SYSTEMATIC REVIEW

Diagnostic potential of various laboratory tests for Irritable Bowel Syndrome (IBS): A systematic review

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Abstract

Objective: To identify possible tests along with their accuracies that may be used to diagnose irritable bowel syndrome.

Method: The systematic review comprised literature search on Cochrane Library, PubMed, Science Direct and Elsevier databases for randomised controlled trials and cohort studies conducted from January 1, 2015, to December 31, 2022, using appropriate key words and Boolean operators. Focus was kept on studies that reported irritable bowel syndrome diagnosis as the primary outcome. The risk of bias was assessed using quality assessment, data abstraction, and synthesis version 2.

Results: Of the 2,798 studies initially identified, 10(0.35%) were analysed in detail. Of them, 4(40%) used enzyme-linked immunosorbent assay kits to test for anti-cytolethal distending toxin B and anti-vinculin levels, 2(20%) used the kits for serum cytokine profiling and serum calprotectin levels, and 4(40%) used either magnetic resonance imaging scans, faecal metabolic profiling, intestinal biopsy analysis with immunostaining or polymerase chain reaction for differential transfer-ribonucleic acid-derived small ribonucleic acid. Out of the 4(40%) studies on anti-cytolethal distending toxin B and anti-vinculin levels, optical densities >1.56 and >1.60 recorded 100% specificity for irritable bowel syndrome with diarrhoea, but sensitivity was 22%. In contrast, rectal biopsies for cell densities of somatostatin and peptide YY showed high sensitivity and specificity for irritable bowel syndrome ranging 80-90%.

Conclusion: Enzyme-linked immunosorbent assay testing for anti-cytolethal distending toxin B and anti-vinculin as well as rectal biopsies for cell densities could be potential diagnostic tests for irritable bowel syndrome.

Keywords: Irritable bowel syndrome, Diagnosis, Laboratory, ELISA. (JPMA 74: 1300; 2024)

DOI: https://doi.org/10.47391/JPMA.10571

Introduction

Irritable bowel syndrome (IBS), as per the latest definition outlined in the Rome IV criteria, is defined as individuals experiencing chronic abdominal pain at least once a week over the preceding 3 months. It commonly manifests as irregularities in bowel movements, such as IBS constipation-dominant (IBS-C), IBS diarrhoea-dominant (IBS-D), or a mixture of both (IBS-M).[1,2]

The clinical epidemiology of IBS reveals notable differences in prevalence across countries and diagnostic criteria.[3] A study in Pakistan showed a 33.20% prevalence of IBS,[4] being a disease of females like the Western population.[5]

Due to the frequent overlap of its symptoms with those of organic diseases, it often presents with overlapping upper and lower gastrointestinal tract (GIT) symptoms, posing a serious diagnostic challenge.[6] In a survey, more than half of the family practitioners[7] were unable to recognise typical symptoms of IBS, and only 40% physicians reported feeling confident in diagnosing IBS during the patient's initial visit.[8] For this reason, many family practitioners prefer to take it as a diagnosis of exclusion that leads to unnecessary testing in patients with overlapping comorbid conditions,[9] further contributing to the wastage of time. At least 3months history is required before ROME IV can be applied[10] burdening financial resources.[11]

An advanced approach is symptom-based Rome criteria which has been widely used and is considered the gold standard for aiding in the positive diagnosis of IBS.[2] The application of the Rome criteria also requires the patient to be symptomatic for at least three months, which adds to the delay in definitive diagnosis and patient discomfort.

Recently, there has been a growing interest in exploring the potential of stool sampling and novel inflammatory and serum biomarkers, including anti-cytolethal distending toxin B (CdtB), vinculin,[12] faecal calprotectin, B-cell activating factor levels (BAFF),[13] serum levels of interleukin-6 (IL-6), IL-8 and tumour necrosis factor-alpha (TNF-α)[14] to be used as a diagnostic tool for IBS. These biomarkers can serve as a supplementary method along with the conventional Rome criteria in evaluating individuals suspected of IBS, and to make diagnosis of IBS more objective. The current systematic review was planned to

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Submission complete: 06-08-2023 **Review began:** 26-09-2023

Acceptance: 09-03-2024 **Review end:** 27-01-2024

identify possible tests along with their accuracies that may be used to diagnose IBS.

Materials and Methods

The systematic review comprised literature search on Cochrane Library, PubMed, Science Direct and Elsevier databases for randomised controlled trials and cohort studies conducted from January 1, 2015, to December 31, 2022, using appropriate key words and Boolean operators (Appendix).

