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Original Article

Effect of Conventional Anti Tubercular Drugs and Azathioprine on Nontuberculous Mycobacteria in Patients of Crohn's Disease

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Abstract

Background: Tuberculosis is a common disease in Bangladesh so dose the nontuberculous mycobacteria (NTM) infection but the pathogenic role of the nontuberculous mycobacteria is yet to be answered but with development of diagnostics modalities and as well as changing trend of disease like Crohn's disease(CD) in Bangladesh the use of immunomodulator has also increased. There is also gape of knowledge regarding the effect of conventional anti tubercular medication on nontuberula mycobacteria. So we need to find whether the anti Tubercular medication as well as immunomodulator like azathioprine has any role in presence of notuberculous mycobacteria(NTM) in patient; got anti TB drugs or getting azathioprine. **Objective:** Objective of this study is to assess the Effect of Conventional Anti Tubercular Drugs and Azathioprine on Nontuberculous Mycobacteria in Patients of Crohn's Disease. **Materials and Methods:** This Study was carried out in the department of Gastroenterology, BSMMU, Bangladesh. Period of study was from January 2016 to September 20017. 34 diagnosed case of Crohn's disease was included in this study. Biopsy for PCR to detect NTM was collected from ulcer in CD patients**.** ITS primer was used to detect NTM. **Results:** Total 34 Crohn's diseases (CD) patient were included in this study. Among them 15 had history of conventional anti tb drugs intake before diagnosis of CD. In this anti TB medication group only 39.3 % were positive for NTM (OR 0.32 with P value0.219). Among the CD patients 14 patients were taking azathioprine and this azathioprine group had only 35.7% NTM positive (OR 0.28 and P value 0.173). It appears none of these were significant. **Conclusion:** Use of anti TB or the azathioprine has no significant effect on the presence of NTM but the whether the bacteria is present during the Anti Tubercular drug therapy or not yet to be find out and the other immunomodulator can affect the bacteria in which manner need to be discover.

Key words: Crohn's disease, Nontuberculous mycobacteria, Azathioprine

Introduction

Inflammatory bowel diseases (IBD) are chronic inflammatory disorders of the gastrointestinal tract marked by episodes of relapse and remission. There are two identified subtypes of the disease, ulcerative colitis (UC) and Crohn's Disease (CD), which differ in patterns of involvement which differ in patterns of involvement. However the exact etiology has still to be found hence come various hypothesis including one involving the gut microbiota

The main bacterial populations in the human intestinal microbiota (>90%) is part of two phyla: the Firmicutes and the Bacteroidetes; the remainders belong to rarer phyla such as Proteobacteria (containing genuses such as Escherichia and Helicobacter) and Actinobacteria as well as viruses, protists, and fungi[1-4]. The composition of the fecal microbiota may temporally vary following exposure to different types of foods, medications, or physical environments, and also from changes in transit time, as microbial composition in the lumen varies from caecum to rectum[1].

Enteric micro flora can stimulate immune responses either by functioning as adjuvant or antigens. As adjuvant they activate innate immune responses, including dendritic cells and other APCs, and as antigens they stimulate the clonal expansion of T cells that selectively recognize the antigen through their

T-cell receptor[5]. Among bacteria Mycobacterium avium subsp paratuberculosis (MAP) has shown high prevalence[6]. It was in 1895 when Johne and Frothingham first identified MAP as the causative agent of chronic inflammation in the gut of a cow [7]. One of the first descriptions of what later became known as "Crohn's disease" [8] was made by Dalziel in 1913[9]. He noted similarities between his cases of "chronic interstitial inflamation" in humans, and Johne's disease in cattle. This johne's disease is caused by Mycobacterium avium subsp paratuberculosis (MAP). He therefore suggested that MAP may have been the cause of the disease which he described. This hypothesis has since been tested extensively by numerous investigators, and evidence has accumulated both in favor and against the theory. MAP has been cultured from CD tissues[10] and peripheral blood of CD patients[11]. The idea that MAP may contribute to some cases of CD is not new. Previous attempts (1982–94) to test this theory involved the treatment of CD patients with anti-tubercular drugs assumed to be efficacious against MAP[12]. Failure to observe significant clinical improvement dampened enthusiasm for the mycobacterial aetiology theory.

