Influence of IL-1ß, Anti-CdtB and Histamine in Irritable Bowel Syndrome

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تأثير IL-1β, Anti-CdtB، والهستامين في متلازمة تهيج القولون

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Abstract

Irritable Bowel Syndrome (IBS) is a common gut disorder that affects approximately 11% of the global population and negatively affects the quality of life of patients and imposes a significant socioeconomic burden. Control-based study was conducted on 42 Iraqi patients with IBS who were presented at private clinics for gastrointestinal diseases (GIDs), along with 20 healthy individuals matched in their age and gender to act as control group. The study aimed to determine the frequency of IBS among Iraqi patients, as well as the influence of biomarkers on disease's initiation, type and severity such as (IL-1β, anti- Cytolethal distending toxin B 'Anti-CdtB', and histamine). The results showed that constipation subtype of IBS is the most frequent among patients, and females constitute the majority of IBS patients with female: male ratio about 2.23: 1. The levels of IL-1β, anti-CdtB, and histamine are significantly elevated in the serum of IBS patients when compared to their levels in control group. In patients' group, anti-CdtB showed significant positive correlation with their level of histamine, while correlation between anti-CdtB and IL-1β as well as between histamine and IL-1β are not significant. It can be concluded that anti-CdtB may act as a key player in IBS pathogenesis after disruption gut microbiota which needs further investigation.

Keywords: IBS, IL-1β, Anti-CdtB, Histamine.

المستخلص

متلازمة تهيج القولون(IBS) هي اضطراب معوي شائع يصيب ما يقرب من 11٪ من سكان العالم ويؤثر سلبًا على نوعية حياة المرضى ويفرض عبئًا اجتماعيًا واقتصاديًا كبيرًا. أجريت الدراسة القائمة على الضبط على 42 مريضاً عراقياً يعانون من القولون العصبي والذين تم تقديمهم في عيادات خاصة لأمراض الجهاز الهضمي (GIDs)، إلى جانب 20 فردًا يتمتعون بصحة جيدة يتناسبون مع أعمارهم وجنسهم للعمل كمجموعة ضابطة. هدفت الدراسة إلى تحديد مدى تكرار الإصابة بمرض القولون العصبي لدى المرضى العراقيين، وكذلك مدى تأثير المؤشرات الحيوية على بداية المرض ونوعه وشدته مثل (IL-1β، و 'Anti-CdtB anti-Cytolethal distending toxin B'، و الهيستامين). أظهرت النتائج أن نوع الإمساك الفرعي من متلازمة القولون العصبي هو الأكثر شيوعًا بين المرضى، وتشكل الإناث غالبية مرضى القولون العصبي مع نسبة الإناث إلى الذكور حوالي 2.23: 1. حيث ان مستويات IL-1β, anti-CdtB والهستامين مرتفعه بشكل ملحوظ في مصل مرضى القولون العصبي مقارنة بمستوياتهم في المجموعة الضابطة. في مجموعة المرضى، أظهرت مضادات CdtB ارتباطًا إيجابيًا معنويًا بمستوى الهيستامين، في حين أن العلاقة بين مضادات CdtB و IL-1β وكذلك بين الهيستامين و IL-1β ليست ذات دلالة. يمكن أن نستنتج أن مضادات CdtB قد تعمل دور رئيسي في التسبب في مرض القولون العصبي بعد تعطيل ميكروبيوتا الأمعاء التي تحتاج إلى مزيد من التحقيق.

الكلمات المفتاحية: هستامين, مضاد CdtB, IL-1B و IBS

Introduction

Irritable bowel syndrome (IBS) is a common gut disorder that affects approximately 11% of the global population (Canavan *et al.,* 2014). Depending on their bowel habits changes predominance, Rome criteria classified subjects with IBS into different sub-groups including IBS with constipation (IBS-C), IBS with diarrhea (IBS-D), Mixed IBS (IBS-M), and Un-subtyped IBS (Lacy and Patel, 2017). IBS is a common gastrointestinal disorder negatively affects the quality of life of patients and imposes a significant socioeconomic burden (Black and Ford, 2020). Although the etiology and underlying pathophysiology of IBS remain incompletely understood, visceral hypersensitivity, impaired gut motility, increased intestinal permeability, emotional disorders, and changes in the immune system are proposed mechanisms involved in its pathogenesis (Holtmann *et al.,* 2016; D'Antongiovanni *et al.,* 2020; Hadjivasilis *et al.,* 2019; Casado-Bedmar and Keita, 2020). Different biomarkers have been investigated to diagnose or classify IBS, among these biomarkers; three types of biomarkers are identified in IBS patients of the present study including; human interleukin-1 beta (IL-1β), anti-cytolethal distending toxin B (Anti-CdtB), and Histamine.