Appendix

Search strategies for different databases

PubMed - run 15/12/2022

("irritable bowel syndrome"[MeSH Terms] OR ("irritable"[All Fields] AND "bowel"[All Fields] AND "syndrome"[All Fields]) OR "irritable bowel syndrome"[All Fields] OR "ibs"[All Fields]) AND ((("linguist approaches biling"[Journal] OR "lab"[All Fields]) AND ("diagnosable"[All Fields] OR "diagnosi"[All Fields] OR "diagnosis"[MeSH Terms] OR "diagnosis"[All Fields] OR "diagnose"[All Fields] OR "diagnosed"[All Fields] OR "diagnoses"[All Fields] OR "diagnosing"[All Fields] OR "diagnosis"[MeSH Subheading])) OR ("diagnostic tests, routine"[MeSH Terms] OR ("diagnostic"[All Fields] AND "tests"[All Fields] AND "routine"[All Fields]) OR "routine diagnostic tests"[All Fields] OR ("diagnostic"[All Fields] AND "tests"[All Fields]) OR "diagnostic tests"[All Fields])) AND ((("serum"[MeSH Terms] OR "serum"[All Fields] OR "serums"[All Fields] OR "serum s"[All Fields] OR "seruma l"[All Fields]) AND ("antigen s"[All Fields] OR "antigene"[All Fields] OR "antigenes"[All Fields] OR "antigenic"[All Fields] OR "antigenically"[All Fields] OR "antigenicities"[All Fields] OR "antigenicity"[All Fields] OR "antigenized"[All Fields] OR "antigens"[MeSH Terms] OR "antigens"[All Fields] OR "antigen"[All Fields])) OR (("faecally"[All Fields] OR "fecally"[All Fields] OR "fecals"[All Fields] OR "feces"[MeSH Terms] OR "feces"[All Fields] OR "faecal"[All Fields] OR "fecal"[All Fields]) AND ("antigen s"[All Fields] OR "antigene"[All Fields] OR "antigenes"[All Fields] OR "antigenic"[All Fields] OR "antigenically"[All Fields] OR "antigenicities"[All Fields] OR "antigenicity"[All Fields] OR "antigenized"[All Fields] OR "antigens"[MeSH Terms] OR "antigens"[All Fields] OR "antigen"[All Fields])) OR ("vinculin"[MeSH Terms] OR "vinculin"[All Fields] OR "vinculin s"[All Fields] OR "vinculins"[All Fields]) OR ("biomarker s"[All Fields] OR

"biomarkers"[MeSH Terms] OR "biomarkers"[All Fields] OR "biomarker"[All Fields]))

NIH run on 15/12/2022

Filters Applied: 1st Jan, 2015 – 15th Dec, 2022; Full text available; Human studies; English Language

("irritable bowel syndrome"[MeSH Terms] OR (irritable[All Fields] AND bowel[All Fields] AND syndrome[All Fields]) OR "irritable bowel syndrome"[All Fields] OR ibs[All Fields]) AND ((("linguist approaches biling"[Journal] OR lab[All Fields]) AND (diagnosable[All Fields] OR diagnosi[All Fields] OR diagnosis[MeSH Terms] OR diagnosis[All Fields] OR diagnose[All Fields] OR diagnosed[All Fields] OR diagnoses[All Fields] OR diagnosing[All Fields] OR diagnosis[MeSH Subheading])) OR ("diagnostic tests, routine"[MeSH Terms] OR (diagnostic[All Fields] AND tests[All Fields] AND routine[All Fields]) OR "routine diagnostic tests"[All Fields] OR (diagnostic[All Fields] AND tests[All Fields]) OR "diagnostic tests"[All Fields])) AND (((serum[MeSH Terms] OR serum[All Fields] OR serums[All Fields] OR "serum s"[All Fields] OR seruma l[All Fields]) AND ("antigen s"[All Fields] OR antigene[All Fields] OR antigenes[All Fields] OR antigenic[All Fields] OR antigenically[All Fields] OR antigenicities[All Fields] OR antigenicity[All Fields] OR antigenized[All Fields] OR antigens[MeSH Terms] OR antigens[All Fields] OR antigen[All Fields])) OR ((faecally[All Fields] OR fecally[All Fields] OR fecals[All Fields] OR feces[MeSH Terms] OR feces[All Fields] OR faecal[All Fields] OR fecal[All Fields]) AND ("antigen s"[All Fields] OR antigene[All Fields] OR antigenes[All Fields] OR antigenic[All Fields] OR antigenically[All Fields] OR antigenicities[All Fields] OR antigenicity[All Fields] OR antigenized[All Fields] OR antigens[MeSH Terms] OR antigens[All Fields] OR antigen[All Fields])) OR (vinculin[MeSH Terms] OR vinculin[All Fields] OR "vinculin s"[All Fields] OR vinculins[All Fields]) OR ("biomarker s"[All Fields] OR biomarkers[MeSH Terms] OR biomarkers[All Fields] OR biomarker[All Fields]))

The Cochrane Library for Cochrane Reviews

Filters Applied: 1st Jan,2015 – 15th Dec,2022; were selected using MeSH terms for irritable bowel syndrome + diagnostic tools + bio-markers in advanced search; source Embase, ICTRP, PubMed, CT.gov

Search Name: ibs review
Last Saved: 15/12/2022 14:05:59

Comment:

