

*Research Article***Colonic Eosinophilia in Irritable Bowel Syndrome Patients****Taha Mohamed Hassanin¹, Al-Shaymaa Ahmed Mohamed Hassanin, Fatama Elzahraa A. S.Mohamed² and Ekhlas Hamed Abdel-Hafeez^{1&3}**¹ Department of Tropical Medicine -Faculty of Medicine- Minia University.² Department of pathology- Faculty of Medicine-Minia University.³ Department of Parasitology-Faculty of Medicine-Minia University.**Abstract**

Irritable bowel syndrome (IBS) is characterized by chronic recurrent abdominal pain and alterations in bowel habits, unrelated to organic causes. Eosinophils have a role in GIT pathology mediated by inflammatory mechanisms and by classical and non-classical food allergy mechanisms. Eosinophils that infiltrate the epithelium in more than occasional numbers, coalesce to form aggregates, or show extensive degranulation are always abnormal and raise a broad differential diagnosis. We aimed in this work, to study the association between colonic mucosal eosinophilia and IBS patients. The study was conducted on 80 patients who met the inclusion criteria of IBS based on Rome IV symptoms questionnaire. Patients were subjected to thorough general and abdominal examination, basic laboratory investigations, abdominal ultrasonography, lower endoscopy (colonoscopy) and biopsy and histopathological examination. Here in this study, IBS patients diagnosed according to Rome IV symptoms questionnaire. The percentage of IBS was higher among females (71.25%) compared to that in males (28.75%). 62.5% patients was IBS- D subtype and 25% patients was IBS-C subtype. Histopathological results of IBS Patients' biopsies were as follow: 10% were mainly lymphocytic infiltrates; 15% were eosinophilic infiltrates mainly; 75% were having very few mixed inflammatory cells. 17.5% female patients' biopsies showed eosinophilic inflammatory cells infiltrates compared to that from males (8.7%), which was statistically insignificant. The current study has some limitations which should be noted. It was a relatively small sample size of population were included.

Key words: Irritable bowel syndrome, eosinophilic infiltrates and colonoscopy.

Introduction

Even though irritable bowel syndrome (IBS) has been known for more than 150 years, IBS remains one of the research challenges of the 21st century (Algera et al., 2019). According to Rome IV diagnostic criteria, IBS is characterized by chronic recurrent abdominal pain and alterations in bowel habits, unrelated to organic causes. Onset of symptoms should start at least 6 months before diagnosis and should present during the last 3 months (Canavan et al., 2014 and Lacy et al., 2016).

Eosinophils are present in the healthy intestinal mucosa, but their recruitment from the blood is increased during inflammatory conditions (Kristjansson et

al., 2004 and Park et al., 2008). Eosinophils have a role in GIT pathology mediated by inflammatory mechanisms and by classical and non-classical food allergy mechanisms (Fritscher-Ravens et al., 2019). The distinction between the upper limit of normal and abnormally increased mucosal eosinophils is not well defined (Kiss et al., 2018). However, eosinophils that infiltrate the epithelium in more than occasional numbers, coalesce to form aggregates, or show extensive degranulation are always abnormal and raise a broad differential diagnosis (Yantiss, 2015).

Therefore, we aimed in this work, to study the association between colonic mucosal eosinophilia and IBS patients.

Patients and Methods

Analytical cross-sectional study was conducted in one year duration from the period of October 2019 to September 2020. This study was carried out at GIT clinic, Endemic Medicine Department, Minia University Hospital, Minia, Egypt. A validated pre-constructed anonymous questionnaire was used to collect baseline information such as socio-demographic (age, gender, occupation, number of siblings, education), The questionnaire consisted of several questions in regard to bowel habits and other gastrointestinal symptoms experienced over the previous 12 months. Individuals who diagnosed as IBS were subjected to the following:

1. Thorough general and abdominal examination.
2. Laboratory investigations:
Complete blood picture (CBC): Total and differential counts, Liver function tests (LFTs): ALT, AST, total albumin and total protein, Kidney function tests (KFTs): Urea and creatinine, C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR).
- 3- Abdominal ultrasonography was done to all patients to detect liver pathology, gall bladder diseases, or other abnormality.
- 4- Lower Endoscopy (colonoscopy) and biopsy:
Colonic Preparation of the patients for colonoscopy was done before colonoscopic examinations During a colonoscopy, a long, flexible tube (colonoscope) is inserted into the rectum. During the colonoscopy multiple biopsies were taken of the apparently intact mucosa from the ascending & descending colon, transverse colon, and rectum, 10 cm distance between colonic biopsies were taken. Biopsy specimens were preserved in 10% formalin until referred to Pathology Department.
- 5- Histopathological examination:
The biopsies were referred to the pathology department. The total numbers

of eosinophils within the epithelium of 4 consecutive crypts and those present in the lamina propria of 4 consecutive intercrypt areas were recorded for each case.