In colonic biopsies from patients with IBS, an increased number of mast cells have been found in close proximity to enteric nerve fibers and have been thought to be key players in intestinal mucosal inflammation that associated with the severity of symptoms (Enck *et al.,* 2016; Wouters *et al.,* 2016). When mast cells undergo degranulation, they release inflammatory mediators (histamine, serotonin, and proteases), resulting in lymphocyte activation and cytokine imbalance. Therefore, patients with IBS were found to have higher levels of proinflammatory interleukin (IL-6, IL-8, IL-1β), tumor

necrosis factor-α (TNF-α) and lower levels of anti-inflammatory IL-10 in both serum and the intestinal mucosa (Choghakhori *et al.,* 2017).

On the other hand, it has been reported that progression to IBS in a rat model was accompanied by the detection of a specific bacterial toxin named cytolethal distending toxin B (CdtB) after infection with Campylobacter jejuni (Pokkunuri *et al.,* 2012). After development of anti-CdtB, it is able to cross-react with vinculin, a host cell adhesion protein present in interstitial cells of Cajal (ICC) and the myenteric ganglia that control the normal activity of the intestinal tract (Pimentel *et al.,* 2015). This autoimmunity may profoundly affect the host immune response to infections with C. jejuni, and subsequently leading to IBS (Rezaie *et al.,* 2017). The serum levels of anti-CdtB and anti-vinculin has been detected to identify patients with IBS-D, and to differentiate it from other IBS subtypes (Morales *et al.,* 2019; Zaki *et al.,* 2021).

Materials and Methods

Case-control study was carried out on 42 Iraqi patients with IBS and 20 normal individuals who were matched in age and gender to the patients to act as the control group. Diagnosis of IBS and its subtypes is confirmed based on Rome IV criteria under supervision of specialists in gastrointestinal diseases. From all patients and normal subjects, the serum levels of biomarkers (IL1β, Anti-CdtB, and Histamine) were determined by using diagnostic kits from (BioSource, USA) for IL1β and histamine, and from (Creative Diagnostics, USA) for Anti-CdtB based on sandwich enzyme-linked immune sorbent assay technology (Sandwich ELISA) (Crowther, 1995). The difference between two independent groups was statistically evaluated by using the t-test, while categorical data were reported as percentage values,

and difference between two groups is performed by Chi square test. Pearson's correlation calculator test examines the relationship between two variables. Any difference at the P level less than 0.05 are regarded as significant.

Results

Results in Table-1 shows that the most frequent subtypes of IBS is constipation subtype which constitutes about 69% of total patients, while those with IBS-d and IBS-m constitute about 16.7% and 14.3% respectively. Also, this table reveals non-significant difference in the age and gender between patients and control groups. However, the age of patients at disease onset is 27.8 ± 12.5 year, and females are more affected with IBS in a female/ male ratio (F/M) about 2.23.

Table-1: Subtypes of IBS patients and their age and gender in corresponding with control group

Medical history		Patients (n=42)	Control (n=20)	P value
IBS subtype (n, %)	IBS-c	29 (69%)		
	IBS-d	7 (16.7%)		
	IBS-m	6 (14.3%)		
Age (year) (M ± SD)	Current	32.5 ± 12.1	33 ± 10.2	0.888
	At disease onset	27.8 ± 12.5		
	Disease duration	5.2 ± 3.3		
Gender (n, %)	Female	29 (69%)	12 (60%)	0.481
	Male	13 (31%)	8 (40%)	
	Female: Male ratio	2.23: 1		

In concerning with IBS-related biomarkers, the M ± SE of serum levels for all biomarkers (IL-1β, anti-CdtB, and histamine) in patients are equal to 183.3 ± 16.8 pg/ml, 2.1 ± 0.07 ng/ml, and 5.31 ± 0.48 ng/ml respectively,

which are significantly higher than those in control group (85.5 ± 3.3 pg/ml, 1.4 ± 0.02 ng/ml, and 1.12 ± 0.04 ng/ml respectively) as shown in Figure-1, 2, and 3.

