ATENA JOURNAL OF PUBLIC HEALTH

https://atenajournals.com

Received: **02/06/2022.** Accepted: **05/08/2022.** Published: **08/08/2022**

Interleukin-2 concentration and C-reactive protein positivity among patients with irritable bowel syndrome in Diyala Province-Iraq

Hiba Hadi Rashid*

Department of Microbiology, College of Medicine, University of Diyala, Iraq.

* Correspondence: Hiba Hadi Rashid; mastermedical55@gmail.com

ABSTRACT

Objectives: To assess the levels of C-reactive protein (CRP) and interleukin-2 (IL-2) concentration in patients clinically diagnosed with irritable bowel syndrome (IBS), and to compare these levels with those in a control group.

Methods: Seventy patients with IBS were diagnosed based on ultrasonography and clinical findings, and an additional 30 seemingly healthy individuals were included in the current study. The study was performed at Baqubah Teaching Hospital, Diyala, Iraq, over one year, from September 2019 to October 2020. IL-2 concentration was determined by enzyme-linked immunosorbent assay (Sino Gene Clon Biotech Co., Ltd., China), and CRP was standardized using particle-enhanced immunoturbidometric latex (Spinreact, Spain).

Results: The results showed a relationship between IL-2 concentration and socio-demographic factors. In IBS patients, the IL-2 concentration was highest in the 30-39 age group, though the variation was not statistically significant. For the control group, significant variation was observed ($P = 0.013$). IL-2 levels were slightly higher in IBS females compared to males, with no significant effect in controls. The C-reactive protein positivity rate (≥ 6 mg/dl) was significantly higher in IBS patients than in healthy controls ($P = 0.048$). IL-2 concentration was insignificantly higher in IBS patients compared to controls ($P = 0.126$).

Conclusions: The study suggests that alterations in CRP positivity and IL-2 concentration in patients with IBS may play a major role in its pathogenesis.

KEYWORDS: Irritable Bowel Syndrome; Interleukin-2; ELISA; CRP

1. INTRODUCTION

Interleukin-2 (IL-2) was discovered, isolated, and subscribe between 1976 and 1983. Interleukin-2 (IL-2) is an interleukin, a type of cytokine signaling molecule in the immune system. It is a 15.5-16kDa protein that participates in regulates the events of white blood cells (leukocyte, often lymphocyte) that are dependable for immunity and considered the first immunotherapy demonstrating clinical efficacy in metastatic cancer[1-2]. IL-2 is part of the body's natural response to microbial infection, and in discernment between foreign ("non-self") and "self" proteins. IL-2 mediates its effects by linking to IL-2 receptors, which are expressed by lymphocytes. The main sources of IL-2 are activated CD4+T cell and activated CD8+ cells [3]. Several immune cells have been shown to secrete IL-2 when activated, including T cell receptor (TCR), natural killer cells (NK) cells, dendric cells (DCs) and mast cells [4].

Irritable bowel syndrome (IBS) is a general chronic and debilitating functional of the gastrointestinal (GI) tract with a predominance rate of ~10%–15% in the western world [5-6]. IBS featured by changed bowel habits linked in the absence of identified structural and biochemical abnormalities and caused symptoms include nausea, vomiting, diarrhea, constipation, difficult passage of food or feces and abdominal disturbance or pain [7-8]. The etiology is unwell understood, and many factors are implicated. IBS pathophysiology involves multiple mechanisms. Previous studies proposed that there are various gastrointestinal and extra-intestinal sides associated with this disease; including variety of intestinal flora, chronic mucosa inflammation, abnormal brain- bowel axis, that most likely is modulated by genetic agents, and that regulate the regional inflammatory, in addition, psychological disorders can be involved in the pathophysiology of IBS [9]. An infectious event can seemingly take part in IBS development in individuals with a genetic and psychosocial, susceptibility, most likely by accommodation a mild grade of intestinal mucosa inflammation that leads to immune, system activation [10-11]. C-reactive proteins (CRP) is an acute phase reactant that arises from the liver, produced by hepatocytes pending the acute phase reaction, mainly by stimulation with interleukin (IL)-6, it has many clinical and biological effects and can be used for the follow-up and diagnosed of various traumatic and inflammatory processes [12].

