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Role of serotonin hormone, TIMP-1, and CXCL-1 in diagnosis and differentiation types of irritable bowel syndrome

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ABSTRACT

Introduction: Irritable bowel syndrome (IBS) is one of the most common diseases of the gastrointestinal tract. The diagnosis of IBS depends primarily on the assessment of symptoms as there are no definitive diagnostic tests. Therefore, the current study aims to fill the gap in the sequence of events mechanism to understand the pathogenesis of IBS.

Methods: The current retrospective study was conducted between January and November 2024 and included 120 adults with IBS. It was divided as follows: 60 adults with type C IBS and 60 adults with type D IBS. CXC motif chemokine ligand 1 (CXCL-1) and tissue inhibitors of metalloprotein 1 (TIMP-1) as well as lipid profile and albumin were evaluated, and the relationships between them were examined to accurately diagnose and differentiate the two syndrome types (IBS type C and type D).

Results: The current results showed a significant difference in high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and triglyceride (TG) levels between IBS with constipation (IBS-C) and IBS with diarrhea (IBS-D). HDL levels were significantly high in IBS-C patients, while VLDL and TG levels were significantly elevated in patients with IBS-D ($p < 0.001$). Serotonin levels do not express a significant difference among IBS subtypes ($p > 0.01$). However, TIMP-1 and CXCL-1 levels were significantly elevated in IBS-D patients compared to IBS-C patients ($p < 0.001$). According to the correlations, a positive, strongly evident correlation between TIMP-1 and CXCL-1 was documented.

Conclusion: CXCL-1 and TIMP-1 could be considered new and important markers for the diagnosis and monitoring of the clinical manifestations of IBS. They can be used to differentiate between the subtypes of IBS and provide insight into the mechanism of IBS development and may eventually help in the development of treatment protocols.

Keywords: Irritable bowel syndrome; serotonin; tissue inhibitors of metalloprotein 1 CXC motif chemokine ligand 1; lipid profile

INTRODUCTION

Irritable bowel syndrome (IBS) is one of the most common diseases of the gastrointestinal (GI) tract (1). About 12% of people who visit a health center are due to IBS, while between 20% and 50% of gastroenterologist visits are for IBS (2,3). The condition is 1.5-2 times more common in women than in men and is most often diagnosed in people under the age of 50 years (1). IBS is a chronic dysfunction of the GI tract characterized by recurrent and relapsing symptoms such as abdominal pain, bloating, and altered bowel habits, with no detectable structural abnormalities (4,5).

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Although no single pathophysiological explanation for IBS has been found and the condition does not correlate with an increased mortality rate (6), it is significantly associated with patients' quality of life (7), ability to work, and social life (8). IBS also represents a significant financial burden on healthcare systems (9).

According to the Rome IV criteria, a diagnosis of IBS is made when there is recurrent abdominal pain accompanied by at least two of the following conditions: (a) pain associated with defecation, (b) changes in stool frequency, or (c) changes in stool consistency. These symptoms must have occurred at least once a week for the previous 3 months and must have been present for at least 3 months, with onset 6 months prior to diagnosis. IBS is classified into four different subtypes depending on the predominant features of stool frequency and consistency: IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), mixed IBS (IBS-M), and unclassified IBS (10).

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According to the diagnosis, there is no definitive test for IBS; therefore, patient diagnosis is primarily based on symptom assessment. Ongoing studies are investigating the effect of antidepressants in improving the symptoms of IBS. These studies have shown that approximately half of patients treated with selective serotonin reuptake inhibitors experienced improvement in symptoms compared to 33% of patients receiving placebo (11). The exact pathophysiology of IBS and the optimal treatment strategies remain unclear (12). The pathophysiology of IBS is thought to be multifactorial, involving abnormalities in GI motility, gene expression of serotonin reuptake transporters, alterations in the human microbiome, psychological stress, dysregulation of the brain–gut axis, and persistent low-grade inflammation (2).

CXC motif chemokine ligand 1 (CXCL-1) is one of the chemokines belonging to the CXC subfamily. It is one of the chemotactic cytokine subfamilies categorized according to their N-terminal cysteine moiety. A defining feature of this subfamily is the conserved CXC motif, which plays a crucial role in the formation of double disulfide bonds (13). Although CXCL-1 – also known as melanoma growth-stimulating activity, and growth-regulated oncogene-α (Gro-α) – is one of the most extensively studied ligands of the CXC motif chemokine receptor 2 (CXCR2), a comprehensive synthesis of the current knowledge of this chemokine is lacking (14).

Metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) exhibit a controlled and harmonized activity configuration that facilitates tissue degradation and remodeling while preventing tissue damage (15). TIMPs, the endogenous inhibitors of MMPs, are widely distributed in various fluids and body tissues. There are four documented members of TIMPs (TIMP-1, TIMP-2, TIMP-3, and TIMP-4). Like MMPs, the expression of TIMPs in tissues is tightly regulated to maintain homeostasis in extracellular matrix (ECM) metabolism (15).

Several previous studies have demonstrated increased levels of TIMP-1 in the mucosa and plasma of people with inflammatory bowel disease (IBD) (16). Furthermore, plasma TIMP-1 levels in patients with ulcerative colitis (UC) are significantly positively correlated with the endoscopic extent of mucosal damage, disease activity, and C-reactive protein levels(17). Therefore, a current study aims to investigate the serum levels of CXCL-1, TIMP-1, serotonin, lipid profile, and albumin in patients with IBS type C and type D and to evaluate their role in the diagnosis and differentiation between the different forms of IBS.

METHODS

The study was approved by the Ethics Committee of Baghdad Medical City/Iraqi Ministry of Health. A case–control study was conducted between January and November 2024, involving 180 adults. They were divided as follows: 60 IBS adults with type C, 60 IBS adults with type D, and 60 adults as a control group who do not suffer from IBS. The patients' serum was collected from the Gastroenterology and Liver Hospital in Baghdad Medical City and several private clinics in Baghdad after the patients were routinely examined, excluding patients with hypertension and diabetes and pregnant women. The age range of the groups was between 18 and 65 years. A blood sample was taken from each patient after they had fasted for 8 h.

Only adult patients aged 18-65 years with a confirmed diagnosis of IBS (IBS-C, IBS-D, or IBS-M) according to the Rome IV criteria, with symptoms for at least 6 months and written informed consent, were included in the study. Exclusion criteria included individuals with other GI disorders, severe psychiatric illness, pregnancy, major GI surgery, certain medications that affect GI function, alcohol consumption, or uncontrolled systemic diseases such as diabetes and hypertension.

All samples were subjected to biochemical testing. The three markers (serotonin hormone, growth-regulated oncogene alpha [CXCL-1], and tissue inhibitors of metalloprotein 1 [TIMP-1]) were measured in serum using the enzyme-linked immunosorbent assay (ELISA) method (ELISA reader type Huma). All solutions and samples were brought to the laboratory and stored at room temperature. The tests were performed without interruption after the start, and the experiment was conducted in the laboratories of Gastroenterology and Liver Hospital/ Medical City/Baghdad. The tests for the biomarkers were performed according to the manufacturer's approved procedure. The kits used for the experiment were serotonin (serotonin/5-hydroxytryptamine) (catalog number: E-EL-0033) (Elabscience, China), human growth-regulated oncogene alpha/CXCL-1 (catalog number: E-EL-H0045) (Elabscience, China), and TIMP-1 (human tissue inhibitors of metalloproteinase 1) (catalog number: E-EL-H0184) (Elabscience, China). Serum lipid profile and albumin content were measured by the spectrophotometric method (Mindray device, BA-88A) according to the manufacturer's instructions (Biomaghreb, Tunis), except for low-density lipoprotein (LDL), which was calculated by cholesterol-high-density lipoprotein-(triglyceride/5) using a special mathematical formula.

GraphPad Prism (version 8.0 for Windows) was used for statistical analysis. Mann–Whitney U test (non-parametric test) was used to perform the comparison between the variables (age, lipid profile, serotonin, TIMP-1, and CXCL-1 levels) between groups C and D of IBS. Fisher's exact test was used to show the variables of gender and age distribution between IBS-C and IBS-D patients. Spearman's rank test was used to show the correlation between the biomarkers. The results are expressed as median and interquartile range (IQR). $p < 0.5$ was considered statistically significant.

RESULTS

In the current retrospective study, we compare the group of IBS-C and IBS-D patients based on various biomarker variables, as shown in Table 1.

Regarding to the age, an age of 30 years (range: 23-37 years) was documented for the group of IBS-C patients and an age of 27.00 years (range: 24.00-38.00) for the group of IBS-D patients, although the difference was not significant ($p = 0.857$). The gender distribution of patients in the present study showed 27 men and 33 women in IBS-C. In IBS-D, there were 38 men and 22 women, with $p = 0.067$.

