RHEUMATOLOGY ADVANCES IN PRACTICE

Rheumatology Advances in Practice 2018;0:1–11
doi:10.1093/rap/rky016

Original article

The colon and terminal ileum in patients with ankylosing spondylitis and controls in Bangladesh: a macroscopic and microscopic study

Nira Ferdous[1], Md. Nazrul Islam[1,2], Shamsuddin Mohammed Ishaque[3], Shabnam Akhter[4], Mohammed Kamal[4] and Johannes J. Rasker[5]

Abstract

Objective. Little is known about gut lesions in AS patients in a developing country, such as Bangladesh.

Methods. Full colonoscopy, including the terminal ileum, was performed in 60 AS patients and 20 controls, without diarrhoea, to study macroscopic and microscopic lesions.

Results. In the colon, in 60 AS patients 17 macroscopic lesions were found, of which 11 were in the rectum; only one lesion was found in 20 controls. The prevalence of microscopic lesions in the ascending colon, sigmoid colon and rectum was 51, 44 and 50 in patients, respectively, and 13, 9 and 8 in controls. In the terminal ileum, macroscopic and microscopic lesions were seen in 21/56 and 43/56 AS patients, respectively, and in 1/20 and 9/20 controls. In the AS group, macroscopic (38.5 *vs* 5%, $P < 0.01$) and microscopic (76.8 *vs* 45%, $P = 0.009$) lesions were more frequent than in controls; no IBD was diagnosed. Findings were comparable in the axial AS group ($n = 25$) and the mainly peripheral group ($n = 35$). In AS patients, marked eosinophilic infiltration was observed in the ascending colon and sigmoid colon but not in the rectum, and this infiltration was more than in controls. The colonic mucosa in controls was otherwise comparable with western studies. Anaemia was seen in 18/60 cases. No association was found between anaemia or HLA-B27 status and gut lesions.

Conclusion. There was an equal percentage of microscopic lesions in the whole gut in AS cases and healthy controls. Previous helminth invasions might have played a role. Lesions differ significantly between AS and controls only in the ileum; therefore, the ileal lesions might be more disease related than the colonic ones.

Key words: microscopic, macroscopic, colonoscopy, large gut, terminal ileum, ankylosing spondylitis, healthy controls

ORIGINAL ARTICLE

Introduction

AS is the prototype of a group of disorders called spondyloarthritides [1], which show familial aggregation, arthritis of sacroiliac and peripheral joints with enthesopathy, a high association with HLA-B27 and absence of RF [2, 3]. Associations between inflammatory gut lesions caused by *Salmonella*, *Shigella*, *Yersina* and reactive arthritis are well established [4–6]. The prevalence of spondyloarthritides, including AS, in Crohn's disease and ulcerative colitis is high [7, 8]. The prevalence rates have been described as 10–15% for sacroiliitis and 7–12% for spondylitis, although the figures are probably higher [7].

Some 10% of patients with IBD attending a gastroenterology unit fulfilled the criteria for AS, and an additional 18% of patients had asymptomatic sacroiliitis detected by conventional X-ray [7]. In contrast, subclinical inflammatory gut lesions were also reported in patients with spondyloarthritides. In Belgian and Scandinavian studies, macroscopic and microscopic changes have been identified in up to 50% of patients with

[1]Modern One Stop Arthritis Care and Research Center® (MOAC&RC®), Dhaka, Bangladesh, [2]Department of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), [3]Department of Gastroenterology, BSMMU, [4]Department of Pathology, BSMMU and [5]Faculty of Behavioural, Management & Social sciences, Psychology, Health and Technology, University of Twente, Enschede, The Netherlands

Submitted 25 September 2017; revised version accepted 24 April 2018

Correspondence to: Md. Nazrul Islam, level 16, Block D, Department of Rheumatology, BSMMU, Shahbag, Dhaka 1000, Bangladesh.
E-mail: islam1nazrul@gmail.com

spondyloarthritides [9–11]. One study in Bangladesh looked at the colon in patients with AS and in normal subjects, with short colonoscopy; the frequency of inflammatory lesions was 50% in AS patients. An additional finding in that study was an eosinophilic infiltration in 85.7% of AS patients and in 80% of controls, probably because of the prevalence of helminthic infections in this part of the world [12].

Anaemia is a common finding in patients with inflammatory arthritis [13]. The exact prevalence of anaemia in patients with AS is unknown, but it is very common in AS [14, 15].

In this study, full colonoscopy was performed in AS patients and in controls to answer the following questions.

(i) What is the prevalence of gut lesions when using full colonoscopy? (ii) Does the frequency of gut lesions differ between mainly axial and mainly peripheral AS patients? (iii) Is anaemia in AS patients related to gut lesions? (iv) Is the eosinophilic infiltration in the rectum lower after deworming than in our earlier study?

Methods

This observational study was carried out in the Departments of Rheumatology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Modern One Stop Arthritis Care and Research Center® (MOAC&RC®), Department of Gastroenterology and Department of Pathology (BSMMU), Dhaka, Bangladesh from July 2011 to June 2012.

Following the purposive sampling method, consecutive AS patients were enrolled who fulfilled the revised New York criteria (1984), were not taking DMARDs or corticosteroids (CSs), and had no history of diarrhoea and dysentery in last 1 month and no contraindication for colonoscopy. A total of 60 consecutive AS patients and 20 age- and sex-matched controls were included in the study after informed consent.

The 20 controls consisted of 10 clinically healthy volunteers and 10 persons visiting the gastroenterologist with upper gastrointestinal problems, with a plan to evaluate the upper gut by endoscopy and having no lower gut complaints and not using NSAIDs in last month, who were invited to participate in the study as controls.

In all AS patients, X-rays were made of the lumbosacral spine including both SI joints (anteroposterior and lateral views), and X-rays of SI joints with oblique view were done to assess the radiological status of the disease. At baseline, complete blood count, CRP and HLA-B27 were done. In this study, subjects having haemoglobin levels of $\leq$10 g/dl were considered to be anaemic [16].

All AS patients were assessed using items of the Assessment of SpondyloArthritis international Society (ASAS) core set [17]: BASDAI for disease activity and visual analog scale (VAS) pain score in the past week. The English version was interviewer administered. Enthesitis was assessed using the Maastricht score [18]. The AS patients without peripheral arthritis were classified as the axial group, and those with tender or swollen peripheral joints on examination were classified as the peripheral group.