ID	Search
#1	MeSH descriptor: [Irritable Bowel Syndrome] explode all trees
#2	(irritable NEXT bowel NEXT syndrome):ti,ab,kw OR (IBS):ti,ab,kw (Word variations have been searched)
#3	#1 OR #2
#4	MeSH descriptor: [Diagnostic Techniques and Procedures] this term only
#5	(diagnosis NEXT (lab" OR tests" OR agent)):ti,ab,kw (Word variations have been searched)
#6	#4 OR #5
#7	#3 AND #6
#8	("bioantigens"):ti,ab,kw (Word variations have been searched)
#9	("calprotectin"):ti,ab,kw (Word variations have been searched)
#10	#3 AND #9
#11	(fecal OR (fecal NEXT antigen)):ti,ab,kw (Word variations have been searched)
#12	#3 AND #11
#13	("lactoferrin"):ti,ab,kw (Word variations have been searched)
#14	#3 AND #13
#15	(Short NEXT Chain NEXT Fatty NEXT acids):ti,ab,kw (Word variations have been searched)
#16	#3 AND #15
#17	("biopsy"):ti,ab,kw (Word variations have been searched)
#18	#3 AND #17
#19	MeSH descriptor: [Biomarkers] explode all trees
#20	("biomarkers"):ti,ab,kw (Word variations have been searched)
#21	#19 OR #20
#22	#3 AND #21

Embase via Elsevier - run 15/12/2022

('irritable bowel syndrome'/exp OR (irritable AND bowel AND syndrome) OR 'irritable bowel syndrome' OR ibs) AND ((('linguist approaches biling':jt OR lab) AND (diagnosable OR diagnosi OR diagnosis/exp OR diagnosis OR diagnose OR diagnosed OR diagnoses OR diagnosing OR diagnosis:lnk)) OR ('diagnostic tests, routine'/exp OR (diagnostic AND tests AND routine) OR 'routine diagnostic tests' OR (diagnostic AND tests) OR 'diagnostic tests')) AND (((serum/exp OR serum OR serums OR 'serum s' OR seruma l) AND ('antigen s' OR antigene OR antigenes OR antigenic OR antigenically OR antigenicities OR antigenicity OR antigenized OR antigens/exp OR antigens OR antigen)) OR ((faecally OR fecally OR fecals OR feces/exp OR feces OR faecal OR fecal) AND ('antigen s' OR antigene OR antigenes OR antigenic OR antigenically OR antigenicities OR antigenicity OR antigenized OR antigens/exp OR antigens OR antigen)) OR (vinculin/exp OR vinculin OR 'vinculin s' OR vinculins) OR ('biomarker s' OR biomarkers/exp OR biomarkers OR biomarker))

Science Direct - run 15/12/2022

Article type, time and subject areas were applied according to inclusion criteria

("irritable bowel syndrome" OR (irritable AND bowel AND syndrome) OR "irritable bowel syndrome" OR ibs) AND ((("linguist approaches biling" OR lab) AND (diagnosable OR diagnosi OR diagnosis OR diagnosis OR diagnose OR diagnosed OR diagnoses OR diagnosing OR diagnosis)) OR ("diagnostic tests, routine" OR (diagnostic AND tests AND routine) OR "routine diagnostic tests" OR (diagnostic AND tests) OR "diagnostic tests")) AND (((serum OR serum OR serums OR "serum s" OR seruma l) AND ("antigen s" OR antigene OR antigenes OR antigenic OR antigenically OR antigenicities OR antigenicity OR antigenized OR antigens OR antigens OR antigen)) OR ((faecally OR fecally OR fecals OR feces OR feces OR faecal OR fecal) AND ("antigen s" OR antigene OR antigenes OR antigenic OR antigenically OR antigenicities OR antigenicity OR antigenized OR antigens OR antigens OR antigen)) OR (vinculin OR vinculin OR "vinculin s" OR vinculins) OR ("biomarker s" OR biomarkers OR biomarkers OR biomarker))

The review was conducted in line with Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA)[15]

Table-1: Exclusion criteria.

Cross-sectional studies, thesis, conference abstracts, book chapters, articles in press
Studies conducted on non-human subjects
Clinical symptomatology-based diagnosis of IBS
Referring to IBS as a diagnosis of exclusion
Studies using the Methane Breath test as an index test
Studies including patients with co-morbidities
Studies conducted on pregnant females

IBS: Irritable bowel syndrome.

Table-2: Characteristics of the studies analysed.

