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Research Article

Colonic Lymphoid Aggregate Histology Is Associated with Gut Microbial Flora Content in Symptomatic Patients with Normal Colonoscopy

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Abstract

Colonic lymphoid aggregates in patients without colonic pathology and normal colonoscopic findings have been associated with persistent diarrhoea in a subset of patients. These lymphoid aggregates are not consistently reported by pathologists. We evaluated lymphoid aggregates in 40 symptomatic patients from otherwise normal colonic biopsies with normal colonoscopy findings to determine the relationship between clinical and pathologic parameters including any associations with normal colonic microbial flora. We found that the presence of gram-negative cocci, short bacilli and long bacilli were associated with larger more well-circumscribed lymphoid aggregates than other microbial flora combinations. For some patients, specific colonic microbial flora content may be associated with lymphoid response and symptoms.

Keywords: Colon; Lymphoid Aggregates; Diarrhoea; Gut Flora; Gram-Negative Bacteria

Introduction

Colonic lymphoid aggregates in patients with normal colonoscopy findings have been associated with persistent diarrhoea in a subset of patients.[1] These lymphoid aggregates are reported by some pathologists and considered 'within normal limits' by others. We evaluated lymphoid aggregates in 40 symptomatic patients from otherwise normal colonic biopsies with normal colonoscopy findings as well as the morphology of microbial flora present in colonic epithelial surface mucin. Data compiled included gender, age, symptoms, site of endoscopic biopsy, and number and size of tissue biopsies. Hematoxylin and eosin-stained sections were reviewed in all cases to determine quantity and size and circumscription of lymphoid aggregates and whether secondary follicles were present. Gram stain was performed and evaluated on recut sections for presence of gram-negative and/or gram-positive microorganisms. Patients were male (16) and female (24) ranging in age from 18 to 83 with a mean of 51 years. Diarrhea was the most common symptom (30). Endoscopic colon biopsies were mostly random (35) and all clinical endoscopic colonic findings were reported as normal. The number of lymphoid aggregates in each biopsy sample ranged from 2 to 12 with a mean of 5. All cases showed the presence of predominantly gram-negative bacteria including long and/or short bacilli and/or cocci in epithelial surface mucin that were listed in order of highest to lowest quantity. It was noted that the presence of all three gram-negative bacterial morphologies was associated to a greater extent with larger, well circumscribed lymphoid aggregates. Other clinical and pathologic parameters including age, symptoms, colonic site, biopsy size, quantity of lymphoid aggregates, presence of secondary follicles and presence or predominance of single morphologic gram-negative bacteria did not reveal any specific associations.

Materials and Methods

Electronic medical record search in Co Path histopathology laboratory information system using key search words "colon biopsy endoscopy" and "lymphoid aggregate" detected 72 cases from January 2021- April 2022. Anonymised histopathology reports were reviewed and 32 cases were omitted due to active or history of inflammatory bowel disease or other mucosal injury including mild focal active colitis, ischaemic colitis, radiation treatment and colonic surgery. Forty cases were available for study. Other data compiled included gender, age, symptoms, site of endoscopic biopsy, and number and size of tissue biopsies. Hematoxylin and eosin-stained sections were reviewed in all cases to determine quantity, size (by graticule measurement) and circumscription of lymphoid aggregates and whether secondary follicles were present. Gram stain was performed and evaluated on recut sections for presence of gram-positive and/or gram-negative microorganisms. Gram-negative bacteria were listed from highest to lowest quantity as per light microscopic review of Gram-stained sections with adequate control. Presence or absence of other microorganisms such as spirochetes or parasites and viral inclusions was also noted.

Results and Discussion

Patients were male (16) and female (24) ranging in age from 18 to 83 with a mean of 51 years. Diarrhea was the most common symptom (30). Other complaints included abdominal pain (6), constipation (4), abdominal bloating (3), bleeding per-rectum (3), frequency (2), cramping (2), nausea/vomiting (2) and unintentional weight loss (2). Endoscopic colon biopsies were random (35), of caecum (1), ascending colon (1), transverse colon (1) and descending colon (1). All clinical endoscopic colonic findings were normal. Biopsies were multiple (> or = 5 pieces) in 33 cases with 5 cases having 4 pieces (4) or 3 pieces (2) in the sample. Sizes of individual biopsies ranged from 0.1cm to 0.8 cm, with mean size = 0.3cm. The number of lymphoid aggregates in each biopsy sample ranged from 2 to 12 with a mean of 5. Twenty-six cases showed well circumscribed lymphoid aggregates and 14 cases showed poorly circumscribed lymphoid aggregates. Secondary follicles with germinal centers were seen in 18 cases, while the remaining 22 had no germinal centers. All cases showed the presence of predominantly gram-negative bacteria including long and/or short bacilli and/or cocci in epithelial surface mucin that were listed in order of highest to lowest quantity. Thirty-seven cases had gram-negative short bacilli identified, 26 cases had the presence of gram-negative long bacilli, and 23 cases revealed gram-negative cocci. Fourteen cases showed the presence of all three morphologies, 14 showed two morphologies and 12 showed the presence of only one. Gram-positive cocci and rods were rare but morphologically compatible with skin contaminates and not quantitated. Parasites, spirochetes or viral cytopathic effect or inclusions were not seen in any cases. It was noted that the presence of all three gram-negative bacterial morphologies was associated to a greater extent with larger, well circumscribed lymphoid aggregates (Figure 1 and Table 1). Other clinical and pathologic parameters including age, symptoms, colonic site, biopsy size, quantity of lymphoid aggregates, presence of secondary follicles and presence or predominance of single morphologic gram-negative bacteria did not reveal any specific associations.