TABLE 1. Comparison of demographic and lipid profile parameters between irritable bowel syndrome-C and irritable bowel syndrome-D patients

Biomarker	IBS-C (n=60)	IBS-D (n=60)	*p*-value	Figure 1
Serotonin (ng/mL)	85.03 (76.45–99.88)	90.78 (80.69–98.00)	0.197	1A
TIMP-1 (ng/mL)	2.334 (2.216–2.822)	3.167 (2.749–3.528)	<0.001	1B
CXCL-1 (ng/mL)	280.1 (231.4–292.8)	514.2 (463.7–563.7)	<0.001	1C

IBS: Irritable bowel syndrome, TIMP-1: Tissue inhibitors of metalloprotein 1, CXCL-1: CXC motif chemokine ligand 1

The lipid profile tests in both study groups were also included in the current study. As shown in Table 1, the group of IBS-C patients had a mean total cholesterol of 172.0 mg/dL (144.0-191.2). In contrast, the group of IBS-D patients had a significantly lower mean value of 152.7 mg/dL (range: 126.0-183.8) (p = 0.012). In addition, the high-density lipoprotein (HDL) cholesterol concentration was higher in IBS-C (50.25 mg/dL; range: 35.00-59.80) than in IBS-D (33.50 mg/dL; range: 30.80-44.50), with $p < 0.001$. While the concentration of LDL cholesterol showed no significant differences between the groups (in the IBS-C group the value was 115.0 mg/dL (range: 89.00-136.0) and in the IBS-D 105.0 mg/dL (range: 84.00-134.0), p = 0.994.

In the patient group with IBS-C, they had a significantly higher mean very LDL (VLDL) of 27.39 mg/dL (range: 21.45-31.30) compared to 19.91 mg/dL (range: 16.00-25.00) in IBS-D ($p < 0.001$). Triglyceride levels were higher in the IBS-C patients (131.9 mg/dL; range: 107.3-165.8) compared to the IBS-D patient group (98.72 mg/dL; range: 83.00-125.0), $p < 0.001$.

Finally, as shown in Table 1, no statistically significant difference was found in albumin levels comparing the two patient groups. In the IBS-C group, the value was 4.50 g/dL (range: 3.50-5.00), and in IBS-D, it was 4.40 g/dL (range: 3.70-4.70), p = 0.755.

The serotonin concentration (ng/mL) in patients with IBS-C was 85.03 ng/mL (IQR: 76.45, 99.88) and with IBS-D was 90.78 ng/mL (IQR: 80.69, 98.00), i.e., no difference was found between them (p = 0.197), as shown in Table 2 and Figure 1A.

The concentration of TIMP-1 (ng/mL) in patients with IBS-C was 2.334 ng/mL (IQR: 2.216, 2.822) and with IBS-D was 3.167 ng/mL (IQR: 2.749, 3.528). In contrast to serotonin, a significant difference was evident between the two groups ($p < 0.001$), as shown in Figure 1B.

The concentration of CXCL-1 (ng/mL) was 280.1 ng/mL in the IBS-C patients (IQR: 231.4, 292.8) and 514.2 ng/mL in the IBS-D patients (IQR: 463.7, 563.7). Similar to TIMP-1, statistical analysis showed a significant difference between the two study groups ($p < 0.001$), as shown in Figure 1C.

Previous information showed significant differences in TIMP-1 and CXCL-1 levels between IBS-C and IBS-D patients, with both biomarkers being elevated in the IBS-D group. These biomarkers could help to differentiate between the subtypes of IBS and in particular highlight the differences in inflammation and tissue remodeling in IBS-D. Serotonin levels, on the other hand, showed no differences, suggesting that it does not help to differentiate between these IBS subtypes.

Serotonin concentration data (ng/mL) are shown in Table 3. Based on gender, male patients with IBS-C had significantly higher serotonin levels (median: 90.06 ng/mL) than females (median: 80.58 ng/mL) (p = 0.018). In patients with IBS-D, serotonin levels were similar in males (median: 90.78 ng/mL) and females (median: 92.28 ng/mL), i.e., without significant differences (p = 0.681), as shown in Figure 2A.

In terms of patient age, serotonin levels in the IBS-C group were similar in patients ≤30 years (median: 82.55 ng/mL) and those over 30 years (median: 89.24 ng/mL), i.e., no significant difference documented between the two age groups (p = 0.265). In IBS-D, younger patients (≤30 years) had a slightly lower median serotonin level (90.30 ng/mL) compared to older patients (>30 years, median: 93.21 ng/mL), but this difference does not reach a significant level (p = 0.063), as shown in Figure 2B.

The distribution of TIMP-1 concentration (ng/mL) by age and gender is also shown in Table 2 and Figure 3A and B. In IBS-C patients, males have lower TIMP-1 levels (median: 2.181 ng/mL) than females (median: 2.822 ng/mL) ($p < 0.001$). In patients with IBS-D, no significant difference was documented between men (median: 3.227 ng/mL) and women (median: 3.111 ng/mL) (p = 0.909). By age: For the biomarker IBS-C, patients ≤30 years had significantly lower TIMP-1 levels (median: 2.239 ng/mL) than patients >30 years (median: 2.605 ng/mL) (p = 0.028). Furthermore, in IBS-D, younger patients (≤30 years) had significantly higher TIMP-1 levels (median: 3.444 ng/mL) than older patients (>30 years, median: 2.719 ng/mL) ($p < 0.001$).