References	Study Design	Study Population	Sample size	Clinical characteristic of the study population	Index test	Reference standard
Seyedmehdi Seyedmirzaee et al. 2016[20]	Original article (RCT)	IBS-D+C+M patients	Total sample= 149 IBS patients: 74 Healthy control: 75	IBS-D: 34 (M=19; F= 15) IBS-C: 29 (M= 5; F= 24) IBS-M: 11 (M= 4; F= 7) Mean age for patients 35.52 ± 11.72yrs **Control group:** 75 (M= 27, F= 48) Mean age= 37.37±12.50yrs	ELISA for serum levels of IL-6, IL-8, and TNF-a measurement	Rome III criteria
Walter Morales et al. 2019[12]	Clinical Randomized Control Trial	IBS-D, IBD patients	Total Sample= 131 IBS-D= 100 IBD= 31	**Anti-CdtB:** IBS-D= 100; Age 52±13yrs; F= 58% IBD=31; Age 38±12yrs; F= 71% **Anti-vinculin:** IBS-D= 100 51±13yrs; F= 59% IBD= 22; Age 39±12yrs; F= 58%	CdtB and vinculin with ELISA testing	Rome III criteria
Yuna Chai et al. 2021(21)	Original article (RCT)	IBS-D patients	Total Sample= 42 IBS-D patients: 31 Control group: 11	Aged 20-60 yrs (after statistics no significant differences in age, sex, height, weight, and BMI)	q-PCR verifications of differential tsRNAs in intestinal mucosa. Then Target gene prediction and bioinformatics analysis of differential tsRNAs	Rome IV criteria.
Nicholas J. et al. 2019[22]	Multicenter RCT	IBS patients and/or functional dyspepsia patients and/or organic gastrointestinal disease patients.	Total Sample= 791 Population-based arm= 331 Clinical based arm= 460	**Population-based arm:** IBS= 63; FD= 61; HC= 246 Age= 62 ± 12yrs; F= 49% **Clinic-based arm:** **Newcastle site:** IBS= 126; FD= 66; Overlap= 2 Organic Controls= 53 Age= 44yrs ± 17; F= 67% **Brisbane site:** IBS= 157; FD= 17; Overlap= 31 Organic controls= 129 Age= 50 ± 16yrs; F= 62%	Serum levels of anti-CdtB/anti-vinculin antibodies by ELISA	Modified Rome III criteria
Mark Pimentel et al. 2015[23]	RCT	IBS-D, IBD, and celiac disease patients	Total Sample= 2681 D-IBS= 2375 IBD= 142 Coeliac disease= 121 Healthy controls= 43	**IBS-D= 2375** Age: 44±12yrs (18–65yrs); F= 68% **Crohn's Disease= 73** Age: 41±11yrs (18–65yrs); F= 56% **UC= 69** Age: 41±12yrs (19–63yrs); F= 55% **Coeliac Disease= 121** Age: 42±12yrs (19–65yrs); F= 76% **Controls= 43** Age: 36±10yrs (22-62yrs); F= 67%	Plasma levels of anti-CdtB and anti-vinculin antibodies determination by ELISA	Rome- III criteria
Magdy El-Salhy et al. 2015[24]	Original article (RCT)	IBS	Total sample=163 IBS patients: 101 Control group: 62	**IBS Patients:** 71 F, 30 M Mean age= 35yrs (range 18–61yrs) All having IBS for >10yrs **Control:** 38 F, 24 M Mean age= 41yrs (range 18–65yrs)	Immunohistochemical staining for PYY + somatostatin in rectal samples taken from 15cm above the anus on colonoscopy	Rome III criteria
Yu Fu et al. 2017[25]	RCT	IBD, IBS patients	Total sample= 146 CD= 44 UC= 49 IBS= 27 Healthy Controls= 26	**CD**= M= 28 F= 16 Age: 29yrs (24–37yrs) **UC**= M= 31 F= 18 Age: 39yrs (34–48yrs) **IBS**= M=34 F= 9 Age:34yrs (27–40yrs) **HC**= M= 15 F= 11 Age:32yrs (27–43yrs)	Faecal calprotectin, B-Cell Activating Factor, and Fecal Occult Blood Test ELISA kit	Rome-III criteria for IBS patients
C. LAM et al. 2016[26]	Clinical trial	Patients with functional constipation (FC), IBS-D patients	Total sample=48 FC patients: 24 IBS patients: 24	45 F, 3 M 21–68 years old	Hourly scans (0-4h) to assess intestinal response following ingestion of PEG and electrolyte solution.	Rome III criteria
Emily B. Hollister et al. 2019[27]	Randomized Control Trial	Preadolescent children with IBS	Total Sample= 45 IBS= 23 Healthy controls= 22	IBS and Healthy Controls were aged 7 to 12yrs with 9% Females in both groups.	Stool samples for fecal metabolomic profiling.	Rome III criteria
Ali Rezaie et al. 2017[28]	Multicenter Clinical Trial	Patients of IBS (all subtypes)	Total Sample= 2473 IBS-D= 2375 IBS-M= 25 IBS-C= 30 Healthy Controls= 43	**IBS-D**= F=68% Age= 44 ± 12yrs (18–65yrs) **IBS-M**= F= 64% Age= 40 ± 14yrs (20–67yrs) **IBS-C**= F= 80% Age= 41 ± 14.yrs (19–70yrs) **Healthy controls**= F= 67.4% Age= 36± 10yrs (22–62yrs)	Determination of Plasma levels of anti-CdtB and anti-vinculin antibodies by ELISA	Rome III criteria

BMI: Body Mass Index, CD: Crohn Disease, F: Female, FC: Functional Constipation, FD: Functional Diarrhoea, HC: Healthy Control, IBS-M: Irritable Bowel Syndrome Mixed Type, M: Male, RCT: Randomised Control Trial, UC: Ulcerative Colitis.

guidelines, and was registered with the International Prospective Register of Systematic Reviews PROSPERO.[16] The search was designed with the aid of Polyglot Search Translator[17] peer-reviewed by the supervisor. Search Filters were applied in accordance with the inclusion and exclusion criteria.