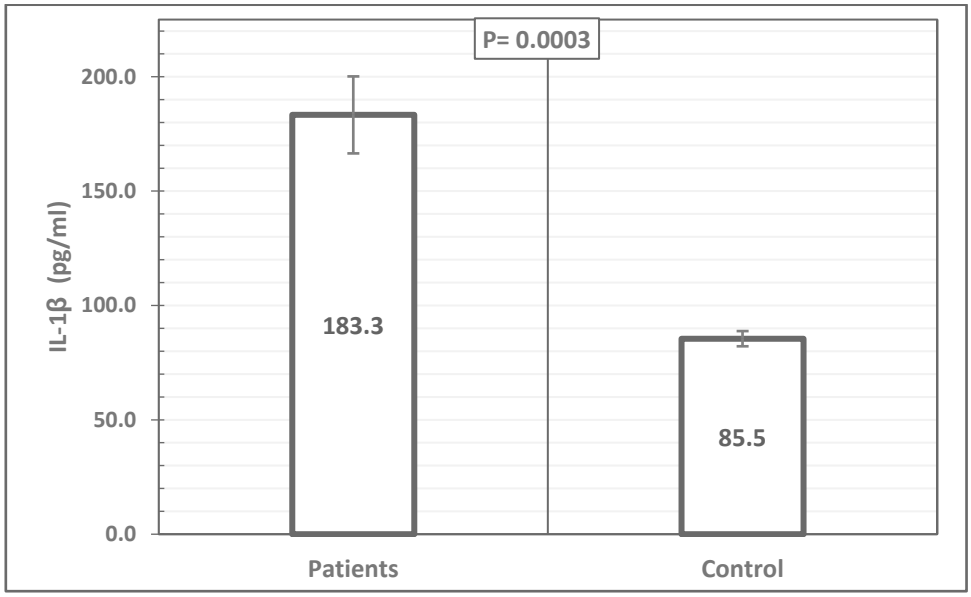

Figure-1: Serum level of IL-1β in IBS patients and control groups

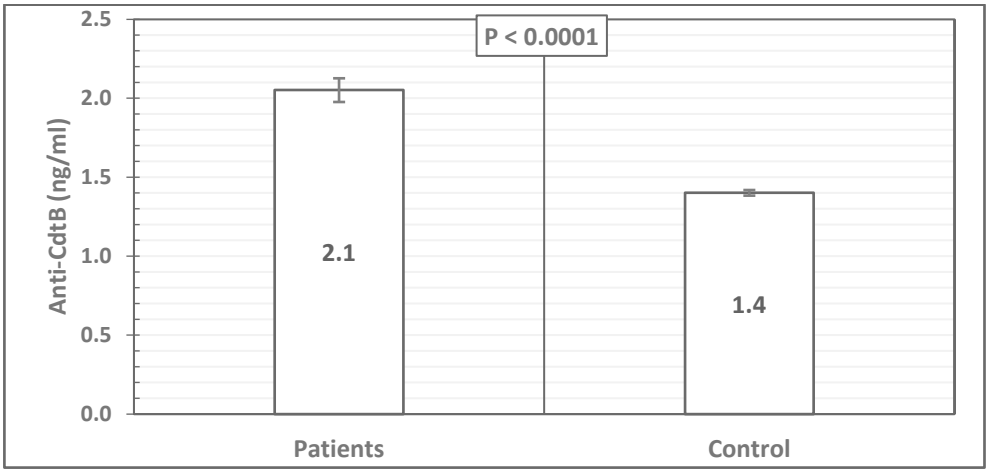

Figure-2: Serum level of anti-CdtB in IBS patients and control groups

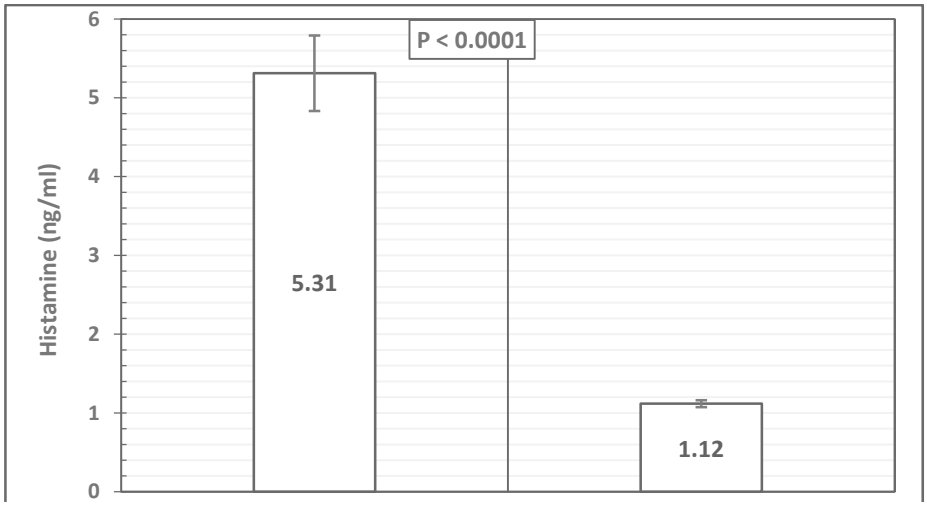

Figure-3: Serum level of histamine in IBS patients and control groups

On the other hand, Pearson's correlation calculator shows significant positive correlation between histamine level in serum of IBS patients and their anti-CdtB level (r= 0.430, P= 0.004) (Figure-4).