Only later was it recognized that MAP is resistant to most first-line tuberculosis (TB) drugs[13]. More recent clinical trials have treated CD patients with combinations of macrolides, rifabutin and clofazimine based on limited MAP antibiotic susceptibility data and on the assumption that MAP should be similar in susceptibility to other members of the M. avium complex (MAC).[14-20] Clinical outcomes of these studies were generally encouraging, but far from conclusive.[21,21]

Objective

To assess the effect of Anti tubercular therapy and azathioprine on nontuberculous Mycobacteria

Materials and Methods

Patient's of CD diagnosed on the basis of consistent clinical features, laboratory investigations like elevated CRP, endoscopic appearance, imaging demonstrating segmental narrowing and gut wall thickening and histological exclusion of caseating granuloma in the biopsy tissue were included in the study Patients who was diagnosed as Crohn's disease at BSMMU during the study period taking Azathioprine 100mg per day or has completed conventional Anti Tubercular therapy with 4FDC for 2 months and 2FDC for 10 months before diagnosis of Crohn's disease having active lesion on colonoscopy; enrolled as cases. Patients who was diagnosed as Crohn's disease at BSMMU during the study period not taking Azathioprine or no history of Anti Tubercular therapy before diagnosis of Crohn's diseae having active lesion on colonoscopy considered as control.

Two bites of biopsy from the lesion in terminal ileum or in colon taken and studied for NTM by PCR. We have used ITS primer for NTM in PCR. We extracted the DNA from tissue for PCR using QIAamp DNA mini kit.

Operational Definitions

Diagnostic criteria of Crohn's disease

Diagnosis of CD was based on morphological (radiological, endoscopic or surgical findings) and pathological criteria.(1) morphological:

(1) discontinuous/ segmental and asymmetrical mucosal involvement, (b) deep mucosal longitudinal fissures/ ulcers, (c) transmural inflammation, (d) rigid and strictured intestinal wall, (e) presence of entero-cutaneous/enteroenteric fistula and/or chronic perianal disease; (2) pathological: (a) normal mucus content in the goblet cells of the inflamed region, (b) lymphocyte aggregation in the mucosa and submucosa, (c) non- caseating granuloma, (d) longitudinal ulcers /fissures, (e) transmural inflammation or inflammation beyond mucosa. For definite diagnosis of CD, the following criteria were used: presence of at least 3 different criteria or presence of non- caseating granuloma on histology with at least 1 other criterion; exclusion of TB (by histology, microbiological and PCR studies) and complete resolution of symptoms and morphological (endoscopic and histological/ microbiological) features after 1-year treatment of corticosteroid and 5-ASA preparations (with or without surgery)[23] .

Results

This observational study was conducted in the department of Gastroenterology, BSMMU where 34 patient of Crohn's Disease (CD) were included in this study.

Table-I: Demographic characteristics of the study population (n=34)

Demographic characteristics	Crohn's Disease patints (n=34)	
	n	%
Age (years)		
≤20	3	8.8
21-30	14	41.2
31-40	10	29.4
41-50	2	5.9
> 50	5	14.7
Mean±SD	32.9±10.9	
Range (min-max)	18-55	
Sex		
Male	24	70.6
Female	10	29.4

Almost one third (29.4%) patients belonged to age 31-40 years. The mean age was found 32.9±10.9 years. Twenty four (70.6%) patients were male. (Table-I)

Figure 1: Pie chart shows history of anti TB drug before diagnosis of Crohn's disease

Fifteen (44.1%) patients were found to have history of anti TB therapy before diagnosis of Crohn's disease. (figure-1)

Table II: Interval Between completion of anti TB therapy and taking biopsy for NTM PCR in case group (n=34)

	Mean SD (In months)	Min-Max (In months)
Interval Between completion of anti TB therapy and taking biopsy for NTM PCR in case group (months)	8.5±7.4	1-24

In case group, mean duration of completion of anti TB therapy before taking biopsy for NMTB PCR was found 8.5±7.4 months with range from 1 to 24 months. ((Table-II)

Table III: NTM detection rate in case with history of anti TB before diagnosis of Crohn disease (n=34)