Systematic Review Accelerator[18] was used to remove duplicate studies. The reference lists of the included studies were checked manually. Two researchers independently screened the titles and abstracts to identify eligible articles, and disputes were resolved in consultation with the third researcher. Full-text screening was done independently by the 3 researchers, and disagreements were resolved by mutual discussion on the reasons for exclusion (Table 1).

The studies analysed in detail were randomised controlled trials (RCTs), clinical trials and cohort longitudinal studies comprising participants regardless of age, gender, severity and subtype of IBS that were published in the English language during the stipulated time period. The primary outcome of the review was to include studies that used one of the following IBS index tests. including colonic biopsy, radiographic imaging, serum or faecal biomarkers. The index test was defined in terms of specificity, positive predictive value (PPV), area under receiver operating characteristic (ROC) curves (AUC) or *p*-values.

Two researchers extracted study data, including type, methods, participant characteristics, index test accuracy and the outcomes. The risk of bias and applicability of primary diagnostic accuracy studies were assessed using the quality assessment, data abstraction, and synthesis tool version 2 (QUADAS-2).[19] The studies were scored independently by 2 researchers across 4 subheadings: index test, reference standard, patient selection, and flow and timing. The rating of the studies was done by an expert in the field on the basis of study characteristics.

Results

Of the 2,798 studies initially identified, 10(0.35%)[12,20-28] were analysed in detail (Figure). Of them, 7(70%) studies were RCTs conducted in a single centre, 2(20%) were multicentre RCTs, and 1(10%) was a clinical trial.[26] There was 1(10%) study that had pre-adolescent females aged 7-12yrs, while 9(90%) included the adult population comprising both genders. IBS was pre-diagnosed with Rome III in 9(90%) studies, and Rome IV in 1(10%) study (Table 2).

Of them, 4(40%) used enzyme-linked immunosorbent assay (ELISA) kits to test for anti-CdtB and anti-vinculin levels, 2(20%) used the kits for serum cytokine profiling and serum calprotectin levels, and 4(40%) used either magnetic

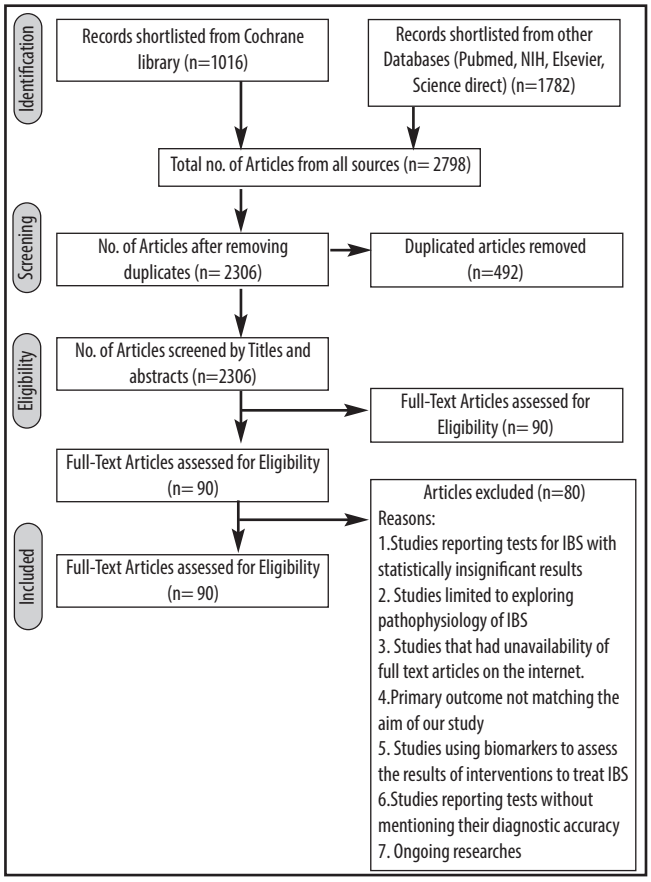

Figure: Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart

resonance imaging (MRI) scans, faecal metabolic profiling, intestinal biopsy analysis with immunostaining or polymerase chain reaction (PCR) for differential transfer-ribonucleic acid-derived small ribonucleic acid (tsRNA).

