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Spectrum of Infection in Patients with Irritable Bowel Syndrome

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Abstract:
Background: *Irritable bowel syndrome is a major chronic gastrointestinal disorder which is also referred as functional bowel disorder. Overall worldwide prevalence ranges from 12%-30%. Etiology of IBS is currently unknown, although infection, genetic, environmental, psychosocial or physiological factors are likely to contribute to the disorder. Bacteria and parasites have been described having a possible role in etiology.* ***Materials and Methods:*** *The study was done to determine the spectrum of infection in patients with irritable bowel syndrome. 117 stool sample from IBD patients and 101 samples from healthy patients were collected. 3 consecutive samples were collected. Macroscopic observation was done followed by microscopy along with culture methods, antigen detection of E. histolytica through ELISA and C. difficile toxin A and b detection by ELISA.****Results:*** *Among the 50 patients included, 39 (78%) were male, whereas 11(22%) were female. The mean age of patients were found to be 35.2 years. Giardia and Entamoeba histolytica were the major causative agents.*

Keywords: *Irritable Bowel Syndrome, Prevalence, Northern India, ELISA, Opportunistic pathogens.*

I. Introduction

Irritable bowel syndrome (IBS) is one of the major common chronic gastrointestinal disorders. IBS has been referred to as functional bowel disorders, which is diagnosed by a characteristic cluster of symptoms in absence of detectable structural abnormalities. Symptomatic overlap occurs between microscopic colitis and IBS and it is necessary to carry out biopsy of colon [1].

The case burden of IBS in the world is variable. Overall worldwide prevalence ranges from 12%-30% [2]. In a multi-centric study in India, the prevalence was found to be 4.2% overall but they show increasing trend of the incidence [2]. Males are affected slightly more (4.3%) than females (4.0%) in contrast to western studies. IBS can affect persons from15 to 65 years of age. It is more prevalent in UK (22%) and USA (20%) and other western countries. Females are affected more than males. The burden in Asia is comparatively less, but it is increasing [3].

The etiology of IBS is currently unknown although infectious, genetic, environmental, psychosocial or physiological factors are likely to contribute to the disorder. Among the infectious causes various intestinal pathogens may play role in the onset and maintenance of IBS given that previous studies have indicated disparities in the micro-biota between sufferers and healthy individuals. Current evidence shows that about 25% sufferers of IBS had history of infectious enteritis [4].Recent study states that inflammation is found in a small subset of IBS. Previous gastroenteritis has been identified as the most important risk factor for IBS [4]. Bacteria like Shigella, Salmonella, Vibrio, Campylobacter and Clostridium difficile may produce post-infectious IBS [5]. Duration of diarrhea and severity of illness is an independent risk factor for the production of IBS [6]. In a report from India, bacterial overgrowth was seen in 7.4% and Shigella was the predominant pathogen of this condition [7].

Recent studies have described a possible role for protozoan parasites, such as Blastocystishominis and Dientamoebafragilis, in the etiology of IBS [8]. The role of B.hominis as an etiological agent of IBS is inconclusive, due to contradictory reports and the controversial nature of B.hominis as a human pathogen [9]. Although, Entamoeba histolytica infections occur predominantly in developing regions of the world, clinical diagnosis of amoebiasis is often difficult because symptoms of patients with IBS may closely mimic those with non-dysenteric amoebic colitis [10]. Clinical manifestations of Giardia intestinalis infections also vary from asymptomatic carriage to acute and chronic diarrhea with abdominal pain [11]. It is essential that all patients with IBS undergo routine parasitological investigations in order to rule out the presence of protozoan parasites as the causative agents of the clinical signs.

In developing country like India, majority of hospitalization is due to infectious causes. Thus, to make routine stool investigations to diagnose common pathogens as well as special investigations for opportunistic pathogens for patients presenting with these conditions becomes important. Therefore, this study was planned with an intention to look through various dimensions on this aspect.

II. Materials and Methods

This prospective study was carried out in Parasitology laboratory of the Department of Microbiology in association with the Department of Gastroenterology, SGPGIMS, Lucknow, a tertiary care center.Stool samples from patients with IBS were collected during a period from April 2010 to May 2012. Attempt was made to collect 3 consecutive samples from each patient. 117 stool samples from IBS patients were collected. 101 stool samples were collected from healthy controls for comparison.