There is powerful guide that CRP is a strong predictor of incident cardiovascular events free of level of LDL cholesterol. Contrarily, some patients do not develop high CRP levels despite their functional disease [13-14]. Although CRP is not disease-specific [15], CRP biomarker that reverses systemic inflammation, which has been estimated for its benefit as a marker of IBS activity. Last studies have also qualified the advantage of other biomarkers in fecal samples, such as fecal immunochemical occult blood test, and mention that these biomarkers inverted more accurately the

local inflammation of the colon in patients with ulcerative colitis [16-17].

2. METHODS

2.1. Study Design and Participants

This cross-sectional, case-control study was conducted from September 2019 to October 2020 at Baqubah Teaching Hospitals in Diyala Governorate. The largest study group comprised 70 patients with irritable bowel syndrome, diagnosed based on clinical findings and ultrasonography results, with ages ranging from 15 to 79 years. This group consisted of 25 (35.7%) males and 45 (64.3%) females. Additionally, 30 apparently healthy individuals participated, including 19 (63%) males and 11 (36.7%) females. Therefore, the sample included 100 individuals, with 70 diagnosed with irritable bowel syndrome and 30 healthy controls. The exclusion criteria were: immunosuppressed patients, diabetic patients, and those who had recently undergone digestive surgery. The Ethics Committee of Diyala University College of Medicine authorized the research.

2.2. Sample collection

About 4-5 milliliters of venous blood was drawn from all participants and placed in a serum sorting gel tube (SST). The blood in gel tube from patients and controls was left for 20 minutes at room temperature. After clotting, the sera were separated by centrifugation at 4000 x for 15 minutes, and then applied for IL-2 concentration limitation by enzyme -linked-immunosorbent assay (Sino Gene Clon Biotech Co., Ltd China) and CRP rate detection by latex particle immunoturbidometry (spin react. Spain).

2.3. Instrument

The questions for all participants were prepared in an Arabic and English languages to reduce any problems by all study groups. The participants' confidentiality was preserved and questionnaire on personal acquaintance was acquired. Data were obtained orally from each participant.

2.4. Statistical Analysis

These data included: age, occupation, education level, and social status. The distribution of sociodemographic data according to IL-2 concentration was compared between IBS patients and the control group, as well as the distribution of IL-2 and CRP positivity between the study groups. The data were analyzed using the statistical package for social sciences (SPSS) version 25. The results were considered significant when the difference between two independent variables using Student-T Test obtained a level of 0.05 and the variation between more than two independent variables using the

ANOVA test reached a level of 0.05.

3. RESULTS

The results in table 1 reveal the association of IL-2 concentration with certain socio-demographic agents in all study groups. Regarding the age, in the IBS patients although the difference among the age groups was statistically insignificant, the higher mean ± DS of IL-2 was among those with 30-39 years old. On the control side, the significant difference (P= 0.013) was due to absence of 60-69 age group.

Regarding gender in IBS patients, although the variation between male and female was no significant effect, the higher mean ± DS of IL-2 was in females. However, other demographic factors: occupation, levels of education, marital status were showed no significant effect on the concentration of IL-2 in both study groups.

