

# Evaluation of Toll-Like Receptor Alteration in Intestinal Biopsies of Patients with Irritable Bowel Syndrome: A Systematic Review

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## ABSTRACT

### Background:

Irritable bowel syndrome (IBS) is a functional disorder with an unknown etiology. Some recent studies report the presence of a microscopic inflammation in people with IBS. This micro-inflammation can be due to an alteration in the pattern of recognition receptors especially in Toll-like receptors (TLRs). In this systematic review, we reviewed an evaluated existing studies related to TLRs and their changes in patients with IBS.

### Materials and Methods:

Three science citation index databases including Web of Science, Pubmed and Embase were searched for related keywords from September 2000 to September 2018. There was no language restriction. Two reviewers screened and extracted data independently.

### Results:

A total of 178 records were retrieved and eight eligible studies (265 patients) were included for data extraction and analysis. The results showed that, compared with the healthy group, the expression of some of the TLRs such as TLR2, TLR3, TLR4 and TLR5 increased whereas the expression of some others including TLR7 and TLR8 decreased significantly.

### Conclusion:

Alteration of TLRs in patients with IBS can be due to changes in microbiota causing a micro-inflammation. This alteration can have a potential role in pathophysiology of IBS.

**Keywords:** Irritable Bowel Syndrome, Toll-Like Receptor, Intestinal Biopsy, Systematic Review

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## INTRODUCTION

Irritable bowel syndrome (IBS) is a common disorder of the gastrointestinal tract, which is associated with abdominal pain or discomfort, disruption of bowel movements and changes in stool consistency (1). Biochemical and structural disorders are not observed in the intestines of these individuals, but recently there have been reports about microscopic inflammation in people with IBS (2). The incidence is different in various parts of the world and it is reported to be between 7% and 21%, and women are more affected than men (3). The disease does

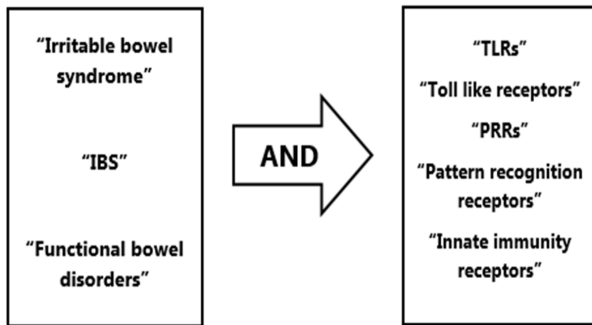


Fig. 1: Keywords for search in publications

not lead to severe and fatal conditions, but it negatively affects the quality of life greatly and imposes high costs on the individual and the health system directly and indirectly (4). The diagnosis is based on the symptoms and responses to a questionnaire, which is based on the ROME criteria (5). The exact etiology of the disease is unknown, but recently researchers have paid attention to the role of gastrointestinal pathogens as well as intestinal microbiota changes as some of the possible reasons (6). Pattern Recognition Receptors (PRRs) play an important role in non-specific identification and understanding the presence of microorganisms, especially at their entry sites into the body including the digestive tract. PRRs are divided into five main categories of Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type Lectin receptors (CLRs), RIG-I like receptors (RLRs), and DNA recognition receptors (7,8). Among these, TLRs have a special role due to their widespread distribution, the ability of dimerization with and identification of various types of microorganisms including viruses, bacteria and fungi. TLRs are the first detected innate immune receptors, and 10 different types of them have been discovered so far (9). Each of these receptors is responsible for identifying a specific type of microorganism structures. TLR1 and 2 are responsible for identifying lipoproteins, TLR3 is responsible for identifying two-stranded RNA, TLR4 detects lipopolysaccharides (LPS), TLR5 detects bacterial flagellum, TLR6 with TLR2 identify lipoproteins, TLR7 and 8 identify single-stranded RNA and TLR9 detects CpG DNA in bacteria and viruses. Due to binding of different types of TLRs to their ligands and signaling, various transcription factors are used, including NF-KB and/or IRFs, and

secretion of inflammatory and antiviral cytokines (10). In recent studies, alteration in cytokine profile has been mentioned in patients with IBS (11). Considering that many studies have suggested the emergence of Post-infection IBS (PI-IBS), and given the role of TLRs in detecting and responding to microorganisms, in this systematic review, we reviewed and evaluated existing studies related to TLRs and their changes in patients with IBS.

## MATERIALS AND METHODS

### Search Strategy

In order to identify all relevant published articles from September 2000 to September 2018, a search was conducted in three medical electronic databases. In order to identify studies about IBS, the following terms were used: IBS, Irritable Bowel Syndrome and functional bowel disorder (as MeSH and free text terms). These were combined with studies which have been identified with the terms TLRs, Toll-like receptors, PRRs, Pattern recognition receptors and innate immunity receptors using AND as a set operator (Figure 1). Reference sections of the selected studies were searched manually in order to identify other appropriate articles. Books were not included. IBS review articles were examined for additional references.