Rome III criteria were used for diagnosis of IBS patients. At least 3 months, with onset at least 6 months previously of recurrent abdominal pain or discomfort associated with 2 or more of the following: improvement with defecation and/or onset associated with a change of frequency of stool and/or onset associated with a change in form of stool [13]. Healthy control included persons without any gastrointestinal symptoms.

3 consecutive stool samples from each patient were collected in a clean wide mouth plastic container avoiding contamination with urine, water disinfectants. These samples were transported to laboratory as soon as possible. A proforma containing details about patients and results were maintained. Sample processing was done using following methods:

1. Macroscopic examination:
2. Microscopy [14]:
i. Direct wet smear
ii. Concentration method
iii. Kinyoun and Modified trichrome staining [for opportunistic parasitic pathogen].
iv. Calcoflour white staining

3. CultureMethods [15]:
i. Aerobic
ii. Anaerobic (for C.difficile)
iii. Micro-aerophilic (for Campylobacter spp.)

4. Antigen detection by ELISA for E histolytica
5. C. difficile toxin A and B detection by ELISA

1. Macroscopic Examination:

Stool samples were examined to look for mucus, blood, consistency, texture, presence of whole worm or presence of segments of parasites.

2. **Microscopic Examinations:**

(a) Direct Wet Mount:The microscopic examination of stool was carried-out with normal saline as unstained preparation and also with Iodine solution as stained preparation. The unstained preparation was performed for the demonstration of actively motile forms (trophozoites). While, the stained-preparation was carried-out for the demonstration of cysts/ova (infective forms) or dead specimens of trophozoites. Besides these, the stained-preparation was also carried-out for the study of nuclear-characters and glycogen-mass (food-storage sites).

(b) Concentration Wet Mount: Formal Ether Concentration Method (Modified from Allen & Ridley, 1970)-Wet Mount, one of the most widely used. It is probably the most useful and convenient of all the concentration procedures.

(c) Staining Methods:The staining procedures used for the diagnosis of opportunistic parasitic pathogens were:

I. Modified Kinyoun's Acid-Fast staining
II. Modified Trichrome staining
III. Calcofluor-White Fluorescent staining

3. Culture-Methods:

(a) Aerobic-Culture Methods:To perform aerobic-culture for the selective isolation of any pathogenic aerobic bacteria in the stool-sample, the stool/faecal sample was cultured on plates by Streak-Plate method on three plates:Mac Conkey Agar, DCA (Deoxycholate Citrate Agar), DSRA (D-Sorbitol Rhamanose Agar). Sample

was also put in GN (Gram-Negative) Broth for enrichment.Fecal-sample was cultured by Streak-Plate culture (Surface-Plating) method that was routinely employed for the isolation of bacteria in the pure-culture.

(b) Anaerobic-Culture Method: Anaerobic culture for the selective isolation of Clostridium difficile was performed using CefoxitinCycloserine fructose agar [CCFA].

(c) Micro-aerophilic Culture:Micro-aerophilic culture for the selective isolation of Campylobacter spp., was performed using Charcoal CefoperazoneDeoxycholate Agar [CCDA].

4. ELISA for Detection of Entamoeba histolytica Antigen [16]:

Principle: It uses antibodies as adhesin. The micro-assay wells contain immobilized polyclonal antibody that binds adhesin of E.histolytica. During the first incubation, E. histolytica antigens present in the stool supernatant are captured by antibodies attached to the wells. In second incubation, monoclonal antibody-peroxidase specific for E.histolytica adhesion were added. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in presence of enzyme-complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

The sample was thawed, if needed. Then sufficient diluted Wash buffer was added to make approximately a 1:4 dilution (1gm or a pea-sized fecal sample to 3 ml diluted Wash buffer) and mixed well.

5. ELISA for Detection of Clostridium difficileAntigen Or Toxin 'A' &'B' [17]:

Principle: During the first incubation, Clostridium difficile antigens present in the stool supernatant are captured by antibodies attached to the wells. In second incubation anti- Clostridium difficile antibody conjugated to peroxidase was added. After washings to remove unbound enzyme, a chromogen is added which develops a blue color in presence of enzyme-complex and peroxide. The stop solution ends the reaction and turns the blue color to yellow.

III. Results

The present study was carried out in the Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS), a tertiary health care center at Lucknow. 50 patients of irritable bowel syndrome (IBS) were included in the study. A total number of 117 stool samples were collected from IBS patients. In addition 101 stool samples from 50 healthy controls were also collected for comparison.