The 4(40%) studies using anti-CtdB and anti-vinculin titers were found to have different optical densities (ODs) for the interpretation of the results. The earliest study was conducted in 2015[23] and it concluded that the sensitivity, specificity and positive likelihood ratio changed upon increasing the OD from >2.49 to >3.04 for anti-CtdB and from >1.68 to >1.80 for anti-vinculin. There were 2(20%) studies conducted in 2017(28) and 2019[22] which independently used OD CdtB >2.80 and for vinculin >1.68. The later[22] reported no statistically significant difference between the serum levels of anti-CtdB and anti-vinculin in IBS subjects compared to healthy controls. Although an earlier study[28] using the same OD did report a significant difference in IBS-D patients compared to healthy controls, but not with IBS-C cases. A study in 2019[12] with OD anti-CdtB >1.56 and anti-vinculin >1.60 recorded 100% specificity and PPV for IBS-D, but a low sensitivity of 22%.

There was 1(10%) study that included tested rectal biopsies

for somatostatin cell and rectal peptide YY cell densities, and concluded high sensitivities of 91% and 89%, respectively, as well as specificities 81 and 87%, respectively for IBS and its subtypes.[24] Faecal ELISA testing for BAFF showed encouraging results in terms of sensitivity 84% and 100% in a study[25] (Table 3).

The risk of bias analysis showed that random sampling, a pre-specified threshold for index test, and avoidance of case-control study were the most important elements in rating the articles, and 9(90%) studies were of high or moderate quality (Table 4).

Discussion

IBS is the most common functional GOT disorder that presents with abdominal pain and changes in bowel habits.[20] Despite the fact that it is highly prevalent, IBS remains a diagnostic dilemma with no definite lab tests currently under practice. Although different diagnostic tests have been under experiment for many years, researchers and clinicians have not reached a unanimous conclusion as to the best diagnostic test. The current review highlighted the tests that can help in making IBS diagnosis more objective and definite in the future.

For diagnostic tests, it is more preferable to use a test which

Table-3: Characteristics of the index test.

References	Index Test	Positive IBS	Clinical characteristic Index Test Diagnostic Value the study population	
Seyedmehdi Seyedmirzaee et al. 2015[20]	ELISA (to determine serum levels of various cytokines IL-6, IL-8, TNF-a)	n=74	**Comparison of IL-6, IL-8, TNF-alpha** 1. IL and TNF level in IBS patients > controls (*p*- value< 0.001) 2. IL, TNF level in IBS-D patients > controls (*p*- value<0.05) 3. IL, TNF level in IBS-C patients > controls (*p*- value<0.05)	
Walter Morales et al. 2019[12]	ELISA for anti-CtdB and anti-vinculin	n=100	**Anti-CdtB (OD>1.56)** Sensitivity%=43.00; Specificity%= 93.50; PPV= 95.60 **Anti-vinculin (OD>1.60)** Sensitivity%= 52.00; Specificity%= 90.90; PPV= 96.30	Both antibodies positive Sensitivity%=22 Specificity%=100 PPV=100
Yuna Chai et al. 2021[21]	q-PCR verifications of differential tsRNA in intestinal mucosa. Then Target gene prediction and bioinformatics analysis of differential tsRNA	n=31	**IBS-D vs controls** tiRNA-His-GTG-001: IBS-D> controls (p<0.05) tRF-Ser-GCT-113 + tRF-GlnTTG-035 IBS-D < Controls (p<0.01)	
Nicholas J. et al 2019[22]	ELISA for anti-CdtB and anti-vinculin antibodies	n=346	**Anti-CdtB (OD≥2.80)** IBS vs HC (p=0.06) not significant **Anti-vinculin (OD≥1.68)** IBS vs Organic disease (p=0.60) not significant Anti vinculin+ anti **CtdB ROC= 0.58** Anti vinculin+ Anti-CtdB+ 2 markers (stress, anxiety)	
Mark Pimentel et al. 2015[23]	ELISA for anti-CdtB and anti-vinculin (at two different optical densities)	n=2375	**Anti-CdtB (OD>2.49)** Sensitivity%=43.70; Specificity%= 91.60; Positive likelihood ratio=5.20 **Anti-Vinculin (OD>1.68)** Sensitivity%=32.6; Specificity%= 83.8; Positive likelihood ratio=2.0	**Anti-CdtB (OD>3.04)** Sensitivity%=28.30; Specificity%= 95.80 Positive likelihood ratio=6.70 **Anti-Vinculin (OD>1.80)** Sensitivity%=28.90; Specificity%= 84.50 Positive likelihood ratio=1.80
Magdy El-Salhy et al. 2015[24]	Immunostaining of rectal biopsy samples for PYY and somatostatin	n=101	**Somatostatin cell density IBS%** Sensitivity=91; Specificity=81 **IBS-D%** Sensitivity=87; Specificity=81 **IBS-C%** Sensitivity=93; Specificity=81	**PYY cell density IBS%** Sensitivity=89; Specificity=87 **IBS-D%** Sensitivity=87; Specificity=87 **IBS-C%** Sensitivity=88; Specificity=87
Yu Fu et al. 2017[25]	ELISA for fecal BAFF, calprotectin and FOBT	n=20	**Transit time** IBS< FC(p-value<0.01) **Mean time to first bowel movement** IBS<FC (p- value<0.01) **Bowel movements after 24h** IBS> FC (p-value<0.01) **Fasting and baseline total colonic volumes 120min after start of Moviprep** IBS< FC(p-value<0.01)	
Emily B. Hollister et al.2019 [27]	Stool samples for metabolomic profiling	n=23	Presence of specific bacteria, higher metabolites, and metabolic functions differentiated IBS from HC. **Area under the ROC curve=0.93** **Classification accuracy= 0.80**	
Ali Rezaie et al. 2017[28]	ELISA for anti-CtdB and anti-vinculin	n=2430	**anti CdtB OD ≥ 2.80** IBS-D > healthy controls (p-value=0.005) IBS-C = healthy (not significant) **anti vinculin OD ≥ 1.68** IBS-D > controls (p- value=0.002) IBS-C > controls (not significant)	