### Study Selection Criteria

The titles of all citations identified through the literature searches were reviewed. The abstract of relevant studies were skimmed. The selection criteria were applied. We ensured that the eligible studies included all of the following conditions: 1) results were published in full articles (or had adequate data available); 2) the research design was case-control; 3) studies conducted only on humans and adults; 4) studies that used ROME criteria to identify patients with IBS (Figure 2).

### Data Extraction and Analysis

Eligible articles were independently reviewed by two investigators (M.A and A.Y). Eligible abstracts were reviewed and abstracts of full articles meeting inclusion criteria were evaluated with an agreement of greater than 95%. Disagreement in data extraction was resolved through discussion and consensus. The

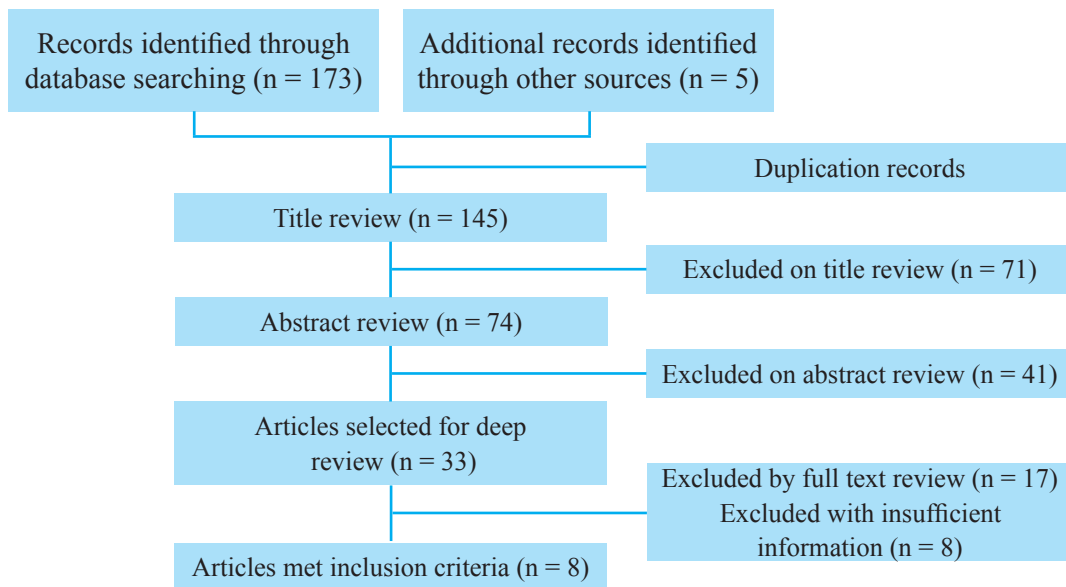


Fig. 2: Retrieving process for eligible studies in the systematic review

following information was recorded for each study: first author, year of publication, region, number of patients and controls, mean age and/or age range, percentage of male/female, IBS subtypes, diagnostic criteria to determine IBS, kinds of TLRs that were studied and the method of detection of TLRs. Extracted data were converted to tabular form.

## RESULT

178 findings (Embase:  $n = 138$ , Pubmed:  $n = 26$ , Web of Science:  $n = 9$ , other:  $n = 5$ ) were obtained by adopting the defined strategy. By reviewing the titles, duplicates were removed and eight articles were selected by reviewing the abstracts and the full texts (if available) and searching for the relation and selection criteria (Figure 2). All studies were conducted between 2011 and 2018, among which seven were in English and one was in Chinese. Four were conducted in Europe, three in Asia and one in the United States. ROME II ( $n = 2$ ) and ROME III ( $n = 6$ ) indexes were used in order to identify patients with IBS and all studies were conducted based on the results of colon biopsy. In one study, sampling was done only on the women and in another study, the sex distribution of patients was not mentioned, but in the rest of

the studies, patients were selected from both sexes. All selected studies measured at least two different TLRs and a study examined all known human TLRs. TLR2 and TLR4 were evaluated in six studies, TLR3, TLR5 and TLR9 were evaluated in three studies, and TLRs 1 and 8 were evaluated in two studies. The rest of the TLRs were examined only in one study. In the IBS subgroups, two studies included only type D subgroup, three included type C and D, and three included more subgroups. Only one study evaluated TLRs in all IBS subgroups (based on the ROME III criteria). Regarding the methods used to identify and evaluate TLRs in case and control groups, researchers used only one method in five studies and in three studies they used more than one method. Among these methods, qRT-PCR, which had the most application, was used in six studies, immunofluorescence and immunohistochemistry were used in two studies, and microarray, Western blotting and flow cytometry were used in one study. The extracted data and the findings for each of the papers are listed in Table 1.