After assessment, all AS patients and controls were de-wormed with mebendazole 100 mg every 12 hourly for 3 days and after 10 days by albendazole 400 mg for total eradication. This de-worming was done in patients and controls, because in our previous study with short colonoscopy a high eosinophil count was found [12], and in Bangladesh helminthes are the most common cause of gut eosinophilia.

After 14 days, subjects were prepared for full colonoscopy. The colon was prepared with 20% mannitol; no premedication was used. Macroscopic lesions were graded as follows: grade 0, normal; grade 1, redness and oedema of mucosa; grade 2, small ulceration of mucosa; and grade 3, mucosal oedema, ulcerations and haemorrhage [12]. Biopsy specimens were taken from the colon and rectum of all subjects. Two specimens were taken from all subjects irrespective of the presence or absence of macroscopically evident lesions, and another two specimens were taken from macroscopically evident lesions, if available. One biopsy was taken from the proximal colon and two from the distal colon. The biopsy sites of each subject were recorded. Histological features were graded from 0 to 3 in the specimens following the Cuvelier *et al.* [19] grading. The higher grading represents more chronic inflammation, as follows: grade 0, normal; grade 1, lymphoid hyperplasia, increase in chronic inflammatory cell content in the lamina propria, with or without eosinophilia, but no evidence of cryptitis or epithelial abnormalities; grade 2, diffuse increase of inflammatory cells in the lamina propria with partial villous flattening, crypt distortion and reactive hyperplasia of crypt cell epithelium, infiltration of crypt cell epithelium with neutrophils, crypt abscesses); and grade 3, aphthous ulcerations with or without epithelioid granulomas. Stage I lesions were considered to be a part of the spectrum of normal terminal ileal histology. All measurements and observations were done at $\times$400 magnification ($\times$10 ocular and $\times$40 objective lens). For eosinophilic infiltration, the cell count was done by calculating the mean from three high-power fields (HPFs) of each biopsy specimen [20].

Data analysis

Data were entered and calculated using SPSS 17.1 version for Windows. Frequencies of macroscopic and microscopic lesions were calculated as percentages. Associations between different parameters were analysed by Chi-square test (χ^2) test and Fisher's exact test, and P value $<$ 0.05 implies statistical significance.

Ethics

The study was approved by the Ethics Committee of Bangabandhu Sheikh Mujib Medical University Shahbagh, Dhaka, Bangladesh. The study was

performed following the principles of the Declaration of Helsinki, and informed consent was obtained from all participants before enrolment.

Results

A total of 65 subjects in the AS group and 35 subjects in the control group were invited to participate in this study. Four females and one male in AS group, nine females and six males in control group refused to participate, leaving 60 AS patients and 20 controls in the study.

In this study, the male-to-female ratio was 3:1 in the AS group and 7:3 in controls. The mean (S.D.) age of the AS group was 30.4 (9.6) years and of controls 33.0 (12.0) years. Of the AS patients, 44 (73.3%) had a family history of low back pain, enthesitis was seen in 51.67% and only one patient had uveitis. The axial group consisted of 25 cases (male 20, female 5) and the predominantly peripheral group of 35 cases (male 25, female 10).

HLAB-27 was tested in 37 cases; of these, 20 (59%) were HLA-B27^{+} (14 male, female 6) and 17 were HLAB-27^{-} (male 13, female 4).

The disease duration of the AS patients ($n = 60$) was $<$12 months in 14 (23.3%), 12–24 months in 11 (18.3%), 24–48 months in 11 (18.3%) and $>$48 months in 24 (40%). Further demographics are summarized in Table 1.

Treatment

Among 60 patients, 43 had no history of use of DMARDs; in the past, 15 had been on SSZ for some time, 1 had taken MTX and 1 HCQ; in total, 9 patients had been on CSs for some time in the past. None had ever taken a biological. Among mainly peripheral AS patients, 11/35 had taken SSZ in the past and 1 HCQ and 9/35 took CSs for some time previously. Among the mainly axial AS cases, 4/25 had taken SSZ and 1/25 had taken MTX previously. All had stopped these treatments $\geq$3 months before the start of the study. Other baseline characteristics of the study subjects are shown in Table 2.

Baseline clinical and laboratory characteristics

The mean score of the VAS pain score was 45.2, the BASDAI score 3.8, haemoglobin 13.0 g/dl, ESR 31.5 mm in the first hour, mean corpuscular volume 28 fl, mean corpuscular haemoglobin 32.2 pg, blood eosinophil count 3.7% and CRP 22.6 mg/dl. Details are shown in Table 3.

Colonoscopic findings

Colonoscopy was performed in the AS group ($n = 60$) and controls ($n = 20$). In four patients, colonoscopic evaluation of the terminal ileum was not performed. The investigator failed to pass the probe through the ileocoecal junction.

In the ascending colon and sigmoid colon, macroscopic lesions were unremarkable in both groups, but microscopic lesions were more often observed in the AS group than in the control group, although the difference was not statistically significant (Table 4).

In the rectum, both macroscopic and microscopic lesions were more frequently seen in the AS group than in the control group, but the difference was again not statistically significant (Table 4). In the terminal ileum, in AS patients ($n = 56$) macroscopic and microscopic lesions were found in 21/56 and 43/56, respectively, and in controls in 1/20 and 9/20, respectively. In the AS group, both macroscopic (38.5 *vs* 5%, $P < 0.01$) and microscopic (76.8 *vs* 45%, $P = 0.009$) lesions were significantly more frequent than in controls (Table 4).