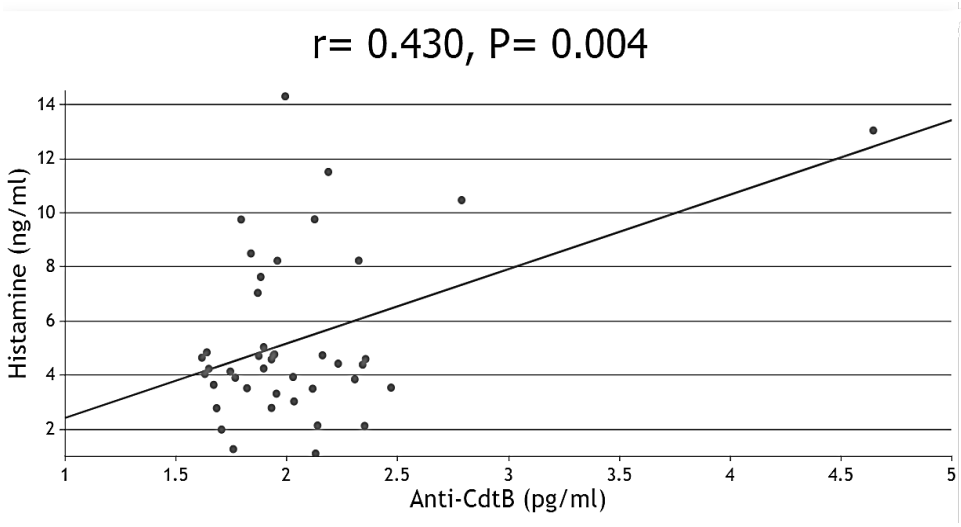

Figure-4: Correlation of anti-CdtB level in serum of IBS patients with their histamine

However, the correlations between histamine and IL-1β (r= 0.251, P= 0.108) as well as between anti-CdtB and IL-1β (r= 0.096, P= 0.545) are non -significant as shown in Figure-5 and Figure-6 respectively.

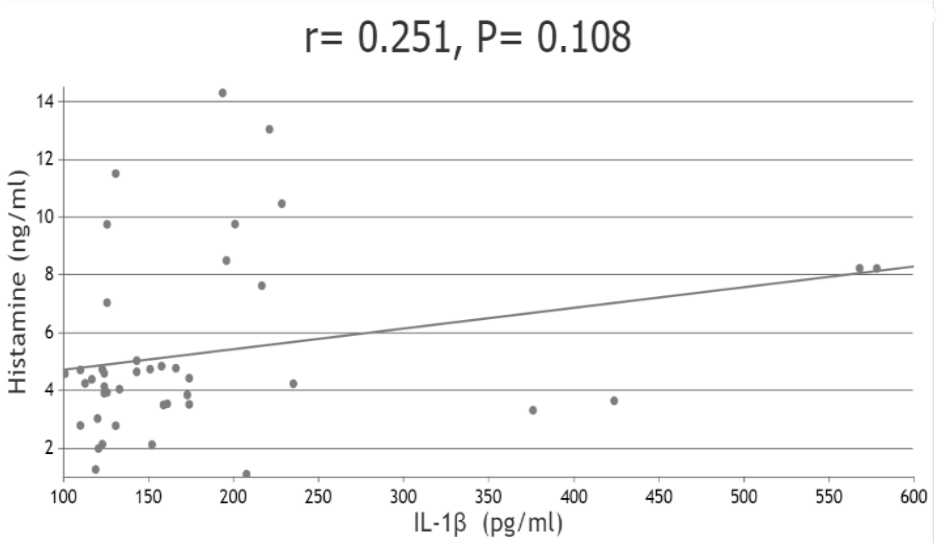

Figure-5: Correlation of IL-1β level in serum of IBS patients with their histamine