## DISCUSSION

IBS is a common disorder of the gastrointestinal tract, which is associated with abdominal pain and/or

**Table 1: Characteristics of the TLR screening studies**

First Author (Year of publication)	Region	Subjects		Gender of cases	Age of cases (Average or Range)	Diagnostic Criteria	IBS subtypes	Samples	Methods	Assessed TLRs	Findings
		Control	Case								
Brint (12) (2011)	Ireland	26	19	All females	18 - 65	ROME II	D and C	Colonic biopsies	qRT-PCR, Microarray, Immunofluorescence and Western blot	TLR1-10	Upregulation of TLR2, TLR4 and TLR5 and down regulation of TLR7 and TLR8 in IBS patients
Dlugosz (13) (2017)	Sweden	22	14	17 F 5 M	18 - 66	ROME II	D and C	Jejunum and sigmoid biopsies	qRT-PCR, Immunohistochemistry	TLR2,4,5 and 9	Upregulation of TLR4, 5, and 9 in IBS patients. No deference in TLR2 level.
Belmonte (14) (2012)	France	48	31	38 F 11 M	14 ± 49	ROME III	D, C and M	Descending colon biopsies	qRT-PCR, Immunofluorescence and flow cytometry	TLR2 and 4	Upregulation of TLR2 and TLR4 in IBS patients
Koçak (15) (2016)	Turkey	51	15	43 F 8 M	18 - 65	ROME III	D and C	Colonic biopsies	ELISA	TLR2 and 4	Upregulation of TLR2 and TLR4 in IBS patients.
Camilleri (16) (2016)	USA	15	7	13 F 2 M	2.3 ± 40.3	ROME III	D	Colonic biopsies	qRT-PCR	TLR1,3 and 8	Upregulation of TLR3 and down regulation of TLR1 and TLR8 in IBS patients
Guo W (17) (2016)	China	20	20	13F 7M	12.5 ± 42.9	ROME III	D	Colonic biopsies	qRT-PCR	TLR2 and 4	Upregulation of TLR2 and TLR4 in IBS patients
Mohammadi (18) (2016)	Iran	36	30	20 F 16 M	18 - 65	ROME III	D, C, M and U	Colonic biopsies	Immunohistochemistry	TLR3 and 9	Upregulation of TLR3 and TLR9 in IBS patients
Ghoshal (19) (2017)	India	47	30	Unmentioned	Unmentioned	ROME III	D, C and U	Colonic biopsies	qRT-PCR	TLR2,4,5	Upregulation of TLR2 and TLR5

discomfort, impaired defecation and changes in stool consistency (1). Although, the exact etiology of the disease is unknown, various studies have indicated the presence of microscopic inflammation in the intestines of patients with IBS (2). TLRs are regarded as one type of pattern recognition receptors in innate immunity that can detect microorganisms followed by activation and signaling, and can trigger inflammation by expressing pro-inflammatory cytokines (10). The present study is a systematic review of all published papers from September 2000 to September 2018 existing in Embase, Pubmed and Web of Science databases concerning TLR alteration in IBS colonic biopsy samples compared to healthy controls. According to our knowledge, this study is the first study of this type.

Eight studies were selected and examined carefully by adopting a specific strategy. Among these, most studies (six studies) were performed on TLR2 and TLR4. All studies have shown increased levels of

TLR2 and TLR4 molecules in colonic biopsies of patients with IBS compared to healthy subjects. Only one study showed no significant difference in TLR4 and TLR2 between case and control groups. These two TLRs have previously been studied in inflammatory bowel disease (IBD). A study by Cario and colleagues on colonic biopsy samples in patients with IBD showed that TLR4 protein levels increased significantly in these individuals compared to the control group (20). Also, a study by Szebani and colleagues indicated a significant increase in TLR2 and TLR4 levels in colon mucosa in children with IBD (21).

TLR2 and TLR4 mostly play a role in identifying bacterial lipopeptides and lipoproteins (LPS), respectively. Dlugosz and colleagues studied the serum levels of LPS in patients with IBS and observed that this level was increased significantly in the subgroup of IBS-D compared with the control group (22).

Since the intestinal environment is rich in various microbiota, an increase in LPS levels suggests the hypothesis of a change in the microbiota of patients with IBS, and is considered as one of the possible etiologies of the disease mentioned in many studies (23-27). Therefore, using probiotics has been recommended to reduce the symptoms associated with IBS (28,29).