I. Ibs Patients

- **Distribution of patients in relation to age:** Patients were divided according to different age groups. Number of patients in different age groups were 3 (6%), 14 (28%), 15 (30%), 13 (26%), 4 (8%), 1 (2%) in 10-19,20-29,30-39,40-49,50-59,60-69 respectively. The mean age of the patients were found to be 35.2 years.

Table 1: Age wise distribution of IBS patients (n=50)

Age (In Years)	Number (%)
10-19	3(6%)
20-29	14(28%)
30-39	15(30%)
40-49	13(26%)
50-59	4(8%)
60-69	1(2%)
Total	**50**

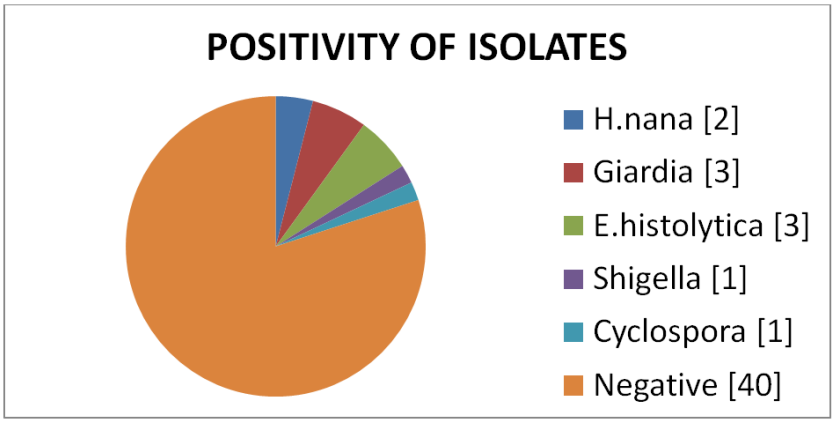
- **Sex-wise distribution of patients with IBS:** Out of 50 patients 39(78%) were male and 11(22%) were female.

Table 2: sex-wise distribution (n=50)

Age (In Years)	Male (%)	Female (%)
10-19	2(4%)	1(2%)
20-29	11(22%)	3(6%)
30-39	12(24%)	3(6%)
40-49	9(18%)	4(8%)
50-59	4(8%)	0(0%)
60-69	1(2%)	0(0%)
Total	**39(78%)**	**11(22%)**

- **Positivity of microorganisms in IBS patients:** Overall positivity of microorganisms combining all diagnostic methods was 10(20%). Different microorganisms isolated were: H.nana in 2 (4%) patient, Giardia in 3 (6%) patients, and Cyclospora in 1 (2%) patient. All of the microorganisms were detected in microscopy. Shigellaflexneri was isolated in culture from 1 patient (2%). Antigen ELISA for Entamoeba histolytica was positive was positive in 3 (6%) patients.

Fig 1: Chart showing positivity of IBS patients (n=50)



- **Comparison of direct microscopy and concentration method:** All samples were first examined by direct microscopy and then by concentration method. 4 samples were positive for Giardia in direct microscopy. 2 samples were positive forH.nana. Total positivity by direct microscopy was 6 (5%). All samples which were positive in direct microscopy were also positive in microscopy after concentration. After concentration, 1 sample was found to be positive for Giardia which was not seen in direct microscopy. Total positivity after concentration was 7 (5.9%). 1 sample was positive for Cyclospora in Kinyoun's staining and calcoflour white staining.

Table 3: Comparison of direct microscopy and microscopy after concentration

Microscopy	**Direct (N=117)**		**After Concentration (N=117)**			
	NS	**Iodine**	**NS**	**Iodine**	**K**	**CW**
Positive	H.nana (2) + Giardia (4)	H.nana(2) + Giardia(4)	H.nana (2) +Giardia (5)	H.nana (2) + Giardia (5)	Cyclospora (1)	Cyclospora (1)
Total	6 (5%)	6 (5%)	7 (5.9%)	7 (5.9%)	1	1(0.8%)
Negative	111 (95%)	111 (95%)	110 (94.1%)	110 (94.1%)	116	116 (99.2%)

- **Comparison of direct microscopy and antigen detection for Entamoeba histolytica in IBS patients:** No Entamoeba histolytica was detected in direct microscopy. Antigen ELISA for E.histolytica was positive for 3 samples which was negative by direct microscopy.