Table 1 Demographics of AS patients ($n = 60$) and controls ($n = 20$)

Characters	Cases ($n = 60$) *n* (%)	Controls ($n = 20$) *n* (%)
Sex		
Male	45 (75)	14 (70)
Female	15 (25)	6 (30)
Marital status		
Married	37 (61.70)	17 (85)
Unmarried	23 (38.30)	3 (15)
Occupation		
Housewife	12 (20)	6 (30)
Business	6 (10)	5 (25)
Service	14 (23.30)	5 (25)
Others	28 (46.70)	4 (20)

Table 2 Other baseline characteristics of the AS patients ($n = 60$)

Characteristics		Total *n* (%)
Previous use of DMARDs		
Yes (%)	17 (28.40)	60 (100)
No (%)	43 (71.60)	
Previous use of CS		
Yes (%)	9 (15)	60 (100)
No (%)	51 (85)	
Irritable bowel syndrome		
Yes (%)	14 (23.30)	60 (100)
No (%)	36 (76.70)	
HLA-B27		
Positive (%)	20 (33.30)	60 (100)
Negative (%)	17 (28.30)	
Not done (%)	23 (38.40)	
Anaemia		
Yes (%)	18 (30)	60 (100)
No (%)	42 (70)	
Pattern of joint involvement		
Axial (%)	25 (41.67)	60 (100)
Predominantly peripheral (%)	35 (58.33)	

AS axial and peripheral subgroups

The ascending colon, sigmoid colon and rectum were studied in the axial group ($n = 25$) and the predominantly peripheral group ($n = 35$). In the ascending colon, sigmoid colon and rectum, macroscopic lesions were unremarkable. Microscopic lesions were seen in both groups similarly (mainly grades 1 and 2 in ~70–80%) in the ascending colon and sigmoid colon, but this difference was not statistically different (Table 5).

Table 3 Baseline clinical and laboratory characteristics of AS patients ($n = 60$)

Clinical characteristics	Range	Mean (s.d.)
Pain last week, visual analog scale (0–100)	0–80	45.20 (17.50)
BASDAI score	0–8.70	3.80 (1.80)
Haemoglobin (g/dl)	9–16.8	13.00 (1.80)
ESR	2–97	31.50 (24.20)
Mean corpuscular volume (fl)	58.7–95.8	86.20 (7.90)
Mean corpuscular haemoglobin (pg)	20.2–31.6	28.00 (2.60)
Mean corpuscular haemoglobin concentration (mg/dl)	24.2–35.8	32.20 (1.70)
Blood eosinophil count (%)	0–12	3.70 (2.70)
CRP (mg/dl)	0.9–151	22.60 (30.00)

In the terminal ileum, the frequency of macroscopic lesions was similar in the axial and peripheral groups. The microscopic lesions were slightly more frequent in the mainly peripheral group (62.5%) than the axial group (88.5%), $P = 0.05$. No IBD was observed (Table 5).

Anaemia present ($n = 18$) and absent ($n = 42$) in the AS group

The frequency of macroscopic lesions did not differ between patients with (59%) and without (68%) anaemia (Table 6). In the terminal ileum, the frequency of macroscopic lesions did not differ between patients with (59%) and without (68%) anaemia. The frequency of microscopic lesions was comparable in the anaemia present group (88%) and the anaemia absent group (72%) (Table 6).

Eosinophil infiltration in different sites of the large gut

In our earlier study, eosinophilic infiltration in the rectum was found in 85.7% of AS patients and in 80% of the controls [12].

In AS patients even more than in controls, a marked eosinophil infiltration was observed in the ileum (8.5 *vs* 6.5, respectively), the ascending colon (median 12.5 *vs* 7.5, respectively) and the sigmoid colon (median 7.0 *vs* 5.5, respectively) but not in the rectum (median 5.7 *vs* 3.0, respectively). The differences with controls were not

Table 4 Grading of macroscopic and microscopic lesions in the large gut in AS patients and controls

	Macroscopic finding			Microscopic finding		
	Patient ($n = 60$)	Control ($n = 20$)	*P*-value	Patient ($n = 60$)	Control ($n = 20$)	*P*-value
Ascending colon	*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Grade 0	56 (93.30)	20 (100)	0.31	9 (15)	7 (35)	0.1
Grade 1	0 (0)	0 (0)	–	40 (66.70)	13 (65)	0.55
Grade 2	4 (6.70)	0 (0)	0.57*	9 (15)	0 (0)	0.1
Grade 3	0 (0)	0 (0)	–	2 (3.30)	0 (0)	0.50*
Sigmoid colon						
Grade 0	58 (96.70)	20 (100)	1	15 (25)	11 (55)	0.03
Grade 1	1 (1.70)	0 (0)	1*	38 (63.30)	9 (45)	0.19
Grade 2	1 (1.70)	0 (0)	1*	6 (10)	0 (0)	0.32
Grade 3	0 (0)	0 (0)	–	1 (1.70)	0 (0)	1.0*
Rectum						
Grade 0	49 (81.70)	19 (95)	0.27	10 (16.70)	12 (60)	0.001
Grade 1	10 (16.70)	1 (5)	0.27	40 (66.70)	8 (40)	0.63
Grade 2	1 (1.70)	0 (0)	1.0*	9 (15)	0 (0)	0.43
Grade 3	0 (0)	0 (0)	–	1 (1.70)	0 (0)	0.56*
Terminal ileum ($n = 56$)						
Grade 0	35 (62.5)	19 (95.0)	0.01	13 (23.2)	11 (55)	0.009
Grade 1	5 (8.9)	0	0.31*	33 (58.9)	9 (45)	0.28
Grade 2	16 (28.6)	1 (5.0)	0.03	6 (10.7)	0	0.33
Grade 3	0	0	–	4 (7.1)	0	0.56*

*Fisher's exact test; χ^2 test.

significant (Table 7). The figures found for the rectum are much lower than in our previous study.

There was a wide range of eosinophil counts in different sites of the gut in both AS patients and controls (0–40/HPF *vs* 1–22/HPF in the ascending colon, 0–24/HPF *vs* 2–12/HPF in the sigmoid colon and 0–13/HPF *vs* 0–11/HPF in the rectum, respectively); the mean eosinophil count was higher in AS patients with a disease duration of 12–24 months. In some of these cases, a 'massive' or 'heavy' eosinophilic infiltration was found, whereas others showed 'patchy infiltration'.

The mean BASDAI score, VAS pain score and eosinophil count showed no correlation with macroscopic or microscopic grading; only in the ascending colon a non-significant tendency to increase along with microscopic grading was observed. For details, see Table 8.

HLA-B27 was done in 37 AS patients; 20 were positive and 17 negative for the antigen.

Macroscopic or microscopic lesions did not differ in HLA-B27$^+$ and HLA-B27$^-$ cases in the ascending colon and sigmoid colon or rectum (Table 9).