Studies show that the levels of stress and depression are higher in patients with IBS than in healthy people, and anxiety and high levels of stress can make symptoms worse (30). Corticotrophin-releasing factor (CRF) plays a central role in response to stress and by influencing the activity of the hypothalamic-pituitary-adrenocortical axis (HPA) causes inflammation, increases visceral sensitivity and makes changes in intestinal movements (31,32). Increasing number of studies suggest the signaling of TLR2 and TLR4 and the developing microscopic inflammation in patients with IBS (33). On the other hand, recent studies have shown that depression is a disease which is associated with microscopic inflammation, in which inflammatory cytokines will be increased (32). Therefore, it seems that stress and depression can cause microscopic inflammation through increasing the levels of CRF and intestinal TLR2 and TLR4, which results in developing symptoms of IBS.

Jizhong and colleagues in a study on patients with depression and IBS showed a significant increase in TLR2, TLR4, and CRF mRNA expression compared to the control group. Also, IL-6 mRNA expression level was higher in patients with DP-IBS than that of control group but against IL10 mRNA decreased significantly (32). These changes confirm the above mentioned hypothesis and suggests using pro-inflammatory cytokine antagonists as a therapeutic approach in order to reduce the symptoms of the disease. Studies show that using tumor necrosis factor (TNF) antagonists reduce the symptoms associated with depression in patients with IBD (34).

Among the selected papers, three studies have evaluated the level of TLR5 in patients with IBS; all of them reported an increase in the level of this receptor. Since the TLR5 recognizes bacterial flagella, the change in regulating its levels in patients with IBS is another indication regarding the role of microbiota

changes and partial inflammation in IBS disease.

The study of Schoepfer and colleagues conducted on 112 patients with IBS and 111 patients with IBD showed that flagellin antibody level increased significantly in both groups compared with healthy group although this increase is higher in patients with IBD (35). Another study showed that people with PI-IBS are more likely to have flagellin antibodies in their serum versus those who have not had infection and basically, it is possible that the presence of these antibodies is only important in the PI-IBS group. According to a study by Spiller, roughly a quarter of people with IBS have had an acute gastrointestinal infection before the onset of the disease (36).

Another study also noted an increase in the level of antibodies against flagellin in the serum of patients with IBS, especially IBS-D, but it did not show any significant difference in patients with PI-IBS and IBS (22). This contradiction explains the need for further studies on this issue.

Among the selected studies, two studies showed a significant increase in TLR3 levels and two studies showed a significant increase in TLR9 levels in patients with IBS compared to healthy group. Since TLR3 is an innate immunity receptor for detecting double-stranded RNA of viruses and TLR9 detecting CpG DNA from bacteria and viruses, the hypothesis of viral involvement is introduced in the development of IBS specifically PI-IBS. Zanini and colleagues, in a study investigating the epidemic of viral gastroenteritis caused by drinking water contaminated with a Norovirus, suggested this infection as a possible cause of IBS creating IBS-related symptoms (37). Also, Marshal and colleagues in another study highlighted the role of Noroviruses in an epidemic of food-borne gastroenteritis and subsequent symptoms of IBS (38). If the hypothesis of Norovirus involvement in the development of IBS is correct (since Noroviruses are classified into a single-stranded RNA gastrointestinal virus group), TLR7 and TLR8 are expected to increase significantly in the colon biopsies of patients with IBS while contrary to this, these two receptors have decreased compared to the control group. Mohammadi and colleagues in a study that exclusively evaluated the virus-detecting receptors of innate immunity system in colonic biopsies of patients with IBS showed

significant increases in retinoic acid-inducible gene 1 (RIG1) levels, along with TLR3 and TLR9, which reinforces the hypothesis of viral involvement in IBS (18). Since RIG1 is responsible for identifying double-stranded RNA viruses, it seems that an enteric virus with a double-stranded RNA such as Rotavirus can be considered as one of the possible viruses. In addition, the role of other enteric DNA viruses such as adenoviruses may not be ruled out in this process. Since none of these viruses, to the best of our knowledge, and even other similar enteric viruses has ever been studied in patients with IBS, further studies are recommended in this regard.

### CONCLUSION

This systematic review showed that some TLRs have been changed significantly in patients with IBS compared to healthy group. This alteration can be due to a change in the microbiota of the intestine and leads to the formation of a micro-inflammation in the intestine of patients with IBS. In addition to bacteria, some gastrointestinal viruses can also play a role in etiology of IBS, which requires further studies. Proving the role of each virus can offer new therapeutic strategies.

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### Authors' Contributions

MA contributed to conception and design of the study. MA and AY designed and carried out search strategies and drafted the manuscript. MMM and MJZ screened the content expertly and helped in writing and editing the article. All authors read, revised and confirmed the manuscript.

### CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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