Table 4: Comparison of ELISA for antigen and direct microscopy

ELISA for Antigen	**Direct microscopy (n=117)**	
	Positive	**Negative**
Positive	0	3
Negative	0	114
Total	0	117

IV. Healthy Controls

- **Distribution of cases in relation to age:** The controls were divided according to different age groups. Number of patients in different age groups were 0 (0%), 2(4%),6(12%), 30(60%),12(24%),0(0%),0(0%), 0(0%) in 0-9,10-19,20-29,30-39,40-49,50-59,60-69,70-79 age groups respectively.

Table 5: age wise distribution of healthy controls (n=50)

Age In Years	**Number (%)**
0-9	0 (0%)
10-19	2 (4%)
20-29	6 (12%)

30-39	30 (60%)
40-49	12 (24%)
50-59	0 (0%)
60-69	0 (0%)
70-79	0 (0%)
Total	**50**

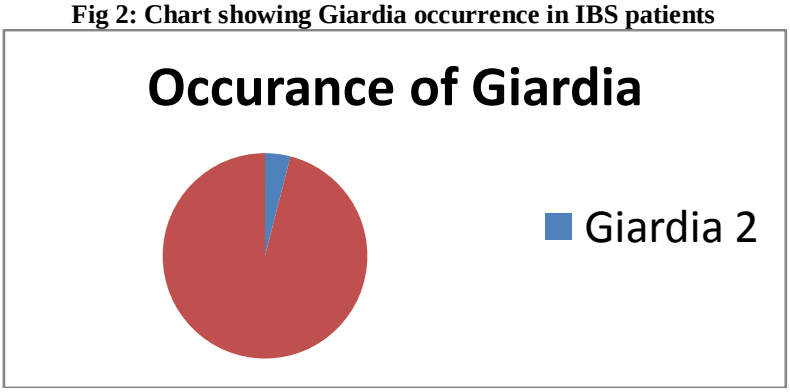
- **Sex-wise distribution of controls:** Total of 50 controls was taken. Out of this 48(96%) were males and 2(4%) were females.

Table 6: sex-wise distribution (n=50)

S.No	Age (In Years)	Male (%)	Female (%)
1	0-9	0	0
2	10-19	0	2(4%)
3	20-29	6(12%)	0
4	30-39	30(60%)	0
5	40-49	12(24%)	0
6	50-59	0(0%)	0
7	60-69	0(0%)	0
8	70-79	0(0%)	0
	Total	**48(96%)**	**2(4%)**

- **Positivity of microorganisms in healthy controls:** Giardia was found in 2(4%) of patients.

Fig 2: Chart showing Giardia occurrence in IBS patients



- **Comparison of direct microscopy and concentration method:** 5(5%) samples were positive for Giardia in direct microscopy. Same 5 (5%) samples were positive after concentration.

Table 7: Comparison of direct microscopy and microscopy after concentration

Microscopy	Direct (N=101)		After Concentration(N=101)				
	NS	Iodine	NS	Iodine	K	Au	KW
Positive	Giardia(5)	Giardia(5)	Giardia(5)	Giardia(5)	0(0%)	0(0%)	0(0%)
Total	5(5%)	5(5%)	5(5%)	5(5%)	0(0%)	0(0%)	0(0%)
Negative	96(95%)	96(95%)	96(95%)	96(95%)	0(0%)	0(0%)	0(0%)

Table 8: Comparison of positivity of IBS, IBD and control

Positivity	IBS (N=50)	Control (N=50)
E.histolytica	3	0
H.nana	2	0
Shigella	1	0
Cryptosporidium	0	0
Isospora	0	0
Cyclospora	1	0
Giardia	3	0

V. Discussion

Approximately 15%-30% of patients with IBS report onset of disease after an enteric infection [18]. This observation implicates that IBS may at least in a subpopulation be an inflammatory disorder. Apart from gastroenteritis, different factors such as altered gut flora, increased permeability, genetic susceptibility and

unrecognized food reactions have all been suggested as possible mediators of an inflammatory response in IBS patients. The incidence of post infection IBS after enteric infections have been reported to vary between 3% to approximately 35%

There are different demographic data about Irritable bowel syndrome. It can affect both sexes. In most part of the world, IBS is more common in females than males [19,20]. In our study males outnumbered females, by showing results of 39(78%) and 11(22%) respectively. Similar findings were reported from India [2,21]. However in all the studies, reporting were voluntary and hospital based .So male patients seeking treatment more than females may be possible cause. This suggests that male preponderance with chronic functional lower GI symptoms is common in India.