Statistical analysis:

All results were collected, recorded, tabulated, and finally analyzed and assessed. The SPSS statistical package version 22 (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. All the data were presented as the mean \pm standard deviation (SD) for continuous variables and as a frequency or percentage for categorical variables. Univariate analysis was performed using independent sample t-test for the comparison of continuous variables and a chi-square test for categorical variables. Values of $P < 0.05$ were considered statistically significant.

Results

This cross-sectional study was conducted on 80 patients who met the inclusion criteria of IBS based on Rome IV symptoms questionnaire. The studied patients were 57 (71.25%) females, and 23 (28.75%) males as in table (1). IBS- D subtype was higher than IBS-C subtype among the studied IBS patients: 62.5% patients had IBS- D, 20 (25%) patients had IBS-C as in table (2).

Histopathological results of the IBS Patients' biopsies were as follow; 12 (15%) of Patients' biopsies were eosinophilic infiltrates mainly; 8 (10%) of Patients' biopsies were sever lymphocytic infiltration and aggregation and 60 (75%) were having very few mixed inflammatory cells ie: Normal finding for microscopy. Colonic biopsies from female patients showed higher eosinophilic infiltrates (17.5%) compared to that from males (8.7%), which were statistically insignificant ($P = 0.5$) as in table (3).

Table (1): Demographic characteristics of the study patients:

Studied variable	N (%)or Mean \pm SD
Age	
Mean \pm SD	34.6 \pm 9
Range	(18-50)
Sex	
Male	23 (28.75%)
Female	57 (71.25%)
Residence	
Rural	24 (30%)
Urban	56 (70%)
Occupation	
Housewife	36(45%)
Student	16(20%)
Employee	28 (35%)
Marital status	
Married	62 (77.5%)
Single	18 (22.5%)
Smoking	20 (25%)
History of allergic disease	12 (15%)

The data were shown as N (%)or Mean \pm SD

Table (2): Clinical characteristics of the study patients:

Studied variable	N (%)
IBS- D	50 (62.5%)
IBS-C	20 (25%)
IBS-M	10 (12.5%)
Abdominal pain	
Mild	34 (42.5%)
Moderate	46 (57.5%)

The data were shown as N (%).

Table (3): Relation between demographic characteristics and histopathological findings:

Studied variable N (%)	Eosinophilic infiltrate 12 (15%)	p-value
Sex		
Male	23 (28.75%)	2 (8.7%)
Female	57 (71.25%)	10 (17.5%)
Occupation		
Housewife	36(45%)	3 (8.3%)
Student	16(20%)	5 (31.2%)
Employee	28 (35%)	4 (14.3%)
Marital status		
Married	62 (77.5%)	10 (16.12%)
Single	18 (22.5%)	2 (11.11%)
Smoking	20 (25%)	2 (10)
History of allergic disease	12 (15%)	8 (66.67%)
		0.0001*

* Significant level of p- value is < 0.05

* p value was calculated by using Chi-square test

Discussion

Irritable bowel syndrome (IBS) is a functional bowel disorders (FBD) characterized by chronic recurrent abdominal pain and alterations in bowel habits, unrelated to organic causes. Onset of symptoms should start at least 6 months before diagnosis and should present during the last 3 months (Canavan et al., 2014 and Lacy et al., 2016). Eosinophils have a role in IBS pathology mediated by inflammatory mechanisms and by classical and non-classical food allergy mechanisms (Fritscher-Ravens et al., 2019).

Eosinophils are gaining increasing attention as the cells of unique properties among leukocytes, which can damage or repair surrounding tissue and modulate the activity of immune cells (Bandeira-Melo and Weller, 2005). Eosinophils are present in low levels in the GIT, particularly in the lamina propria of the stomach, small intestine, cecum, and colon (Rothenberg and Hogan, 2006).

In this work, we aimed to study the association between colonic mucosal eosinophilia and IBS patients. Here in this study, IBS patients were diagnosed according to Rome IV symptoms questionnaire. The percentage of IBS was higher among females (71.25%) compared to that in males (28.75%), table 1.

These data matched with other studies. Choung et al., (2011) reported that women are 2-4 times more likely to develop IBS compared to men. Furthermore in Cairo-Egypt, Ahmed (2011) reported a high prevalence rate of IBS in females (52.7%) compared to that in males (47.3%) among the studied group. Also, Elhosseiny et al., (2019) in Cairo-Egypt reported a high prevalence rate of female gender who was suffering from IBS among of the studied group. Additionally, there was a study conducted in Saudi Arabia showed a high prevalence rate of IBS in female gender (Ashaalan, 2011). Further, Chatila et al., (2017) in Lebanon showed a high prevalence rate of IBS in female gender among.