Table 1. Distribution of IL-2 concentration (pg/ml) according to socio-demographic variables

Variables	**IL-2 concentration (pg/ml)**			
	IBS patients		**Healthy control**	
	No.	**Mean ± SD**	**No.**	**Mean ± SD**
Age				
< 20	2	553.0 ± 120.21	1	848.0 ±
20---29	16	883.63 ± 757.41	14	554.43 ±124.44
30---39	16	1260.31±1044.8	8	763.0 ± 278.62
40---49	8	1169.25 ± 871.64	4	723.0 ± 282.08
50---59	4	1070.5 ±1156.3	2	728.0 ± 42.43
60---69	11	543.45 ± 271.49	-	-
≥ 70	13	632.62 ± 286.35	1	1338.0±222.34
P value		0.175		0.013
Gender				
Male	25	885.72±634.33	19	671.68±202.44
Female	45	913.42±849.06	11	694.36±315.13
P value		0.887		0.811
Occupation				
Students	5	1084.00±1010.21	1	848.00±
Official. employee	10	798.30±494.01	11	631.64±239.09
Housewife	41	933.22±883.21	8	736.75±365.69
Free work	13	844.15±537.38	10	671.00±127.37
P value		0.905		0.739
Levels of education				
Illiterate	35	787.71±816.54	7	798.00±311.23
Primary	17	907.06±539.19	3	801.33±247.86
Secondary	5	1438.00±1217.03	3	498.00±101.49
College & Higher	13	1005.15±713.66	17	642.12±215.67
P value		0.341		0.221
Social status				

Single	16	962.37±715.93	9	613.56±155.97
Married	54	886.09±796.56	21	708.48±272.30
P value		0.732		0.339

Results in Table 2 show that the positive C-reactive protein concentration (≥ 6 mg/dl) was significantly higher among IBS patients (44.3%) compared to that of healthy control group (23.3%), (p= 0.048). Additionally, the results found that mean ± SD of IL-2 concentration in IBS patients was 903.53 ± 774.5 (pg/ml) which was insignificantly higher than that of the health control, 680.0 ± 244.6 (pg/ml) (P= 0.126).

Table 2. Distribution of IL-2 and CRP positivity among study groups group

Variables	**IBS patients**		**Healthy control**		**p value**
	No.	**%**	**No.**	**%**	
C-Reactive protein					
< 6 mg/dl	39	55.7	23	76.7	0.048*
≥ 6 mg/dl	31	44.3	7	23.3	
IL-2 (pg/ml)					
Mean ± SD (Range)	903.53 ± 774.5 (228-4368)		680.0 ± 244.6 (408-1338)		0.126

Table 3 shows the distribution of IL-2 concentration (ng/ml) in both IBS patients and apparently healthy control group. The mean ± SD of IL-2 in IBS patients was 903.52 ±774.49 which was clearly higher than that of control 680.00±244.54. The 75%, 95% and 99% concentration of IL-2 in IBS patients (1098.0, 2798.0 and 4368.0) were respectively higher than their counterparts in control group (808.0, 1178.0 and 1338.0).

Table 3. Distribution of IL-2 concentration among IBS patients and control

IL-2 concentration (pg/ml)	IBS patients	Healthy controls
Mean ± SD	903.529±774.497	680.000±244.546
Standard Error of Mean	92.570	44.648
Range	228-4368	408-1338
Percentile 05	348.0	408.0
25	498.0	478.0
50 (Median)	573.0	623.0
75	1098.0	808.0
95	2798.0	1178.0
99	4368.0	1338.0