Discussion

In this observational study of patients with AS and controls, the entire colon was evaluated for macroscopic and microscopic lesions and eosinophil infiltration in the lamina propria. To our knowledge, this is the first study of its kind in South-East Asia.

In a developing country, such as Bangladesh, the possibility for diagnosing AS following western criteria is restricted. Most patients cannot afford MRI studies, and testing for HLA-B27 is restricted owing to the cost. For that reason, we could not perform this test in all patients. Infestation of the gut with parasites is very common; as shown in our earlier study, in the rectum an eosinophilic infiltration in 85.7% of AS patients and of 80% in controls was found [12]; for that reason, we had to de-worm the patients before this study. For religious reasons, four women refused colonoscopy because this was done by a male doctor. This is, in general, not a problem in western countries. In Bangladesh, 57% of the adult population are illiterate, so obtaining informed consent has to be done orally in most cases, but this has never been a problem in any of our research projects. The same holds true for questionnaires, such the BASDAI and pain VAS, which have to be clarified and filled in by assistants.

The male-to-female ratio in the present study was 3:1, comparable with other studies [21]. In our series, 25 (41.7%) had only axial involvement and 35 (58.3%) had both axial and peripheral (mixed) involvement. These findings are comparable with those of previous Belgian studies [22, 23].

Table 5 Macroscopic and microscopic lesions in the large gut in the axial group ($n = 35$) and in the predominantly peripheral group of AS patients ($n = 25$)

	Macroscopic finding			Microscopic finding		
	Axial group ($n = 25$)	predominantly peripheral ($n = 35$)	*P*-value	Axial group ($n = 25$)	predominantly peripheral ($n = 35$)	*P*-value
Ascending colon	*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Grade 0	24 (96)	32 (91.40)	0.63	5 (20)	4 (11.40)	0.47
Grade 1	0 (0)	0 (0)	–	16 (64)	24 (68.60)	0.78
Grade 2	1 (4)	3 (8.60)	0.63*	4 (16)	5 (14.30)	1
Grade 3	0 (0)	0 (0)	–	0 (0)	2 (5.70)	0.50*
Sigmoid colon						
Grade 0	24 (96)	34 (97.10)	1	3 (12)	12 (34.30)	0.73
Grade 1	0 (0)	1 (2.90)	1.00*	18 (72)	20 (57.10)	0.28
Grade 2	1 (4)	0 (0)	0.41*	3 (12)	3 (8.60)	0.68*
Grade 3	0 (0)	0 (0)	–	1 (4)	0 (0)	0.41*
Rectum						
Grade 0	21 (84)	28 (80)	0.74	2 (8)	8 (22.90)	0.17
Grade 1	3 (12)	7 (20)	0.49	17 (68)	23 (65.70)	1
Grade 2	1 (4)	0 (0)	0.41*	5 (20)	4 (11.40)	0.47
Grade 3	0 (0)	0 (0)	–	1 (4)	0 (0)	0.41*
Terminal ileum ($n = 56$)						
Grade 0	15 (62.5)	20 (62.5)	1	9 (37.5)	4 (12.5)	0.05
Grade 1	0 (0)	5 (15.6)	0.06	11 (45.8)	22 (68.8)	0.1
Grade 2	9 (37.7)	7 (21.9)	0.24	2 (8.3)	4 (12.5)	0.69*
Grade 3	0 (0)	0 (0)	–	2 (8.3)	2 (6.3)	1*

*Fisher's exact test; χ^2 test.

Table 6 In the large gut, macroscopic and microscopic lesions with anaemia in patients and controls

	Macroscopic finding			Microscopic finding		
	Anaemia present ($n = 18$)	Anaemia absent ($n = 42$)	*P*-value	Anaemia present ($n = 18$)	Anaemia absent ($n = 42$)	*P*-value
Ascending colon	*n* (%)	*n* (%)		*n* (%)	*n* (%)	
Grade 0	16 (88.90)	40 (95.20)	0.34	5 (27.80)	4 (9.50)	0.08
Grade 1	0 (0)	0 (0)	–	9 (50)	31 (73.80)	0.07
Grade 2	2 (11.10)	2 (4.80)	0.58*	3 (16.70)	6 (14.30)	0.54
Grade 3	0 (0)	0 (0)	–	1 (5.60)	1 (2.40)	0.51*
Sigmoid colon						
Grade 0	18 (100)	40 (95.20)	0.35	6 (33.30)	9 (21.40)	0.32
Grade 1	0 (0)	1 (2.40)	0.70*	10 (55.60)	28 (66.70)	0.41
Grade 2	0 (0)	1 (2.40)	0.70*	2 (11.10)	4 (9.50)	0.59*
Grade 3	0 (0)	0 (0)	–	0 (0)	1 (2.40)	0.70*
Rectum						
Grade 0	15 (83.30)	34 (81.00)	0.82	5 (27.80)	5 (11.90)	0.13
Grade 1	3 (16.70)	7 (16.70)	0.66	10 (55.60)	30 (71.40)	0.23
Grade 2	0 (0)	1 (2.40)	0.70*	2 (11.10)	7 (16.70)	0.45
Grade 3	0 (0)	0 (0)	–	1 (5.60)	0	0.30*
Terminal ileum ($n = 56$)						
Grade 0	10 (58.8)	25 (68.4)	0.708	2 (11.8)	11 (28.2)	0.16
Grade 1	2 (11.8)	3 (7.7)	0.634*	12 (70.6)	21 (53.8)	0.376
Grade 2	5 (29.4)	11 (28.2)	0.927	2 (11.8)	4 (35)	0.598*
Grade 3	0 (0)	0 (0)	–	1 (5.9)	3 (7.7)	0.647*

*Fisher's exact test; χ^2 test.

Table 7 In study groups, eosinophil infiltration in different sites of large gut in AS patients and controls

	Patient (count per high-power field) ($n = 60$)		Control ($n = 20$)		*P*-value
	Mean (S.D.)	Median	Mean (S.D.)	Median	
Ascending colon	18.5 (21.5)	12.50	11.7 (9.9)	7.50	0.17
Sigmoid colon	11.4 (12.5)	7.00	6.6 (4.6)	5.50	0.09
Rectum	5.7 (6.8)	4.00	4.8 (5.9)	3.00	0.61
Terminal ileum	15.6 (19.6)	8.5	8.8 (8)	6.5	0.14

*Two mean test.