The concentration of CXCL-1 (pg/mL) showed that the women in the IBS-C patient group had significantly higher CXCL-1 levels (median: 292.0 pg/mL) than the men (median: 240.2 pg/mL) (p = 0.008), while no significant difference in CXCL-1 levels was documented between males (median: 523.0 pg/mL) and females (median: 505.1 pg/mL) in the IBS-D group (p = 0.749), as shown in Table 2 and Figure 4A.

According to age, older patients (>30 years) had significantly higher CXCL-1 levels (median: 292.4 pg/mL) compared to younger patients (≤30 years) (median: 236.3 pg/mL) in IBS-C ($p < 0.001$). In IBS-D, CXCL-1 levels showed no significant differences between younger patients (≤30 years, median: 513.9 pg/mL) and older patients (>30 years, median: 515.3 pg/mL) (p = 0.812), as shown in Figure 4B.

Table 4 shows the correlations between various factors (age, gender, TC, HDL, LDL, VLDL, and albumin) with serotonin, TIMP-1, and CXCL-1. The results showed that there was a positive correlation between serotonin, TIMP-1, and CXCL-1, but significance was only found between TIMP-1 and CXCL-1 (r = 0.55, $p < 0.01$). In the correlations of age with gender and other biomarkers, positive correlations were found with serotonin (r = 0.181, $p < 0.01$) and CXCL-1 (r = 0.129, $p > 0.05$), while there was a negative relationship for TIMP-1 (r = –0.040, $p > 0.05$). The correlations between the genders with the biomarkers are

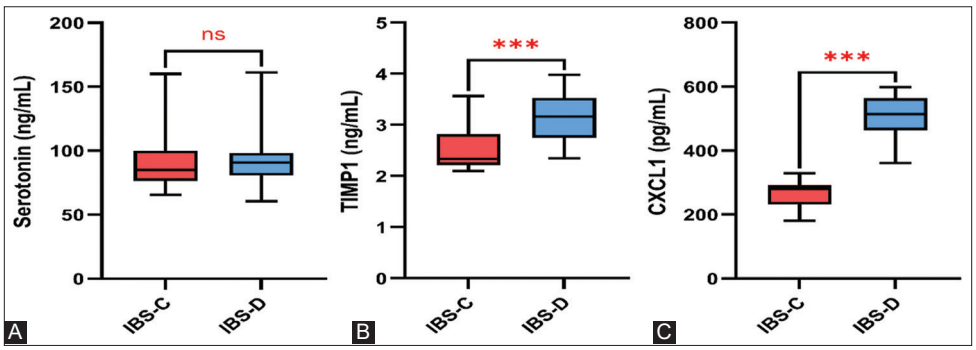

FIGURE 1. (A-C) concentrations of serotonin (ng/mL), tissue inhibitors of metalloprotein 1 (ng/mL), and CXC motif chemokine ligand 1 (ng/mL) in both irritable bowel syndrome (IBS)-constipation and IBS-diarrhea patient groups. Data were presented as median (interquartile range).

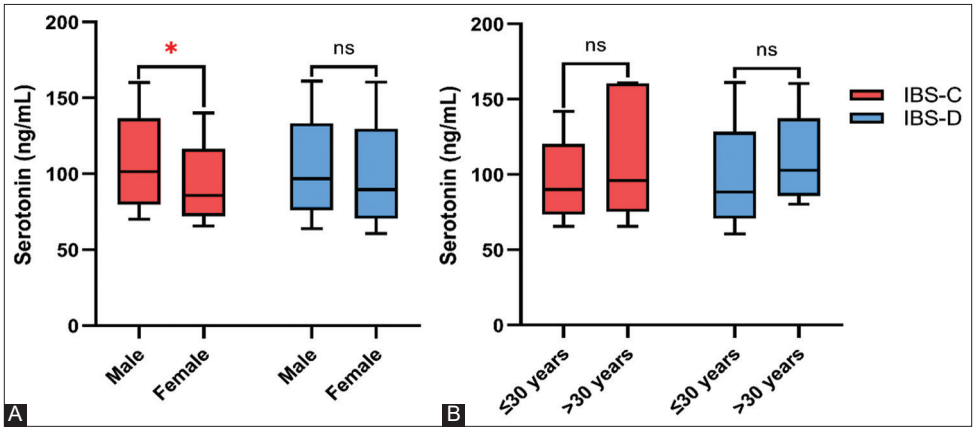

FIGURE 2. The concentration of serotonin in patients with irritable bowel syndrome (IBS)-constipation and IBS-diarrhea between males and females (A) and during age intervals (B).