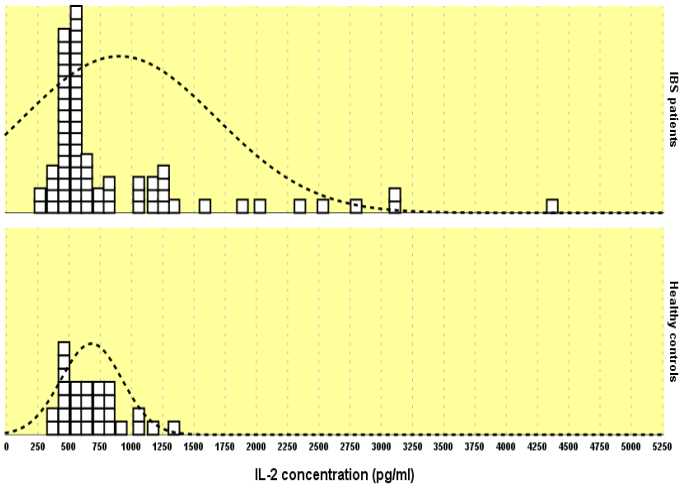

Figure 1. Mean IL-2 concentration (pg/ml) in patients and control group

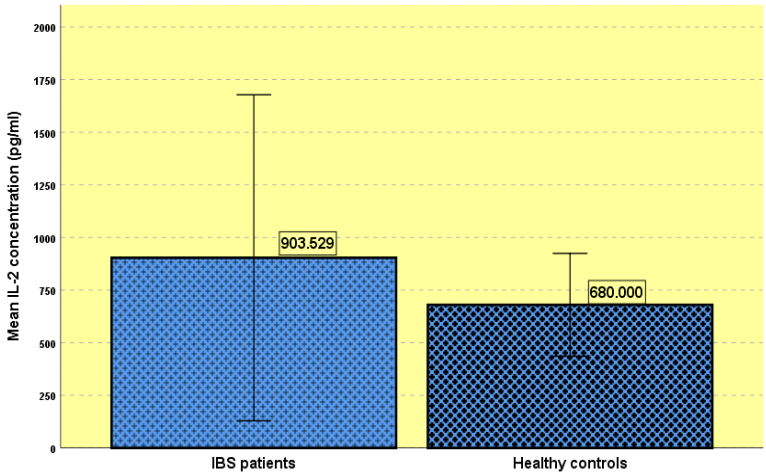

Figure 2. Mean IL-2 concentration (pg/ml) in patients and control group

4. DISCUSSION

The objective of the current study was to demonstrate the several socio-demographic infiltrators that association with IL-2 concentration in study groups and was located rate of CRP and IL-2 concentration in both groups. IBS and its kinds were influenced by age and sex. Most previous studies for IBS explained there was no definite grade for age was dominant [18-19]. Our study documented the mean of IL-2 concentration in females higher than in males. Meta-analysis study appeared that IBS patients differ between men and women based on inflammatory cytokines. TNF-α leves were higher in women with IBS than in men [20]. Another study evaluating inflammatory cytokine in men and women with IBS showed an increase in pro-inflammatory cytokines (IL-17 and TNF-α) and a decrease in anti-inflammatory cytokine (IL-10) lowering in women and men[21]. Moreover, women with IBS revealed excess in number of colonic mucosal mast cells and reduced numbers of CD3+ and CD8+T cells, proposing that mucosal immune enhancement in IBS individuals is sex dependent [20-22]. Numerous

women accordingly men influenced by irritable bowel syndrome, proposing assignment of hormones in the pathophysiology of IBS. These hormones can also alter the susceptibility to stress, which animated agent in IBS apparition and sign riskiness, for proverb, estrogen-dependent hyper-responsiveness to stress can induce immune induction or failure of barrier mission [23].

However, other demographic factors namely: occupation, levels of education, marital status showed no significant effect on the concentration of IL-2 in both study groups. The divergence in these results was due to small size of pattern that recruited for the study.

This study observed the positivity rate of CRP in IBS patients significantly raised than controls. These results are similar to the study which refers that the levels of high sensitive CRP in group with IBS were significantly higher than healthy group [24]. Another previous study in China showed the level of CRP was significantly different in active irritable bowel disease than inactive patients (P<0.001) [25]. Previous lines revealed reasonable path mechanical position for variation in intestinal immune mission and low grade inflammation in several patients with IBS [26-27]. Other studies that reviewed systemic immune mission in patients with IBS have elucidated that implicit inflammatory response can also be specified in peripheral blood. For instance, increase proportion of pro inflammatory cytokines (e.g., IL-6, IL-1β, and TNF-α) from peripheral blood mononuclear cells and incremented in number of activated T lymphocytes in peripheral blood in IBS patients simile to controls [28-29]. For another side, a meta-analysis study explained IBS patients having in normal range levels of CRP was minimal than 1% [30]. However, in order to the CRP consider non- specific biomarker, it can rise in situations other than IBS, like systemic disease and infections. Contrariwise, several patients do not promote high CRP levels regardless of their active infections[31-32].