In the same way, a Pakistani study reported that females had a significant increase in prevalence of IBS, compared to males. Also, a Malaysian study reported a similar result (Naeem et al., 2012). Similarly, a study in Japan reported 41.5% of females had IBS symptoms compared to 13.8% of males (Okami. et al., 2011). An Indian study reported the same findings, IBS symptoms were being more in females (Basandra et al., 2014). On the same way, an Australian study reported the same findings (Parthiepan and Rode, 2011). Also, in Korea Lee et al., (2013) reported that IBS symptoms were being more in females.

Various hypotheses could explain the higher prevalence of IBS in females. Firstly, higher serotonin synthesis in the brain of female gender (Park et al., 2010). The effect of female sex hormones' on gastrointestinal motility (Wald et al., 1981) and the association between IBS and an anti-conceptive mechanism (Unruh, 1996). Also, female gender, suffering from anxiety which was the main associated factor for IBS (Elhosseiny et al., 2019).

On the other hand, in Korea Jung et al., (2011) found that the prevalence of IBS was more in males than females, being 41% and 25%, respectively. Another study in Pakistan stated that IBS higher in males compared to that in females (Jafri et al., 2005). On the other hand, Qureshi et al., (2016) reported that there is no exact effect of gender on the prevalence of the disease among studied groups

Moreover, in this study, 62.5% patients were IBS- D subtype and 25% patients was IBS-C subtype (Table 2). These data matched with an Australian study reported the same findings, IBS-D subtype was being more than that of IBS-C subtype (Parthiepan and Rode, 2011).

On the other hand, these data were dissimilar to other studies. Elhosseiny et al., (2019) reported that 26.6% was IBS-D subtype and 37.4% was IBS-C subtype

among studied groups in the Faculty of Medicine -Ain Shams University-Cairo-Egypt. Also, Ibrahim et al., (2013) reported the same data for a study was performed in Saudi Arabia, King Abdulaziz University in Jeddah. Moreover, Agrawal et al., (2008) reported that 31% of IBS-D subtype and 49% of IBS-C subtype among studied groups University Hospital of South Manchester-United Kingdom.

Furthermore in this work, histopathological results of IBS Patients' biopsies showed that 10%. of these biopsies were mainly lymphocytic infiltrates. These data were unusual finding to the expected histopathological results of IBS Patients' biopsies. Thus, these biopsies most probably considered as a microscopic colitis. These data were matching with many researchers (Pardi et al., 2002; Fernandez-Banares et al., 2003; Olesen et al., 2004 and Lee et al., 2013). MC is diagnosed on the basis of histological criteria (by the presence of intraepithelial lymphocytosis and a mixed inflammatory infiltrate in the lamina propria) (Pardi et al., 2004). While IBS is diagnosed by symptom-based criteria. Both have similar endoscopic appearance, the mucosa appears grossly normal or nearly normal at colonoscopy (Pardi et al., 2002; Fernandez-Banares et al., 2003; Olesen et al., 2004 and Lee et al., 2013).

Further in this study, histopathological results of IBS Patients' biopsies showed that 15%. of these biopsies were mainly eosinophilic infiltrates. Colonic eosinophils have been less studied in the context of IBS. Although some studies reported an increased colonic eosinophil in IBS patients. Rothenberg, (2004) have demonstrated an increased number of eosinophils in functional GIT diseases. Furthermore, Parthipan and Rode, (2011) and Willot et al., (2012) founded that eosinophils number were significantly high in colonic biopsies obtained from IBS patients compared to control. On the same way, several publications were reported an elevated numbers of eosinophils in colonoscopic biopsy from IBS patients

(Walker et al., 2009; Powell et al., 2010; Yantiss, 2015; Walker et al., 2018 and Katinios et al., 2020).

On the other hand, some studies, reported that there is no differences in eosinophil density with IBS patients compared to that of the control (Kristjánsson et al., 2004; Ford et al., 2011; De Silva et al., 2012; Lee et al., 2013 and Emmanuel et al., 2016).

In this research, 17.5% female patients' biopsies showed eosinophilic inflammatory cells infiltrates compared to that from males (8.7%), which was statistically insignificant Table (3). An elevated numbers of eosinophils in colonoscopic biopsy samples of IBS patients were reported in several publications (Walker et al., 2009; Yantiss, 2015; Walker et al., 2018 and Katinios et al., 2020). On the other hand, other publications reported that colonic eosinophils do not appear to be increased across the different groups of population of IBS patients (Kristjánsson et al., 2004; Lee et al., 2013; Ford et al., 2011; De Silva et al., 2012; Emmanuel et al., 2016). However, increased colonic numbers of eosinophils does not appear to be affected by sex (Chernetsova et al., 2016; Grzybowska-Chlebowczyk et al., 2017 and Lee et al., 2018).

The current study has some limitations which should be noted. It was a relatively small sample size of the population were included

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