TABLE 2. Comparison of serotonin, tissue inhibitors of metalloprotein 1, and CXC motif chemokine ligand 1 levels between irritable bowel syndrome-C and irritable bowel syndrome-D patients. Data are shown as median values with interquartile ranges

Variables	IBS-C	IBS-D	*p*-value
Age (years)	30.00 (23.00–37.00)	27.00 (24.00–38.00)	0.857
Sex (male/female)	27/33	38/22	0.067
TC	172.0 (144.0–191.2)	152.7 (126.0–183.8)	0.012
HDL-C	50.25 (35.00–59.80)	33.50 (30.80–44.50)	<0.001
LDL-C	115.0 (89.00–136.0)	105.0 (84.00–134.0)	0.994
VLDL-C	27.39 (21.45–31.30)	19.91 (16.00–25.00)	<0.001
Triglycerides	131.9 (107.3–165.8)	98.72 (83.00–125.0)	<0.001
Albumin	4.50 (3.50–5.00)	4.40 (3.70–4.70)	0.755

IBS: Irritable bowel syndrome, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very LDL

also shown in Table 4, which shows a negative significant dependence between genders for serotonin, TIMP-1, and CXCL-1 (r = –0.227, $p < 0.05$, r = –0.278, $p < 0.01$, and r = –0.240, $p < 0.01$, respectively).

Based on the correlation analysis between lipid profile components and biomarkers, a notable negative correlation was observed between cholesterol and serotonin (r = –0.097, p > 0.05), LDL and CXCL-1 (r = –0.107, p > 0.05), serotonin and LDL (r = –0.062, p < 0.01), as well as serotonin and VLDL (r = –0.050, p > 0.05). A negative but non-significant association was observed between cholesterol and TIMP-1 (r = –0.124, p > 0.05), as well as between cholesterol and CXCL-1 (r = –0.156, p > 0.05). Similarly, negative correlations were found between HDL and serotonin (r = –0.128, p > 0.05), TIMP-1 and CXCL-1 (r = –0.041, p > 0.05), and HDL and CXCL-1 (r = –0.205, p < 0.05). Additionally, LDL was negatively correlated with TIMP-1 (r = –0.155, p > 0.05), while VLDL showed negative correlations with both TIMP-1 (r = –0.156, p > 0.05) and CXCL-1 (r = –0.278, p < 0.01). Finally, correlation analysis between albumin and the measured biomarkers revealed a weak negative association with serotonin (r = –0.239, p > 0.01), and weak, non-significant positive correlations with TIMP-1 (r = 0.059, p > 0.05) and CXCL-1 (r = 0.163, p > 0.05).

DISCUSSION

The pathophysiology of IBS is not yet fully understood (18). However, serotonin plays an important role in the regulation of intestinal motility and is closely linked to the pathophysiology of IBS. Serotonin is essential for the maintenance of intestinal distension, motility, and visceral sensitivity. Serotonin is now thought to be related to various mental illnesses such as depression while also regulating peripheral bowel function (19).

In the current study, a significant decrease in serotonin levels was found in IBS types C and D compared to healthy people. These findings are consistent with a previous study that examined rectal biopsies from healthy controls and patients with UC, IBS-D, and IBS-C. The study showed a significant reduction in serotonin concentration in the intestinal mucosa of UC, IBS-C, and IBS-D samples compared to healthy controls (20).

Serotonin plays various roles in digestion by modulating GI secretion, peristalsis, and absorption. It also centrally regulates

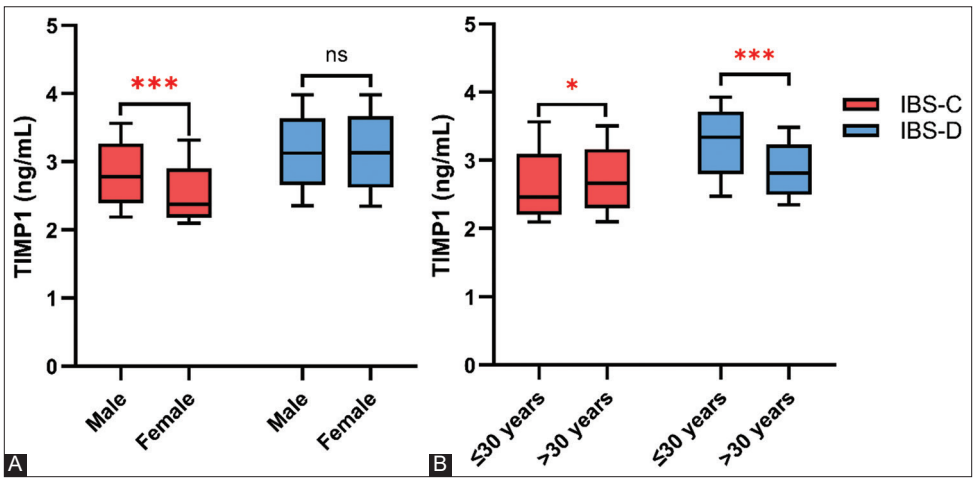

FIGURE 3. The concentration of tissue inhibitors of metalloprotein 1 in patients with irritable bowel syndrome (IBS)-constipation and IBS-diarrhea between males and females (A) and during age intervals (B).