The age range in our study was from 10- 70 years. This was in accordance to recent WHO data which reported age range to be 15 to 65 years [3]. Majority of patients were in age group 20-40 years (58%). Mean age of patients in our study was 35.2 years. It is close to findings of Ghoshal et al (39.4 years) [2]. This further strengthens the view that IBS is usually a disease of male adults especially in India.

Infectious basis of Irritable bowel syndrome has gained much interest in recent years. 25% of IBS patients have an infectious etiology as suggested in literature [22]. As suggested by MaKendrik and Read, the occurrence of IBS following bacteriologically confirmed gastroenteritis increases significantly [23]. Host-bacterial interaction, interbacterial interaction are major area of study about IBS. Small intestinal bacterial overgrowth syndrome (SIBO) is a condition where abnormally high bacterial population is found in gut. Significant association of SIBO was found in a study. 11% of IBS patients showed the condition in contrast to 1% in control group [24].

Bacteria like Salmonella and Shigella have been found to be associated with IBS [5]. Patients with episode of diarrhea have increased risk of IBS. In a study 31% of patients developed new IBS symptoms after Salmonella food poisoning [12]. Post- infectious IBS is now main area of study and Shigella was found to be associated with 8% of these conditions [20]. In our study 1(2%) patient was found to be positive for Shigellaflexneri which is the major species in India. This finding is in concordance with other Asian study by Gwee et al who reported 14.9% of post-infectious IBS due to Shigella [25]. No other pathogenic bacteria were found in IBS like Salmonella and Campylobacter. More number of sample size or more stringent transport conditions may have produced better isolation.

People of developing countries harbor parasites more than their developed counterparts. Parasite pathogenesis and host immunity may have a major role in development of IBS. Intestinal helminthes shift the immune system towards a Th2 response, which may be associated with reduced chance of protracted GI inflammation.Hence, a high frequency of helminthic infestation may explain the low frequency of IBS in tropical countries, such as India, Bangladesh and Thailand despite a high frequency of bacterial GI infections. In a studyby Tungtronchitr A et al on 59 patients of IBS, Blastocystis was found in 13.6% of patients, Strongyloidesstercoralis in 1.7% and Giardia in 1.7% of patients [9]. The findings of our study support this theory. H.nana (4%), Giardia (6%) were found in significant number than control group. Similar findings were found in a study which suggested nematodes to be associated with IBS. Trichinellaspiralis was implicated in another study [20]. It necessitates further study to find out roles of parasites in IBS.

Entamoeba histolytica was also implicated as a pathogen in IBS. Symptoms of IBS closely resemble non-dysenteric amoebic colitis. In our study, 6% IBS patients were found to be positive for E.histolytica as compared to 0% in controls. Study from India, by Chaudhary et al [10] reported 25% of IBS had amoebic or bacillary dysentery etiology. Another study by Anand et al [26] showed E,histolytica prevalence of 18% . So this parasite infection which is common in India may have a role in IBS. This discordance may be due to more prevalence of chronic cyst passers than invasive amoebiasis in our study.

Giardia is proven by numerous studies to be the most common infectious etiology in IBS. In general, persistent infection with Giardia is expected to cause chronic diarrhea, irregular bowel movement and abdominal discomfort, which may be diagnosed as IBS by a symptom-based criterion. In our study, 6% IBS patients were found to be positive for Giardia as compared to 0% in control groups.A study from Norway by Kurt et al showed 12% Giardia etiology [27]. A study from India by U.C.Ghoshal et al on 78 IBS patients showed 54% prevalence of Giardia[7]. However, there are scanty data from Asian countries where this infection is expected to be more common. Hence, more studies evaluating the role of Giardia lamblia in Asia are needed.

VI. Conclusion

Most IBS patients were middle aged persons. Males are affected more than females in contrast to western data.Bacteria like Shigella are associated with IBS.Clostridium difficile association IBS need further study with large sample size.Parasitic agents like H.nana, Giardia, and Entamoeba histolytica are found in higher numbers in IBS. They may have role in initiation, maintenance and relapse of both the diseases.Samples of IBS patients should be routinely evaluated for infectious agents. Special staining should be routinely done in them.Concentration methods are helpful in isolating more infectious agents from stool samples.

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