Despite the fact that IL-2 is the main activator of T-cell generation, our result did not show significantly elevated levels IL-2 in IBS. This proposes that plasma IL-2 changes in IBS are conventional and required further realization. Cytokines such as IL-2 play a role beyond the activation of an inflammatory response. IBS may not be the only reason for causing IBS, a biomarker program would be useful to classify the different subtypes of IBS. IL-2 levels may not change significantly in IBS cases and controls, but could produce a significant change when the population is separated into more defined subgroups.

This study found a clearly higher mean of IL-2 in IBS patients than in controls. A previous study documented significantly increased levels of IL-2 and IL-8 in IBS patients in contrast to healthy controls [33]. In another study, the source of s IL-2R in patients was found to be with inflammatory bowel disease was significantly higher than in healthy volunteers [34]. Meanwhile, another study observed no altered levels of IL-2 in patients with IBS and not correlated with fatigue [35].

The result of our study could also be affected by implied systemic disease that may affect IL-2 output. Some authors have proposed that increased intestinal permeability may allow access of bacterial and luminal antigens to submucosa, which could induce inflammation and different classes of release immune cells such as CD3+ and CD5+ lymphocytes have been demonstrated; Indicating the presence of the IL-2 receptor, a marker of activated lymphocytes [36,37,38,39,40]. Previous studies support the assumption of immune induction in adults with this syndrome: immune cell infiltration into the intestinal mucosa of IBS patients [41] and a major expression of proinflammatory, cytokines, such as interleukin (IL)-6, IL-8, tumor necrosis factor (TNF)-α, and IL-12, with low levels of IL-10 [28-42]**.**

5. CONCLUSIONS

The study suggests that the alteration in CRP positivity and IL-2 concentration in patient with IBS may have a major role in its pathogenesis.

6. REFERENCES

1. Arenas-Ramirez N, Woytschak J. Interleukin-2: Biology, design, and application. Trends Immunol. 2015;36:763–77.
2. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol. 2011;23(5):598–604. doi:10.1016/j.coi.2011.08.003.
3. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: New insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Curr Opin Immunol. 2011;23(5):598–604. doi:10.1016/j.coi.2011.08.003.
4. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012;12(3):180–90. doi:10.1038/nri3156.
5. Farup PG, Ueland T, Rudi K, Lydersen S, Hestad K. Functional bowel disorders are associated with a central immune activation. Gastroenterol Res Pract. 2017;2017(27):1-9. doi:10.1155/2017/1642912.
6. Frandemark A, Jakobsson Ung E, Tornblom H, Simren M, Jakobsson S. Fatigue: a distressing symptom for patients with irritable bowel syndrome. *Neurogastroenterol Motil.* 2017;29(1):1-9.
7. Agarwal N, Spiegel BM. The effect of irritable bowel syndrome on health-related quality of life and health care expenditures. Gastroenterol Clin North Am. 2011;40:11–9.
8. Gulewitsch MD, Enck P, Hautzinger M, Schlarb AA. Irritable bowel syndrome symptoms among German students: prevalence, characteristics, and associations to somatic complaints, sleep, quality of life, and childhood abdominal pain. Eur J Gastroenterol Hepatol. 2011;23:311–6.
9. Coss-Adame E, Rao SSC. Brain and gut interactions in irritable bowel syndrome: new paradigms and new understandings. Curr Gastroenterol Rep. 2014;16(4):379-89.
10. Thabane M, Marshall JK. Post-infectious irritable bowel syndrome. World J Gastroenterol. 2009;15(29):3591–6. doi:10.3748/wjg.15.3591.
11. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut. 2003;52(1):91–3.

