory cytokines and chemokines (Miranda-Ribera et al, 2019).

In comparison to non-PI-IBS patients and controls, we observed a significantly higher percentage of IL-17A+ cells in PI-IBS patients. Th17 cells play a critical role in both chronic inflammation mediation and Defence against bacterial extracellular infections (Chen et al, 2010). Our investigation revealed that the PI-IBS group exhibited higher Th17 cell counts and IL-17 levels than the control group. It is hypothesised that Th17 secretion primarily contributes to the elevation of IL-17 in peripheral blood. Through binding with IL-17R, it not only mediates and promotes the occurrence and progression of inflammatory

diseases but also recruits and enriches neutrophils to mediate inflammatory responses in affected tissues (Pérez et al, 2019). Th17 cells can secrete IL-17 to sustain and amplify the inflammatory response mediated by Th17 cells (Hwang, 2010). According to our research, peripheral blood Th17 cells showed an increasing trend. For individuals with PI-IBS, the continuous activation of the intestinal mucosal immune system largely relies on the differentiation of CD4+ T cells into Th17 cells. Therefore, Th17 cells are involved in the immune regulation of IBS, although the precise mechanisms through which Th17 cells influence the occurrence and progression of IBS require further investigation. Our results from flow cytometry and enzyme-linked immunosorbent assay (ELISA) suggest that the immune barrier is compromised in PI-IBS.

Research indicates that alterations in gastrointestinal motility, visceral inflammation, and tissue damage intensify signals from the ascending visceral afferent pathway, influencing brain activity and leading to heightened pain intensity as well as emotional and mental issues such as despair and anxiety (Chey et al, 2015). According to our study, PI-IBS patients exhibited higher anxiety and depression scores compared to non-PI-IBS patients, while both IBS patient groups showed higher anxiety and depression scores compared to the control group. In PI-IBS patients, the percentages of anxiety and depression reached as high as 56.52% and 47.83%, respectively.

Given the bidirectional nature of the brain-gut axis, factors such as stress, intestinal flora imbalance, anxiety, and depression trigger the release of inflammatory mediators, resulting in an imbalance of IL-17 and IL-10 (Sibelli et al, 2016; Abautret-Daly et al, 2018; Wang and Fang, 2021; Fairbrass et al, 2022). This imbalance contributes to alterations in gastrointestinal motility, increased visceral sensitivity, and heightened intestinal mucosal permeability, thereby exacerbating clinical symptoms in PI-IBS patients.

Studies have demonstrated that both IBS and PI-IBS involve alterations in intestinal flora (Canakis et al, 2020; Berumen et al, 2021). Acute gastrointestinal infections can disrupt the balance of intestinal flora and impact intestinal microecology. While the intestinal microecology typically returns to normal after the infection resolves, some patients may experience incomplete restoration (Barbara et al, 2019). The intestinal flora influences changes in the intestinal mucosal barrier (including ultramicroscopic and microstructural changes) and mucosal immune responses (including cytokine alterations and Th2/Th1 shifts) (Chao and Zhang, 2020; Wang et al, 2020; Staudacher et al, 2021). In response to IL-17, inflammatory cells in the intestinal mucosal barrier proliferate, leading to barrier disruption and the development of mild, persistent chronic inflammation in the intestinal tract, ultimately resulting in PI-IBS.

The microbiome-gut-brain axis, comprising gut flora, intestinal barrier function, and psychological factors, plays a significant role in PI-IBS. Dysregulation of this axis can contribute to the onset of anxiety and depression (Wang et al, 2022). The interaction between anxiety, depression, and the aforementioned factors may further exacerbate intestinal inflammation in PI-IBS patients, prolonging intestinal symptoms (Guo et al, 2021). Promising avenues for therapeutic intervention include targeting visceral hypersensitivity, restoring microbial balance, and enhancing barrier function.

While our study provides valuable insights, the sample size remains insufficient and warrants further expansion. Additionally, the role of changes in intestinal microecology in the onset and progression of PI-IBS requires further exploration.

Conclusion

Compared with non-PI-IBS patients, PI-IBS patients exhibit increased levels of IL-17 and decreased levels of IL-10. These alterations may contribute to the impairment of the intestinal mucosal barrier and the manifestation of corresponding clinical symptoms.

Key points

- The microstructure, ultrastructure, cytokine profiles, clinical symptoms, GSRS scores, and anxiety and depression scores of PI-IBS patients differ from those of non-PI-IBS patients, indicating potential differences in the pathogenesis of PI-IBS and non-PI-IBS.
- Our study suggests that influenced by IL-17, there is an increase in inflammatory cells within the intestinal mucosal barrier, leading to its damage and the development of long-lasting low-grade chronic intestinal inflammation, thereby contributing to the onset of PI-IBS.

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Availability of data and materials

All data included in this study are available upon request by contact with the corresponding author.

Author contributions

JDZ: data curation, formal analysis, methodology, writing—original draft, writing—review and editing. YHD: data curation, methodology, writing—original draft. JT: data curation, investigation. LL: data curation, investigation. XCZ: conceptualisation, data curation, project administration, supervision, writing—review and editing. All authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics approval and consent to participate

All subjects provided written informed consent for inclusion before they participated in the study. This study was conducted in accordance with the Declaration of Helsinki of 1975 and approved by the institutional research ethics committee of Chongqing Medical University (Ethical approval numbers: 2019301).

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Conflict of interest

The authors declare that they have no competing interests.

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