History of anti TB before diagnosis of Crohn's disease	NTM Detected				OR (95%CI)	P value
	Yes (n=28)		No (n=6)			
	n	%	n	%		
Yes	11	39.3	4	66.7	0.32 (0.03-2.65)	0.219^{ns}
No	17	60.7	2	33.3		

ns= not significant

P value reached from chi square test

Eleven (39.3%) patients were found to have history of anti TB drug therapy before diagnosis of Crohn disease in NMTB detected group and 4(66.7%) in without NTM detected group. The difference was not statistically significant (p>0.05) between two groups. (Table-III)

Table IV: Azathioprine intake before taking biopsy for NTM PCR (n=34)

Azathioprine intake before taking biopsy	Number of patients	Percentage
No	20	58.8
Yes	14	41.2
Duration of azathioprine Intake before taking biopsy for NTM PCR (months)	2.6±2.1	
Range (min-max)	1-9	

Fourteen 14(41.2%) patients were taking azathioprine before taking biopsy and their duration of azathioprine intake before taking biopsy for NTM PCR was 2.6±2.1 month with range from 1-9 months. (Table-IV)

Table-V: Distribution of NTM detected in azathioprine taking patients Crohn's disease patients (n=34)

Azathioprine taking patients	NTM Detected				OR (95%CI)	P value	
	Yes (n=28)		No (n=6)				
	n	%	n	%			
Yes	10	35.7	4	66.7	0.28 (0.03 | - 2.29)	0.173	ns
No	18	64.3	2	33.3				

ns= not significant
P value reached from chi square test

Ten (35.7%) patients were found to taken azathioprine in whom NTM was detected group and 4(66.7%) in without NTM detected group. The difference was not statistically significant (p>0.05) between two groups. (Table-V)

Disscusion

This observational study was conducted in the department of Gastroenterology, BSMMU during the periode of April 2016 to August 2017. The objective of the study was to assess the association of antitubercular therapy and azathioprine with non tubercular mycobacteria (NTM) in patients with Crohn's disease (CD). We included 34 patients of Crohn's disease (CD) diagnosed on the basis of slandered criteria.

In the present study the mean age of the CD patients was 32.9±10.9 years and mean age of the Control was 38.2±12.1 years which are almost similar to the study of Clarkston wk et al. 199824.

The patient treated with Anti TB chemotherapy shows there is no effect of anti TB chemotherapy on non tubercular mycobacteria. Out of 15 patient of CD who got anti TB therapy 11 patients were found to have NTM. With OddS ratio 0.32 (95 % CI 0.03-2.65) and non significant P value (0.219) . The mean duration of end of treatment with anti TB and PCR was 8.5± 7.4 months (with range 1-24 months)

Most of the NTM except M.kansasii are inherently resistant or partially susceptible to the standard anti-tubercular drug25. Though its difficult to say whether at the end of therapy there was any NTM or not.

The major limitations for effective therapy were the absence of antimicrobial agents with low toxicity and good in vivo activity against the organism. Most first-line anti-TB drugs have 10 to 100 times less in vitro activity against MAC isolates than against M. tuberculosis 26. Relapses after medical therapy with anti-TB treatment regimens were common, and the best outcomes were frequently in those patients who underwent resectional surgery27 . Out of 14 patients of CD who got Azathioprine treatment 10 was positive for NTM and 4 was negative for NTM with OddS ratio 0.28 (95% CI 0.03-2.29) and non significant P value (0.173). Mean duration of azathioprine intake was 2.6 ± 2.1 months (Range 1-9 months)

It appears that it has no significant effect on NTM but some studies shows it has inhibitory effect. In a vitro study suggests that 6-mercaptopurine may be synergistic with macrolides and rifamycin derivatives against MAP. The activity of clarithromycin against NTM (specially MAP) seems to be enhanced by rifampicin 28.

Among 34 patient of CD 11 had mild disease among them 9 were positive for NTM (OR 0.95 and P value 0.65). Twenty patient had moderate disease with 17 positive for NTM (OR 1.55 and P value 0.482) and 3 had severe disease but 2 were positive for NTM (OR 0.38 and 0.453) . And It appears that None were significant.

Conclusion

It appears that the presence or absence of non tubercular mycobacteria is not affected by the use of anti tubercular medication or the azathioprine. Though this study has limitation to say definitely now but we can study further with more sample size and measure the bacteria at the end of drug as well as during taking of the medication.

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