12. Black S, Kushner I, Samols D. C-reactive protein. J Biol Chem. 2004;279(47):48487–90. doi:10.1074/jbc.R400025200.
13. Henderson P, Kennedy NA, Van Limbergen JE, Cameron FL, Satsangi J, Russell RK, Wilson DC. Serum C-reactive protein and CRP genotype in pediatric inflammatory bowel disease: influence on phenotype, natural history, and response to therapy. Inflamm Bowel Dis. 2015;21(3):596–605. doi:10.1097/MIB.0000000000000296.
14. Fu Y, Wu Y, Liu E. C-reactive protein and cardiovascular disease: From animal studies to the clinic (review). Exp Ther Med. 2020;20(2):1211–9. doi:10.3892/etm.2020.8840.
15. Chenillot O, Henny J, Steinmetz J, Herbeth B, Wagner C, Siest G. High sensitivity C-reactive protein: Biological variations and reference limits. Clin Chem Lab Med. 2000;38:1003–11.
16. Schoepfer AM, Beglinger C, Straumann A, Trummler M, Renzulli P, Seibold F. Ulcerative colitis: correlation of the Rachmilewitz Endoscopic Activity Index with fecal calprotectin, clinical activity, C-reactive protein, and blood leukocytes. Inflamm Bowel Dis. 2009;15(12):1851–8. doi:10.1002/ibd.20986.
17. Mak WY, Buisson A, Rubin DT. Fecal calprotectin in assessing endoscopic and histological remission in patients with ulcerative colitis. *Dig Dis Sci.* 2018;63(5):1294–301.
18. Al-Damarchi AT, Al-Talakani GA. Association of Helicobacter pylori and irritable bowel syndrome. *Indian J Public Health Res Dev.* 2018;9:486–91.
19. Odhar HA, Hashim AF, Obaid DH, Majeed RS, Habeeb OA, Abdalhadi NM. Exploration of potential link between prevalence of irritable bowel syndrome and seropositivity for Helicobacter pylori. *Indian J Public Health Res Dev.* 2019;10(10):2236–40. doi:10.5958/0976-5506.2019.03187.5.
20. Cremon C, Gargano L, Morselli-Labate AM, Santini D, Cogliandro RF, De Giorgio R, Stanghellini V, Corinaldesi R, Barbara G. Mucosal immune activation in irritable bowel syndrome: gender-dependence and association with digestive symptoms. *Am J Gastroenterol.* 2009;104(2):392–400. doi:10.1038/ajg.2008.94.
21. Choghakhori R, Abbasnezhad A, Amani R, Alipour M. Sex-related differences in clinical symptoms, quality of life, and biochemical factors in irritable bowel syndrome. *Dig Dis Sci.* 2017;62:1550–60.
22. Houghton LA, Heitkemper M, Crowell M, et al. Age, gender, and women's health and the patient. *Gastroenterology.* 2016;150:1332–43.
23. Meleine M, Matricon J. Gender-related differences in irritable bowel syndrome: potential mechanisms of sex hormones. *World J Gastroenterol.* 2014;20(22):6725–43. doi:10.3748/wjg.v20.i22.6725.
24. Hod K, Ringel-Kulka T, Martin CF, Ringel Y, Faculty S, Aviv T, Carolina N, Health C, Hill C, Carolina N, Diseases L, Aviv T, Aviv T. High levels of fecal calprotectin in irritable bowel syndrome. *Mayo Clin Proc.* 2017;50(3):227–32. doi:10.1097/MCG.0000000000000327.
25. Chen Y, Wang L, Feng S, Cai W, Chen X, Huang Z. The relationship between C-reactive protein/albumin ratio and disease activity in patients with inflammatory bowel disease. *Gastroenterol Res Pract.* 2020.
26. Barbara G, De Giorgio R, Stanghellini V, Cremon C, Salvioli B, Corinaldesi R. New pathophysiological mechanisms in irritable bowel syndrome. *Aliment Pharmacol Ther.* 2004;20(Suppl. 2):1–9. doi:10.1111/j.1365-2036.2004.02036.x.
27. Camilleri M. Peripheral mechanisms in irritable bowel syndrome. *N Engl J Med.* 2012;367(17):1626–35. doi:10.1056/nejmra1207068.