In our series, the frequency of enthesopathy was 51.7% (31/60), comparable with some European studies [10, 24] and slightly lower than in other series [9, 25]. Uveitis is not a frequent presentation in AS; only one patient in our series and two in that of Mielants *et al*. [22], had uveitis.

HLAB-27 was tested in 37 of the 60 cases; of these, 20/37 (59%) were HLA-B27$^+$. This percentage is lower than in most western series. The incidence/prevalence of HLA-B27 in the general population or in SpA patients has not been studied in Bangladesh, to our knowledge. In one study regarding 71 patients with enthesitis, there were 49 AS cases and all were HLA-B27$^+$, but in that study only patients with HLA-B27 were included so that was not a representative series [26].

In our study, in symptom-free controls the colonic mucosa in the lower gut did not differ from that found in western [19] studies, except for the presence of frequent mild to moderate eosinophilic infiltration in the lamina propria, which was found both in patients and in controls.

Prior to our study, several groups investigated the relationship between spondyloarthropathies and inflammatory lesions of the gut. There are differences between these investigations regarding sample size, study design, the way they evaluated gut lesions and the method of grading of the lesions. Almost all previous studies included a mixture of all kinds of seronegative spondyloarthropathies as study subjects (i.e. reactive arthritis, AS, undifferentiated spondyloarthropathies).

Table 8 Comparison of AS disease activity (BASDAI score and VAS) and eosinophil count in different macroscopic and microscopic lesions in large gut

Microscopic grading	Number	BASDAI score	VAS score	Eosinophil count/HPF
Ascending colon ($n = 60$)	*n*	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Grade 0	9	3.82 (1.85)	42.2 (17.8)	13.4 (8.4)
Grade 1	40	3.59 (1.76)	44.0 (17.8)	20.9 (24.0)
Grade 2	9	4.02 (1.52)	48.9 (14.5)	9.4 (14.6)
Grade 3	2	6.75 (2.75)	65.0 (21.2)	35.5 (24.7)
Sigmoid colon ($n = 60$)				
Grade 0	15	3.32 (1.27)	40.0 (11.9)	16.9 (18.4)
Grade 1	38	4.02 (1.98)	46.1 (19.2)	10.5 (9.8)
Grade 2	6	3.61 (2)	48.3 (16.0)	4.3 (3.7)
Grade 3	01	3.20	70	05
Rectum ($n = 60$)				
Grade 0	10	4.04 (1.93)	47.0 (13.3)	3.5 (2.2)
Grade 1	40	3.83 (1.92)	44.0 (19.7)	5.8 (5.4)
Grade 2	09	3.53 (1.30)	48.9 (11.7)	7.4 (13.5)
Grade 3	01	2.40	40	05

HPF: high-pwer field;VAS: visual analog scale.

Table 9 Frequency of macroscopic and microscopic lesions in the large gut ($n = 60$) in HLA-B27 patient groups

	Macroscopic finding				Microscopic finding			
	HLA-B27$^+$ ($n = 20$)	HLA-B27$^-$ ($n = 17$)	HLA-B27 not done ($n = 23$)	*P*-value	HLA-B27$^+$ ($n = 20$)	HLA-B27$^-$ ($n = 17$)	HLA-B27 not done ($n = 23$)	*P*-value
Ascending colon	*n* (%)	*n* (%)			*n* (%)	*n* (%)		
Grade 0	19 (95)	16 (94.1)	21 (91.3)	0.879	2 (10)	4 (23.5)	3 (13)	0.489
Grade 1	0 (0)	0 (0)	0 (0)	–	13 (65)	11 (64.7)	16 (69.6)	0.932
Grade 2	1 (5)	1 (5.9)	2 (8.7)	0.879	4 (20)	2 (11.8)	3 (13)	0.741
Grade 3	0 (0)	0 (0)	0 (0)	–	1 (5)	0 (0)	1 (4.3)	0.66
Sigmoid colon								
Grade 0	19(95)	17 (100)	22 (95.7)	0.66	5 (25)	1 (5.9)	9 (39.1)	0.056
Grade 1	1 (5)	0 (0)	0 (0)	0.362	14 (70)	13 (76.5)	11 (47.8)	0.133
Grade 2	0 (0)	0 (0)	1 (4.3)	0.441	1 (5)	3 (17.6)	2 (8.7)	0.427
Grade 3	0 (0)	0 (0)	0 (0)	–	0 (0)	0 (0)	1 (4.3)	0.441
Rectum								
Grade 0	14 (70)	14 (82.4)	21 (91.3)	0.197	3 (15)	2 (11.8)	5 (21.7)	0.684
Grade 1	5 (25)	3 (17.6)	2 (8.7)	0.356	14 (70)	12 (70.6)	14 (60.9)	0.754
Grade 2	1 (5)	0 (0)	0 (0)	0.362	3 (15)	2 (11.8)	4 (17.4)	0.886
Grade 3	0 (0)	0 (0)	0 (0)	–	0 (0)	1 (5.9)	0 (0)	0.276
Terminal ileum								
	($n = 18$)	($n = 15$)	($n = 23$)		($n = 18$)	($n = 15$)	($n = 23$)	
Grade 0	9 (50)	11 (73.3)	15 (65.2)	0.364	4 (22.2)	2 (13.3)	7 (30.4)	0.471
Grade 1	2 (11.1)	1 (6.7)	2 (8.7)	0.904	9 (50)	10 (66.7)	4 (60.9)	0.607
Grade 2	7 (38.9)	3 (20)	6 (26.1)	0.461	3 (16.7)	2 (13.3)	1 (4.3)	0.417
Grade 3	0 (0)	0 (0)	0 (0)	–	2 (11.1)	1 (6.7)	1 (4.3)	0.703