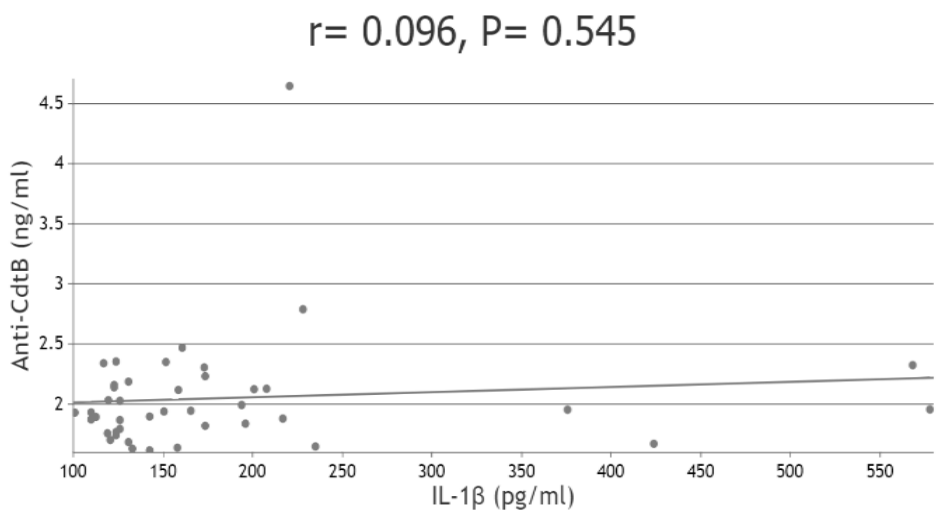

Figure-6: Correlation of IL-1β level in serum of IBS patients with their anti-CdtB

Discussion

Since the majority of IBS patients in the present study are females (F/M ratio= 2.23), the most subtype of IBS recorded is constipation subtype (Table-1). This finding is comparable with those obtained by other studies which indicated that IBS predominantly affects women at a ratio of 2:1, with female patients presenting with more constipation and abdominal pain, rather than diarrhea (Harris *et al.,* 2016; Flanagan and Spangler, 2021).

Concerning with biomarkers, the present results indicated a high level of IL-1β in serum of IBS patients compared to healthy controls as shown in Figure-1, which is in agreement with Güven *et al.* (2022), who found a substantial rise in the rate of IL-1β in IBS patients. It is well understood that IL-1β is a powerful pro-inflammatory cytokine that is essential for host-defense responses to infection and injury (Dinarello, 1996). According to some research, IBS patients had higher blood cytokine levels (Dinan *et al.,* 2006). Also, the production of cytokines from peripheral blood mononuclear cells (PBMCs) from the blood frequently reflects the severity of inflammation, particularly IL-1β, which is thought to reflect disease activity because peripheral immune cells interact with intestinal epithelial cells and cytokines released by PBMCs to modulate intestinal barrier function. As a result, impaired mucosal barrier function may be related to PBMC-mediated cytokine release, implying a role for immunological activation in a subset of IBS patients (Holtkamp *et al.,* 1995; Liebregts *et al.,* 2007).

According to anti-CdtB, the present result demonstrated a high serum level of Anti-CdtB in IBS patients compared to healthy peoples. This biomarker is recognized to play a significant impact on the development of IBS symptoms (Smith and Bayes, 2006). In agreement with present results,

numerous researchers also reported higher level of anti-CdtB in IBS patients when compared with healthy controls (Pimentel *et al.,* 2015; Rezaie *et al.,* 2017; Talley *et al.,* 2019; Zaki *et al.,* 2021). Moreover, the present results demonstrated a considerable rise in serum this is in agreement with Cenac *et al.* (2007) who observed that the release of histamine and the tryptase enzyme are already increased in IBS due to activation of mast cells. While Buhner *et al.* (2009) indicated increased histamine levels at disease sites such as IBD and IBS are still not understood.

In concerning with its correlation with other biomarkers, the current study found that the serum level of histamine is increased with increasing anti-CdtB level (Table-2). This result may indicate that anti-CdtB play a crucial role in stimulating degranulation process in basophil and mast cells. Previous studies reported that microbial dysbiosis within the gut has been implicated in intestinal barrier dysfunction, visceral hypersensitivity, impaired gastrointestinal motility, and altered immune response. Therefore, the gut microbiota has emerged as a potential factor that contributes to the pathophysiology of IBS (Quigley, 2018; Mari *et al.,* 2020). Moreover, various studies have consistently shown the efficacy of microbiota-directed therapies and dietary changes in alleviating IBS symptoms (Herndon *et al.,* 2020). Recently, it has been thought that antibiotics seem to contribute to all aspects of IBS pathogenesis because they act as a major disruptor of the gut microbiota (Mamieva *et al.,* 2022).

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