28. Liebregts T, Adam B, Bredack C, Röth A, Heinzel S, Lester S, Downie-Doyle S, Smith E, Drew P, Talley NJ, Holtmann G. Immune activation in patients with irritable bowel syndrome. *Gastroenterology*. 2007;132(3):913–20. doi:10.1053/j.gastro.2007.01.04.
29. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA*. 2004;292:852–8.
30. Menees SB, Powell C, Kurlander J, Goel A, Chey WD. A meta-analysis of the utility of C-reactive protein, erythrocyte sedimentation rate, fecal calprotectin, and fecal lactoferrin to exclude inflammatory bowel disease in adults with IBS. Am J Gastroenterol. 2015;110:444–54.
31. Suk Danik J, Chasman DI, Cannon CP, Miller DT, Zee RY, Kozlowski P, Kwiatkowski DJ, Ridker PM. Influence of genetic variation in the C-reactive protein gene on the inflammatory response during and after acute coronary ischemia. Ann Hum Genet. 2006;70:705–16.
32. Henderson P, Kennedy NA, Van Limbergen JE, Cameron FL, Satsangi J, Russell RK, Wilson DC. Serum C-reactive protein and CRP genotype in pediatric inflammatory bowel disease: influence on phenotype, natural history, and response to therapy. Inflamm Bowel Dis. 2015;21(3):596–605. doi:10.1097/MIB.0000000000000296.
33. Patel S, Singh A, Misra V, Misra SP, Dwivedi M, Trivedi P. Levels of interleukins 2, 6, 8, and 10 in patients with irritable bowel syndrome. Indian J Pathol Microbiol. 2017;60(3):385–9. doi:10.4103/IJPM.IJPM_544_16.
34. West GA, Fiocchi C, Ferraris L, Klein S. Receptors in inflammatory bowel disease. 2006–2014.
35. Vara EJ, Brokstad KA, Hausken T, Lied GA. Altered levels of cytokines in patients with irritable bowel syndrome are not correlated with fatigue. Int J Gen Med. 2018;11:285–91. doi:10.2147/IJGM.S166600.
36. Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology*. 2002;122(7):1778–83. doi:10.1053/gast.2002.33579.
37. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. *JAMA*. 2004;292:852–8.
38. Marshall JK, Thabane M, Garg AX, Clark W, Meddings J, Collins SM. Intestinal permeability in patients with irritable bowel syndrome after a waterborne outbreak of acute gastroenteritis in Walkerton, Ontario. *Aliment Pharmacol Ther*. 2004;20:1317–22.
39. Spiller R, Campbell E. Post-infectious irritable bowel syndrome. *Curr Opin Gastroenterol*. 2006;22:13–7.
40. Grover M, Herfarth H, Drossman DA. The functional-organic dichotomy: postinfectious irritable bowel syndrome and inflammatory bowel disease-irritable bowel syndrome. *Clin Gastroenterol Hepatol*. 2009;7(1):48–53. doi:10.1016/j.cgh.2008.08.032.
41. Ohman L, Isaksson S, Lindmark AC, Posserud I, Stotzer PO, Strid H, Sjövall H, Simrén M. T-cell activation in patients with irritable bowel syndrome. *Am J Gastroenterol*. 2009;104(5):1205–12. doi:10.1038/ajg.2009.116.
42. O'Mahony L, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EMM. Lactobacillus and Bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology*. 2005;128(3):541–51. doi:10.1053/j.gastro.2004.11.0

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research received no external funding.

COPYRIGHT