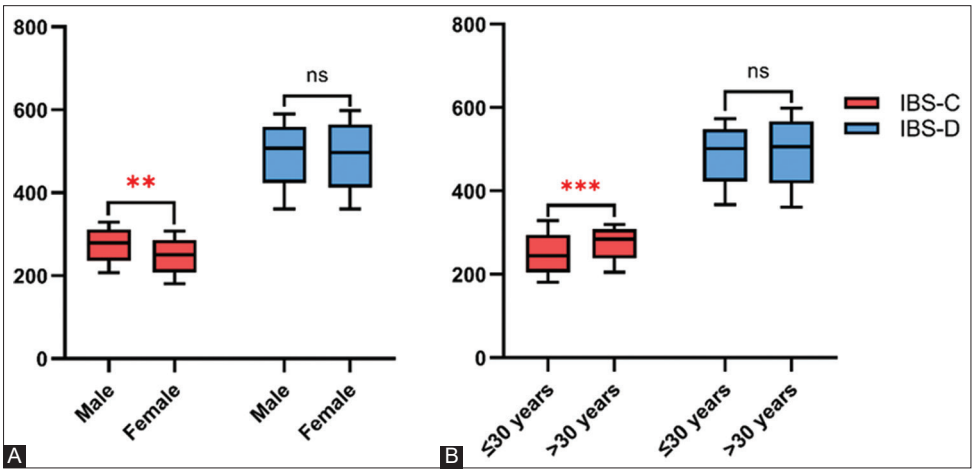

FIGURE 4. The concentration of CXC motif chemokine ligand 1 in patients with irritable bowel syndrome (IBS)-constipation and IBS-diarrhea between males and females (A) and during age intervals (B).

TABLE 3. A subgroup analysis of the levels of biomarkers (serotonin, tissue inhibitors of metalloprotein 1, and CXC motif chemokine ligand 1) in irritable bowel syndrome-C and irritable bowel syndrome-D groups, analyzed by sex and age

Biomarker	Group	Based on sex				Based on age			
		Male	Female	*p*	Figure	≤30 years	>30 years	*p*	Figure
Serotonin (ng/mL)	IBS-C	90.06 (79.91–128.1)	80.58 (71.33–89.32)	0.018	2A	82.55 (76.63–89.30)	89.24 (76.45–101.9)	0.265	2B
	IBS-D	90.78 (84.29–98.19)	92.28 (80.25–98.12)	0.681		90.30 (72.55–97.85)	93.21 (88.98–98.88)	0.063	
TIMP-1 (ng/mL)	IBS-C	2.181 (2.219–2.533)	2.255 (2.822–3.123)	<0.001	3A	2.239 (2.202–2.741)	2.605 (2.282–3.100)	0.028	3B
	IBS-D	3.227 (2.688–3.507)	3.111 (2.749–3.541)	0.909		3.444 (2.849–3.687)	2.719 (2.474–3.194)	<0.001	
CXCL-1 (ng/mL)	IBS-C	240.2 (213.5–292.1)	292.0 (248.2–309.4)	0.008	4A	236.3 (212.9–289.2)	292.4 (279.6–307.4)	<0.001	4B
	IBS-D	523.0 (442.8–551.2)	505.1 (475.6–564.7)	0.749		513.9 (473.7–563.7)	515.3 (444.2–580.4)	0.812	

Data were presented as median (IQR). The data are presented as median values with interquartile ranges and *p*-values indicating statistical significance.
IBS: Irritable bowel syndrome

behavior and important mental processes (21). Experimental exogenous serotonin delivery triggers a range of physiological responses due to the wide distribution and diversity of 5-HT receptors (22). Fourteen different 5-HT receptors have been identified and classified into seven families based on their signaling pathways. While most are coupled to G proteins, the 5-HT3 receptor functions as a ligand-gated ion channel (23). Although the specific functions of some 5-HT receptors are now known, many of them elicit different or even opposing effects (24). The release of 5-HT from enterochromaffin cells in the intestinal epithelium is primarily stimulated by mechanical and chemical interactions with the intestinal wall as food passes through the gut (25). Normal gut flora significantly influences 5-HT regulation by increasing the expression of the enzyme tryptophan hydroxylase and controlling the function of serotonin transporters (26). Sympathetic adrenergic stimulation, mucosal changes, impaired intestinal motility, and a decrease in luminal pH can also regulate 5-HT release (27). Once released, 5-HT triggers the peristaltic reflex, increases blood flow to the ileum and duodenum, and promotes gastric accommodation via the 5-HT1, 5-HT3, 5-HT4, and 5-HT7 receptors (28). To prevent overstimulation by serotonin, enterocytes take up 5-HT via the serotonin transporter (SERT).