[B cell activating factor, BAFF: B cell activating factor, CdtB: Cytolethal distending toxin-B, FOBT: faecal occult blood test, FC: Functional constipation, HC: healthy controls, IBD: Irritable Bowel Disease, IBS: Irritable Bowel Syndrome, IBS-C: Irritable Bowel Syndrome constipation dominant, IBS-D: Irritable Bowel Syndrome Diarrhoea dominant, IL: Interleukin, OD: optical density, PPV: Positive predictive value, PYY: Peptide YY, PEG: polyethylene glycol, ROC curve: receiver operating characteristic curve, TNF: Tumour Necrosis Factor.]

Table-4: Risk of bias assessment.

Author/year	Patient selection				Index test		Reference Standard			Flow n timing			Quality
	consecutive or random sampling	Case-control avoided	avoid inappropriate exclusions	incl patients match the review Qs	Blinding	threshold specified	applicability to review Qs	likely to correctly classify the condition	blinding	applicability	interval between index n reference test	all received reference test	all received same reference test	all patients analyzed	
Seyedmehdi Seyedmirzaee et al. 2015[20]	Consecutive	Yes	yes	yes	no	No	yes	Yes	no	yes	no	yes	yes	yes	Moderate
Walter Morales et al. 2019[12]	consecutive	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	yes	Moderate
Yuna Chai et al. 2021[21]	Consecutive	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Low
Nicholas J. et al. 2019[22]	Random	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	High
Mark Pimentel et al. 2015[23]	Random	yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	High
Magdy El-Salhy et al. 2015[24]	Consecutive	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Moderate
Yu Fu et al. 2017[25]	Consecutive	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Moderate
C. LAM et al. 2016[26]	Consecutive	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	High
Emily B. Hollister et al. 2019[27]	Random	Yes	Yes	Yes	No	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	High
Ali Rezaie et al. 2017[28]	Random	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	High

is both sensitive and specific, but this is not often possible. Typically, there is a trade-off; since increasing sensitivity will decrease the specificity, and vice versa. Hence, for less-prevalent acutely developing diseases, public health practitioners recommend using tests with higher sensitivities to increase the rate of true positives. On the other hand, IBS, being a highly prevalent chronic disease in lower- and middle-income countries (LMICs) (33.20% prevalence in Pakistan[4]) and increasing continuously in high-income countries (HICs), it is advisable to take specificities into consideration to first exclude true negative patients. This will help with decreasing the burden on health facilities in managing false positive patients.

However, this is not a fact, and can be chosen according to the prevalence of IBS in a particular country within the context of the resources available. For the ease of clinicians to decide which test to implement in their clinical setting, the current review summarised both tests having high sensitivity and specificity separately.

ELISA testing for anti-CdtB and anti-vinculin, similarly for faecal calprotectin and BAFF, could be a potential candidate for aiding IBS diagnosis. The tests showed good specificity for anti-CdtB and anti-vinculin. ELISA testing has the advantage of being non-invasive and patient-friendly, and is not a financial burden on the health system and the patient, as opposed to the current IBS workup.[29] Although ELISA testing for antibodies showed the best specificity, inter-study differences could be observed that may be due to the discrepancies in sample collection, storage and even processing.[30] Some researchers have shown concern over the proposed use of anti-vinculin and anti-CdtB because of its overlap with other organic diseases, like systemic sclerosis and functional diarrhoea[31] but evidence regarding this is low. The role of anti-CdtB in campylobacter infection may also interfere with results while testing for IBS[28] but it has been proposed that a major mechanism for the development of IBS is prior gastroenteritis.[32] The drawback of these markers, however, is their low sensitivity, which warrants more research and insight into the potential use of these antibodies in facilitating the diagnosis of IBS in a clinical setting.