Macroscopic lesions

In this series, we had enrolled only AS cases and controls with no history of diarrhoea and dysentery in the last month. In a prospective study, Mielants *et al.* [22] had performed total colonoscopy in 211 patients (75 AS, 32 reactive arthritis and 104 undifferentiated spondyloarthropathies) and 65 controls. Gut lesions were present in 30% (24/75) of the AS patients and in none of the controls. Simenon *et al* [24] found macroscopic gut lesions in 64.9% (37/57 of the AS patients) in a retrospective study involving 96 subjects with AS, reactive arthritis and other spondyloarthropathies; Altomonte *et al.* [27], in their prospective study, included 38

patients with spondyloarthopathy, but none of them fulfilled the criteria of definite AS; macroscopic lesions were found in 65%. In earlier studies, Meuwissen *et al.* [28] and Costello *et al*. [29] studied the gut mucosa by proctosigmoidoscopy and conventional X-rays. The frequencies of macroscopic lesions were lower (5–10%) than those observed in more recent studies using total colonoscopy. Our previous study with short colonoscopy showed macroscopic lesions in 14.28% [12]. In the present series, we observed macroscopic lesions in 25% (15/60), among which were 3 in the ascending colon, 2 in the sigmoid and 11 in the rectum. This frequency was comparable with the findings of Meuwissen *et al.* [28] and Costello *et al*. [29].

Microscopic lesions

Multiple biopsies allow detection of higher numbers of abnormalities than as seen by colonoscopy alone [12]. Accordingly, microscopic lesions were more frequent than macroscopic gut lesions. In our present study, 51 (85%) had microscopic gut lesions in the ascending colon, 44 (73.3%) in the sigmoid and 50 (83.3%) in the rectum, figures comparable with those of Simenon *et al*. [24]. In most studies in AS patients, microscopic lesions were mostly mild, and the frequency varied from 56 to 66.7% [19, 23, 24]. In a small study, only 25% (7/28) had microscopic lesions [30]. All 38 spondyloarthropathy patients in the series of Altomonte *et al*. [27] had microscopic lesions at total colonoscopy. Microscopic lesions are common in the whole colon, both in AS patients and in controls. It cannot be excluded that previous helminth infestation plays a role. The fact that our findings are comparable with other studies does not support that idea.

Axial *vs* peripheral AS

In our study, microscopic lesions were seen in both groups to a similar extent (mainly grades 1 and 2 in ~70–80%) in the ascending colon and sigmoid. Slightly more microscopic lesions were seen in the ileum in the peripheral group ($P = 0.05$). These findings are comparable with those of De Vos *et al*. [23].

IBD symptoms of spondyloarthropathy may precede or coincide with IBD; even articular involvement may be subclinical. De Vlam *et al.* [31] observed asymptomatic sacroiliitis in 18% of IBD patients; they also observed that frequencies of sacroiliitis increased with the duration of IBD. In a prospective study in 521 IBD patients, Protzer *et al*. [32] observed that symptoms of spondyloarthopathy occurred before IBD in 26.8% of all patients and in 14.4% simultaneously with IBD. In our series, none of the gut lesions was diagnostic of IBD on either macroscopic nor microscopic examination.

Anaemia in AS

There are only a few studies exploring anaemia in AS patients. In the *post hoc* analysis of the Ankylosing Spondylitis Study for the Evaluation of Recombinant Infliximab Therapy (ASSERT) study, nearly 20% of patients had anaemia at baseline. The exact prevalence of anaemia in patients with AS is unknown, but 50% of patients with RA have anaemia [33]. There are multiple potential causes of anaemia in patients with AS, including anaemia of chronic disease. Although chronic inflammation may be an important cause of anaemia, the long-term use of NSAIDs, which is rather common in patients with AS, can lead to blood loss in the gastrointestinal tract [34, 35]. In our series, 30% (18/60) had mild anaemia. There was no statistical difference in the non-anaemic and anaemic group regarding the macroscopic and microscopic lesions in the ascending colon, sigmoid and rectum. We did not analyse the type and cause of anaemia in this series owing to financial constraints.

In AS, eosinophilia is an unexplained phenomenon. A detailed study of endoscopic material was carried out by DeBrosse *et al.* [36], who found a gradient of eosinophil density from the ascending colon to the rectum (20/HPF and 8/HPF, respectively) and a wide range (up to 50/HPF), comparable to the findings of Lowichik and Weinberg [37]. The proximal-to-distal gradient of eosinophil distribution has also been described by Gonsalves [38]. In our study, in AS patients, even more than in controls, a marked eosinophil infiltration was also observed in the ascending colon and sigmoid colon but not in the rectum; the difference from controls was not significant. Geographical variation in eosinophil density in the normal colon has also been reported, and there may also be variations in response to seasonal antigenic changes and subclinical infections [39, 40]. The mean eosinophil count was higher in AS patients with 12–24 months disease. In some of these cases, a massive or heavy eosinophilic infiltration was found, whereas others showed patchy infiltration with varying numbers of eosinophils, comparable with other studies [41–44]. Of 34 papers in English reporting eosinophilic colitis since 1959, eosinophil density was quantified in only seven. In these, the density ranged from >20/HPF anywhere in the colon [45] to >120/HPF [46]. Although at present there are no accepted criteria for distinguishing colonic eosinophil density at the upper range of normal, from a pathological increase diagnostic of primary eosinophilic colitis, many authors suggest a minimal eosinophil density for diagnosis of eosinophilic colitis: 6/HPF as suggested by Odze *et al.* [47] and Hwang *et al.* [48], or 30/HPF as suggested by Lee and Kim [49]. In the present series, the large number of eosinophils counted in some AS patients may suggest eosinophilic colitis. But we cannot exclude the possibility that in some cases parasites still played a role as, for example, *Strongyloides* needs different treatment from that which had been prescribed in our series. Further studies are needed to determine whether eosinophilic colitis could be an AS-related disease or rather a separate pathophysiological entity.

Association with HLA-B27, BASDAI and VAS pain

There was no clear association with HLA-B27 status. We found no clear association of BASDAI, and VAS pain

scores with macroscopic and microscopic grading of lesions in all sites, a finding is comparable with other studies [50, 51].

Limitations

The study was conducted in a tertiary health-care centre in Bangladesh and might therefore not be a reflection of the community. In scoring normal rectal histology, we could not use the whole spectrum of scoring features as proposed by Rubio and Kock (1981) [12] owing to adaptation limitations of the department of histopathology. HLA-B27 could not be tested in 23 patients with AS and in controls owing to resource constraints. The number of controls was restricted because it was not easy to find more people to undergo a voluntary colonoscopy.