TABLE 4. Statistical correlations of serotonin, tissue inhibitors of metalloprotein 1, and CXC motif chemokine ligand 1 with age, sex, and lipid profile

Variables	Correlation with serotonin		Correlation with TIMP-1		Correlation with CXCL-1	
	r	*p*	r	*p*	r	*p*
Serotonin	1	-	0.07	>0.05	0.13	>0.05
TIMP-1	0.07	>0.05	1	-	0.55	<0.01
CXCL-1	0.13	>0.05	0.55	<0.01	1	–
Age	0.181	<0.01-	−0.040	>0.05	0.129	>0.05
Sex	−0227	<0.05	−0.278	<0.01	−0.240	<0.01
TC	0.097	>0.05	−0.124	>0.05	−0.156	>0.05
HDL	−0.128	>0.05	−0.041	>0.05	−0.205	<0.05
LDL	0.062	<0.01	−0.155	>0.05	0.107	>0.05
VLDL	0.050	>0.05	−0.156	>0.05	−0.278	<0.01
Albumin	−0.239	>0.01	0.059	>0.05	0.163	>0.05

IBS: Irritable bowel syndrome, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very LDL, TC: Total cholesterol, TIMP-1: Tissue inhibitors of metalloprotein 1, CXCL-1: CXC motif chemokine ligand 1

Alzoghaibi et al. found that circulating CXCL-1 levels were significantly increased in the CD and UC patient groups compared to the healthy group (29), with the highest statistically significant CXCL-1 levels found in IBS D patients and to a lesser extent in patients with type C, suggesting that CXCL-1 may be a differential marker between patients with IBS type D and type C.

CXCL-1 was labeled as a potential biomarker for UC (29). Gene expression analysis suggests that CXCL-1 is a key gene in this disease (30). In addition, mucosal biopsies from patients with UC show a higher expression of another CXCR2 ligand, CXCL-8/IL-8, suggesting that CXCL-1 interacts with other CXCR2 ligands. A study of perfusates from patients with high levels of CXCL-1 (31) found that CXCL-1 levels were 3 times higher than those of CXCL-8/interleukin-8, emphasizing the prominent role of CXCL-1 among CXCR2 ligands in UC. The expression of CXCL-1 is influenced by various factors present in the inflamed gut. For example, elevated levels of interleukin-17 (32) and tumor necrosis factor-β (33) have been found to increase CXCL-1 expression in colonic mucosal biopsies from patients with GIT disease, which may be due to the synergistic effects of these two cytokines (34).

MMPs and their inhibitors are considered key factors in the pathogenesis and inflammatory processes of IBD (35-37). In this study, TIMP-1 levels were investigated in patients with type C and type D IBS. Statistical analysis revealed significant differences between all study groups, with serum TIMP-1 levels being significantly higher in patients with type D IBS than in patients with type C IBS. This result is partially consistent with the findings of Czajkowska et al. (38). Matrix MMPs and their tissue inhibitors (TIMPs) play a central role in the remodeling of the ECM. In particular, MMP-9 enhances the production of pro-inflammatory cytokines, while TIMP-1 modulates cell function by acting as a cytokine regulator (39,40). In addition, MMP-9 contributes to increased intestinal permeability without inducing apoptosis in intestinal cells (39,41,42). Under normal physiological conditions, these enzymes maintain a delicate balance and prevent tissue damage. However, in the inflammatory milieu of IBD, overexpression of MMP-9 and TIMP-1 enhances enzymatic activity (43). Disruption of MMP-9 regulation impairs cell adhesion, stimulates the release of cytokines, and recruits neutrophils to the intestinal epithelium, thereby increasing inflammation (41,42,44).

In a comprehensive systematic review and meta-analysis that included 53 studies, the association between IBD and serum lipid profiles was meticulously examined. Chen et al. reported a significant reduction in total cholesterol (TC), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) in individuals with IBD compared to those without the disease (45). This is not entirely consistent with the current study, because in the current study, there were no significant differences between the groups at the LDL level, but the difference was in the levels of total cholesterol, so there was a statistically significant increase in cholesterol levels in patients with IBS type C compared to IBS type D. When serum VLDL levels were measured, it was found that the levels were significantly increased in patients with IBS type C compared to patients with IBS type D.