Lam C et al.,[26] measuring the transit time through the gut following laxative ingestion proved to be a good test to differentiate IBS from functional constipation (FC). It also measured colonic volumes and used MRI analysis of subjects. Although this can prove to be a promising step in the diagnosis of the disease[33] the technique would require multiple MRI scans to assess different colonic segments which would prove to be time-consuming and costly.[34] In the opinion of the clinical reviewer part of the current study, the delay in the process to get hourly MRIs will be a

hindrance to smooth diagnosis, but can be used in well-equipped setups with lower patient load. Although only a small number of studies have been conducted on the use of imaging to diagnose IBS[35] the initial promising results suggest that it can be used after larger clinical trials have been conducted.

Magdy El-Salhy et al.[24] measured cell densities of peptide YY and somatostatin in rectal biopsies to diagnose IBS. Although rectal PYY levels vary in different gastrointestinal pathologies, the use with somatostatin cell levels helps differentiate IBS from other organic disorders.[36] The site of choice for this procedure is the rectum, but an important finding is the variation in peptide quantities observed between the colon and the rectum[37] and hence, the colon cannot be used for biopsy.

Yuna Chai et al.[21] assessed the levels of different transfer RNAs (tRNAs) in intestinal tissues of IBS-D patients, the values of which could be diagnostic markers for IBS-D. A shortcoming of this test in some clinical settings is, firstly, the non-availability of deoxyribonucleic acid (DNA)/RNA testing facilities, and, secondly, the poor cost-effectiveness which would render it inappropriate as a first-line test.

A systematic review in 2015 aimed at exploring the diagnostic yield of IBS using different diagnostic test markers and their combinations.[6] It compared the likelihood ratios (LRs) of different approaches, such as colonic visceral hypersensitivity, serum levels of ILs and anti-tissue transglutaminase, as well as faecal levels of volatile metabolites and chromogranin. The LR of all these tests was calculated to be <10 which was non-significant to declare these tests as possible diagnostic tests for IBS.[38] Another reason to exclude such tests from the current review was the fact that data for these tests was collected from cross-sectional studies, which did not match the current inclusion criteria of focussing on RCTs and cohort studies.[39]

A meta-analysis in 2015 compared the levels of C-Reactive protein (CRP), erythrocyte sedimentation rate (ESR), faecal calprotectin and lactoferrin in patients with IBS and inflammatory bowel disease (IBD). Although these biomarkers did show elevated levels in patients with IBD, their predictability for IBS remained low.[40] These tests helped differentiate IBD from non-inflammatory gut diseases, but by no means could they provide accurate differentiation between organic diseases. The only test that did show some promising results was for faecal calprotectin, but the PPV was low, causing it to be excluded from the current systematic review.

The strengths of the current systematic review were that it took into consideration articles from 2015 onwards when Rome III and Rome IV criteria had been updated to compare the laboratory testing with the most recent criteria. In contrast to previous studies, which reported different tests, it included newer tests to choose from the latest studies. The strict inclusion/exclusion criteria applied to the systematic review, particularly in terms of study designs given priority (RCT and cohort studies), helped increase the validity and decrease bias in the review. The studies added were all on the basis of the reference standard (Rome III or Rome IV) compared to other reviews which have also been using the older Rome II and Rome I criteria. Lastly, the inclusion of a clinical expert as part of the review gave deeper insight into the practicality of the tests analysed in the current review.

The tests proposed by various studies had the limitation of lacking uniformity and definite results. The tests also needed to be cost-effective and quick to lessen the distress of the patients. Hence, further clinical testing on the above-mentioned tests is recommended to decide on a test that is adequate, accessible and practical to diagnose IBS with ease.

Conclusion

The systematic review provided a thorough insight into all recent diagnostic techniques under observation as a potential definitive test to diagnose IBS. ELISA testing for anti-CdtB and anti-vinculin as well as testing of rectal biopsies for cell densities could be a possible way forward in aiding the diagnosis of IBS. ELISA for antibodies, even with lower ODs provided high specificity. However, low sensitivity warrants further testing and scientific data. Other tests had high diagnostic accuracy, but were time-taking, invasive and had poor cost effectiveness. The findings highlighted that accuracy and future feasibility of these tests were far from incorporation into clinical practice, but the encouraging results reported in literature call for continued research to improve upon the current status.

Acknowledgement: We are grateful to Dr Sana Iftikhar of the Community Medicine Department, Allama Iqbal Medical College, Lahore, for guidance and support through the study, and to Professor of Medicine Dr Faud Shafiq for shortlisting and grading the studies analysed.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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MM: Searching data bases, screening articles, shortlisting and writing.
MHS: Searching data bases, screening articles, shortlisting, compiling results and writing, review registration at PROSPERO.
MI: Extracting data from the final shortlisted article, compiled the result tables and writing.
FM: Assisted in the screening process, compiling result tables, writing the final review.
SI: Supervised the entire project and gave input on the research methodology and technical aspects of writing.
FS: Supervised the entire project, review.