Strengths

This is a large series of AS patients and age- and sex-matched controls. It is the first study in Bangladesh and one of the first on the Asian mainland in AS patients without clinical diarrhoea; before the study, anthelmintics were applied.

Colonoscopy included the whole colon and terminal ileum. Eosinophil count was done by calculating the mean of three HPFs of each biopsy specimen.

Conclusions

In Bangladeshi AS patients and controls, macroscopic lesions are mainly found in the ileum. Microscopic lesions are common in the whole colon, both in AS patients and in controls. The possibility cannot be excluded that previous helminth infestation plays a role, but the fact that our findings are comparable with earlier European studies does not support that idea. It is unlikely that these microscopic or macroscopic lesions are the cause of anaemia. In none of the AS patients was IBD or Crohn's disease found. Lesions are significantly different between AS cases and controls only in the ileum, so the ileal lesions may be more disease related than the colonic ones.

There was no clear association of HLA-B27 status, BASDAI or VAS pain with macroscopic and microscopic grading of the lesions in the large gut.

The colonic mucosa of symptom-free individuals (controls) is similar to that in western studies, except for a frequently found mild-to-moderate eosinophilic infiltration in both patients and controls.

Acknowledgements

We thank all patients and controls for their willingness to participate in the study. All authors contributed in the conception and design of the work the analysis and interpretation of the data, drafting and critically revising the data and all approve of the final version. All are accountable for all aspects of the work. The selection of patients and controls and clinical aspects was done by N.F. and Md.N.I., the colonoscopies were performed by S.M.I., the pathological studies were performed by S.A. and M.K., and supervision was by Md.N.I. and J.J.R. We acknowledge BSMMU authority and different departments for their kind supports for conducting the study smoothly.

Funding: No funding was received from any source and the expenses met up by corresponding author and co-authors as a social work.

Disclosure statement: The authors have no financial or personal relationships or interests regarding this study.

Data sharing statement: The data collected from the participants were analysed and are shown in the manuscript. No additional unpublished data beyond the article are available. If required, the authors are willing to share data with the reviewers.

References

1 Tak Yan Yu D, McGonagle D, Marzo-Ortega H *et al*. Undifferentiated spondylarthritis and reactive arthritis. In: GS Firestein, RC Budd, ED Harris Jr *et al*., eds. Kelley's textbook of rheumatology, 8th edn. Chapter 71. Philadelphia: Saunders/Elsevier; 2008, 1191–1200.

2 Brown MA, Kennedy LG, Darke C *et al*. The effect of HLA DR genes on susceptibility to and severity of ankylosing spondylitis. Arthritis Rheum 1997;41:460–5.

3 Said-Nahal R, Miceli-Richard C, Barthelot JM *et al*. The familial form of spondyloarthropathy: a clinical study of 115 multiplex families. Groupe Français d'Etude Génétique des Spondylarthropathies. Arthritis Rheum 2000;43:1356–65.

4 Keat A. Reiter's syndrome and reactive arthritis in perspective. New Eng J Med 1983;309:1606–15.

5 Mielants H, Veys EM. Inflammation of ileum in patients with B27-positive reactive arthritis. Lancet 1984;1:288.

6 Clain A. Bowel flora and ankylosing spondylitis. Lancet 1986;29:1259.

7 Mielants H, Veys E. Gastrointestinal tract and rheumatic disease. In: MC Hochberg, AJ Silman, JS Smolen *et al*., eds. Rheumatology, 4th edn. Chapter 29. Mosby, USA 2008, 289–96.

8 Mielants H, Veys EM, Cuvelier C, De Vos M, Bottelburghe L. HLA-B27 related arthritis and bowel inflammation. Part 2. Ileocolonoscopy and bowel histology in patients with HLA-B27 related arthritis. J Rheumatol 1985;12:294–8.

9 Leirisalo-Repo M, Turunen U, Stenman S, Helenius P, Seppala K. High frequency of silent inflammatory bowel disease in spondyloarthropathy. Arthritis Rheum 1994; 37:23–31.

10 Mielants H, Veys EM, Cuvelier C, De Vos M. Subclinical involvement of the gut in undifferentiated spondyloarthropathies. Clin Exp Rheumatol 1989;7: 499–504.

11 de Keyser F, Baeten D, van de Bosch F *et al.* Gut inflammation and spondylarthropathies. Curr Rheumatol Rep 2002;4:525–532.

12 Islam MN, Chowdhury MMH, Haq SA *et al*. The colon in patients with ankylosing spondylitis and in normal controls in Bangladesh: a macroscopic and microscopic study. Clin Rheumatol 2010;29:13–18.

13 Mowat AG. Haematologic abnormalities in rheumatoid arthritis. Semin Arthritis Rheum 1971;1:195–219.

14 Zochling J, van der Heijde D, Burgos-Vargas R *et al*. ASAS/EULAR recommendations for the management of ankylosing spondylitis. Ann Rheum Dis 2006;65:442–52.

15 Braun J, van der Heijde D, Doyle M *et al*. Improvement in hemoglobin levels in patients with ankylosing spondylitis treated with infliximap. Arthritis Rheum 2009; 61:1032–6.

16 Nutritional anaemias. Report of a WHO scientific group (WHO technical report series, no. 405). Geneva: World Health Organization, 1968.

17 Van der Heijde D, Calin A, Dougados M *et al*. Selection of instrument in the core set for DC-ART, SMARD, physical therapy, and clinical record keeping in ankylosing spondylitis. Progress report of the ASAS Working Group. Assessment in ankylosing spondylitis. J Rheumatol 1999; 26:951–4.

18 Heuft-Dorenbosch L, Spoorenberg A, van Tubergen A *et al*. Assessment of enthesitis in ankylosing spondylitis. Ann Rheum Dis 2003;62:127–32.

19 Cuvelier C, Barbatis C, Mielants H *et al*. Histopathology of intestinal inflammation related to reactive arthritis. Gut 1987;28:394–401.

20 Bates AW. Diagnosing eosinophilic colitis: histopathological pattern or nosological entity? Scientifica 2012;2012:682576.

21 Smale S, Natt RS, Orchard TR *et al*. Bjarnaso inflammatory bowel disease and spondylarthropathy. Arthritis Rheum 2001;44:2728–36.