The relationship between serum lipids and IBD can be understood through several mechanisms. Apolipoprotein AI (Apo-AI) is usually considered the predominant apolipoprotein of HDL (46). However, during inflammation, pro-inflammatory cytokines such as interleukin (IL)-1, IL-6, and tumor necrosis factor-α trigger the synthesis of substantial amounts of serum amyloid A (SAA) in the liver. Once SAA is released into the bloodstream, it rapidly binds to HDL and displaces Apo-AI as its major apolipoprotein (47). This SAA-enriched HDL is excreted from the bloodstream at an accelerated rate and is preferentially taken up by macrophages rather than hepatocytes, leading to a decrease in HDL-C levels (47). In adipose tissue, glycoproteins on the membranes of fat cells can bind SAA and thus retain HDL in the fat depots. This binding leads to a further decrease in plasma HDL concentration and ultimately contributes to lower HDL-C levels in patients with IBD (48).

In addition, HDL has been shown to have immunomodulatory properties (49). In cellular immunity, major histocompatibility complex (MHC) class II molecules, which play a central role in antigen presentation and signal transduction, are localized in lipid-rich microdomains of antigen-presenting cells. The abundance of these molecules is essential for effective T-cell activation. Lipid rafts – specialized membrane microdomains enriched in cholesterol, proteins, and sphingolipids – have functional properties that are determined by their lipid composition. The loss of cholesterol from these domains can impair several immune cell signaling pathways and impede antigen presentation. In addition, it lowers the antigen threshold required for T-cell activation by bundling MHC–peptide complexes on the surface of antigen-presenting cells (50). HDL facilitates the removal of cholesterol from peripheral cells, which can lead to a reduction in cholesterol levels in lipid rafts. This in turn reduces the amount of MHC class II molecules and thus weakens T-cell activation (51). This explains why HDL levels were lower in the patients of the present study compared to healthy individuals (35-65 mg/dL in men, 35-80 mg/dL in women).

In the results of the present study, we also found an increase in serum triglyceride (TG) levels in patients with IBS type C compared to those with IBS type D. The terminal ileum plays an important role in the absorption of bile acids. When absorption in the small intestine is impaired, significant amounts of bile acids and cholesterol can be lost in the stool, which can lead to decreased lipid levels (52). The small intestine is also an important site for the production of TGs. In the small intestine, bile acids interact with dietary triacylglycerols and facilitate their breakdown into free fatty acids and monoacylglycerols. These components are then reassembled into triacylglycerols in the endoplasmic reticulum (53).

IBS primarily affects the small intestine, which leads to a reduced production of TGs. As a result, TG levels are generally lower. In patients with IBS-D, the values were significantly higher compared to patients with IBS-C. Analysis of the relationships between age, gender, and the markers examined revealed a positive, statistically significant correlation between serotonin levels and age. This indicates that serotonin levels increase with age and decrease with increasing age. In contrast, there was a negative relationship between serotonin, TIMP-1, and CXCL-1 levels and gender.

When examining the relationships between the markers studied, the results also showed a positive correlation between TIMP-1 and CXCL-1, suggesting that as TIMP-1 levels increase, CXCL-1 levels also increase, which correlates with increased bowel symptoms. The study also found a strong, statistically significant positive correlation between serotonin and VLDL, meaning that higher serotonin levels are associated with higher VLDL levels and vice versa. Conversely, there was a strong, statistically significant negative correlation between serotonin and albumin, as well as between CXCL-1 and TG, VLDL, and HDL. To our knowledge, the current study is the first to examine these correlations, although there have been some studies that examined the association between TIMP-1 and CXCL-1 and documented a positive correlation between them (54,55), and these results are consistent with our findings. These results are consistent with our results. However, these studies do not include the current set of biomarkers and this disease (IBS). Therefore, these findings may provide insight into the mechanism of IBS development and help in the design of treatment protocols and ultimately disease management.

As already mentioned, impaired intestinal absorption leads to fat excretion in the stool and thus to a lower fat content. The small intestine plays a key role in the production of TGs, which is directly linked to bile acids and their breakdown to free fatty acids. Any intestinal dysfunction can disrupt this process (56). Therefore, understanding these marker relationships may contribute to a more precise diagnosis and differentiation between the forms of IBS under investigation.

CONCLUSION

The significant decrease in serotonin levels and the increase in CXCL-1 and TIMP-1 levels in the patient groups compared to the control group may provide new insight into the use of these markers in the diagnosis and monitoring of IBS symptoms and in the differentiation between the two types of the syndrome (IBS type C and IBS type D). In addition, these findings may help to understand the mechanism of the development of IBS, and finally, they may shed light on the treatment of IBS.

DECLARATION OF INTERESTS

Authors declare no conflict of interest.

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