22 Mielants H, DeVos M, Cuvelier C, Veys EM. The role of gut inflammation in the pathogenesis of spondyloarthopathy. Acta Clin Belg 1996;51:340–9.

23 De Vos M, Cuvelier C, Mielants H *et al*. Ileocolonoscopy in seronegative spondyloarthopathy. Gastroenterology 1989;96:339–44.

24 Simenon G, van Gossum A, Adler M, Rickaert F, Appelboom T. Macroscopic and microscopic gut lesions in seronegative spondyloarthopathies. J Rheumatol 1990;17:1491–4.

25 Olivieri I, Barozzi L, Padula A. Enthesiopathy: clinical manifestations, imaging and treatment. Baillieres Clin Rheumatol 1998;12:665–681.

26 Siddiq MA, Hasan SA, Rasker JJ. Persistent enthesitis and spondyloarthropathy: a case series of 71 Bangladeshi people. J Back Musculoskelet Rehabil 2015;28:463–71.

27 Altomonte I, Zoli A, Veneziani A *et al*. Clinically silent inflammatory gut lesions in undifferentiated spondyloarthopathies. Clin Rheum 1994;13:565–70.

28 Meuwissen SGM, Dekker-Saeys BJ, Agenant D, Tytgat GNJ. Ankylosing spondylitis and inflammatory bowel disease; I: prevalence of inflammatory bowel diseases in patients suffering from ankylosing spondylitis. Ann Rheum Dis 1978;37:30–2.

29 Costello PB, Alea JA, Kennedy AC, McCluskey RT, Green FA. Prevalence of occult inflammatory bowel diseases in ankylosing spondylitis. Ann Rheum Dis 1980; 9:453–6.

30 Grillet B, de Clerck L, Dequeker J, Rutgeerts P, Geboes K. Systemic ileocolonoscopy and bowel biopsy study in spondyloarthopathy. Br J Rheumatol 1987;26: 338–40.

31 De Vlam K, Mielants H, Cuvelier C *et al*. Spondyloarthopathy is underestimated in inflammatory bowel disease: prevalence and HLA association. J Rheumatol 2000;27:2860–65.

32 Protzer U, Duchmann R, Höhler T *et al*. Enteropathic spondyloarthopathy in chronic inflammatory bowel diseases: prevalence, manifestation pattern and HLA association. Med Klin 1996;91:330–5.

33 Vreugdenhil G, Löwenberg B, Van Eijk HG, Swaak AJ. Tumor necrosis factor alpha is associated with disease activity and the degree of anemia in patients with rheumatoid arthritis. Eur J Clin Invest 1992;22:488–93.

34 Francois RJ, Neure L, Sieper J, Braun J. Immunohistological examination of open sacroiliac biopsies of patients with ankylosing spondylitis: detection of tumour necrosis factor in two patients with early disease and transforming growth factor in three more advanced cases. Ann Rheum Dis 2006;65: 713–20.

35 Thiéfin G, Beaugerie L. Toxic effects of nonsteroidal antiinflammatory drugs on the small bowel, colon, and rectum. Joint Bone Spine 2005;72:286–94.

36 DeBrosse CW, Case JW, Putnam PE, Collins MH, Rothenberg ME. Quantity and distribution of eosinophils in the gastrointestinal tract of children. Pediatric Dev Pathol 2006;9:210–218.

37 Lowichik A, Weinberg AG. A quantitative evaluation of mucosal eosinophils in the pediatric gastrointestinal tract. Mod Pathol 1996;9:110–114.

38 Gonsalves N. Food allergies and eosinophilic gastrointestinal illness. Gastroenterol Clin North Am 2007;36:75–91.

39 Pascal RR, Gramlich TL. Geographic variations of eosinophil concentration in normal colon mucosa. Modern Pathol 1997;10:363–5.

40 Hurrell JM, Genta RM, Melton SD. Histopathologic diagnosis of eosinophilic conditions in the gastrointestinal tract. Adv Anatomic Pathol 2011;18: 335–48.

41 Ohtsuka Y, Shimizu T, Shoji H *et al*. Neonatal transient eosinophilic colitis causes lower gastrointestinal bleeding in early infancy. J Pediatr Gastroenterol Nutr 2007;44: 501–5.

42 Yassin MA, Khan FY, Al-Ani A, Fawzy Z, Al-Bozom IA. Ascites and eosinophilic colitis in a young patient. Saudi Med J 2005;26:1983–5.

43 Karmacharya R, Mino M, Pirl WF. Clozapine-induced eosinophilic colitis. Am J Psychiatr 2005;162:1386–7.

44 Suresh E, Doherty V, Schofield O, Goddard C, Dhillon V. Eosinophilic fasciitis and eosinophilic colitis: a rare association. Rheumatology 2005;44:411–3.

45 Gaertner WB, Macdonald JE, Kwaan MR *et al*. Eosinophilic colitis: University of Minnesota experience and literature review. Gastroenterol Res Practice 2011; 2011:857508.

46 Jiménez-Sáenz M, González-Cámpora R, Linares-Santiago E, Herrerías-Gutiérrez JM. Bleeding colonic ulcer and eosinophilic colitis: a rare complication of nonsteroidal anti-inflammatory drugs. J Clin Gastroenterol 2006;40:84–5.

47 Odze RD, Bines J, Leichtner AM, Goldman H, Antonioli DA. Allergic proctocolitis in infants: a prospective clinicopathologic biopsy study. Human Pathol 1993;24: 668–74.

48 Hwang JB, Moon HP, Yu NK *et al*. Advanced criteria for clinicopathological diagnosis of food protein-induced proctocolitis. J Korean Med Sci 2007;22:213–7.

49 Lee CK, Kim HJ. Primary eosinophilic colitis as an unusual cause of chronic diarrhea. Endoscopy 2010;42: 279–80.

50 Van Praet L, Van den Bosch FE, Jacques P *et al*. Microscopic gut inflammation in axial spondyloarthritis: a multiparametric predictive model. Ann Rheum Dis 2013; 72:414–7.

51 Maleh HC, Bica BE, Papi JA *et al*. Colonoscopic evaluation in patients with ankylosing spondylitis. Rev Bras Reumatol 2014;54:342–8.