Figure 1

Lymphoid aggregates	Mean size	Cases with all three-gram negative bacterial morphologies
Well circumscribed	0.9cm	42% (11/26)
Poorly circumscribed	0.5cm	21% (3/14)

Table 1: Lymphoid aggregate size, architecture and colonic microbial flora morphology.

In previous research, colonic lymphoid aggregates in patients without colonic pathology and normal colonoscopic findings have been associated with persistent diarrhoea in a subset of patients [1]. These lymphoid aggregates are reported by some pathologists and considered 'within normal limits' and insignificant by others. We evaluated lymphoid aggregates in 40 symptomatic patients from otherwise normal colonic biopsies with normal colonoscopy findings to determine the relationship between clinical and pathologic parameters including any associations with normal colonic microbial flora.

Similar to our patient sample, Shah., *et al.* observed chronic diarrhea in 47 patients described as having "non-specific inflammation" and "near normal" biopsy samples with no history of linked diseases, such as Crohn disease. These samples were compared to a control group obtained from normal looking mucosa of colectomy resections of cancer patients. Test samples had an increased level of lymphoid aggregates when compared to the control group. Mast cell and T-Regulatory cell levels were observed to be higher in the sample group when compared with the control group which was expected due to the presence of inflammation. The authors suggested that elevated numbers of lymphoid aggregates correlate with gut mucosal damage and may predict the duration of chronic diarrhea in a sub-set of patients.

There are over 500 species of bacteria that coexist in the human colon. Anaerobic bacteria are approximately ten times more prevalent than aerobic bacteria. The most prominent species found in the human colon include anaerobes such as the gram-negative, rod-shaped *Bacteroides-Prevotella* group [2]. These species play an important role in digestion and homeostasis of the intestine, [3] and a shift in the microbiota balance may cause infection or diseases ranging from bacteraemia to inflammatory bowel disease [4]. These species can also acquire and distribute antibiotic resistance [5].

Intestinal inflammation has been linked to causing diarrhea due to various inflammatory pathways. Cytokine release during inflammation is linked to diarrhea due to downregulation of absorptive proteins and upregulation of secretive proteins. Tumour Necrosis Factor alpha (TNF -α) is involved in secretion of chloride (CL^-) due to its effect on CL^- channels on epithelial cell membranes. There is also an effect on the levels of intracellular sodium (Na^+) and potassium (K^+). TNF -α is thought to inhibit the enzyme Na^+/K^+-ATPase, although the exact mechanism is not known. The changes in these intracellular components due to cytokine release are thought to effect cell permeability in the intestines thus causing diarrhoea [6]. Few large Gram-positive cocci and rods were also seen in biopsy samples during this investigation. Based on size, morphology and location away from surface epithelial mucin, the vast majority of these were deemed skin contaminants introduced when biopsy samples were being prepared. Moreover, gram-positive cocci are mainly found in the small intestine rather than colon [7].

Limitations of our study include relatively small sample size which resulted in smaller subgroups of lymphoid aggregate types and bacterial morphologic groups. A more sophisticated methodology of bacterial assessment and quantification would be more detailed and accurate, such as gene amplification and sequencing. Future research would address these limitations and perhaps include an assessment of inflammatory constituents, such as T-lymphocyte CD4+ and CD8+ subsets, and plasma cells, eosinophils and mast cells.

a

b

Figure 1: a) Well circumscribed lymphoid aggregate (with germinal center), H and E stain, low power.
b) Poorly circumscribed lymphoid aggregate (without germinal center), H and E stain, medium power.

a

b

Figure 3: a) Short bacilli and cocci in colonic surface epithelial mucin, H and E stain, high power.
b) Gram-negative short bacilli in colonic surface epithelial mucin, Gram stain, high power.

Conclusion

Based on our findings, we believe it is reasonable to suggest that increased lymphoid aggregates may result from a particular combination of gram-negative bacteria, which may represent a temporary or more long-standing change, such as imbalance or overgrowth, in an individual patient. The increased lymphoid aggregates, via cytokines and altered intracellular components that lead to changes in cell permeability, may be responsible for diarrhoea and/or other symptoms in this subset of patients. Further characterisation and quantification of bacterial species in these patients would be useful.

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Conflict of Interest

The authors have nothing to disclose.

Bibliography

1. Shah N., *et al.* "Lymphocytic follicles and aggregates are a determinant of mucosal damage and duration of diarrhoea". *Archives of Pathology and Laboratory Medicine* 137.1 (2013): 83-89.
2. Mai V., *et al.* "Colonic Bacterial Flora: Changing Understandings in the Molecular Age". *The Journal of Nutrition* 134.2 (2004): 459-464.
3. Guo P., *et al.* "Clostridium species as probiotics: potentials and challenges". *Journal of Animal Science and Biotechnology* 11.11 (2020):24.
4. Navaneethan U., *et al. "*Mechanisms of infectious diarrhea". *Nature Clinical Practice. Gastroenterology and Hepatology* 5.11 (2008): 637-647.
5. Salyers A.A., *et al.* "Gram-Negative Opportunistic Anaerobes". Friends and Foes. in M. Schaechter (ed.) *Encyclopedia of Microbiology (Third Edition)*. Oxford: Academic Press (2009): 145-152.
6. Musch M.W., *et al.* "T cell activation causes diarrhea by increasing intestinal permeability and inhibiting epithelial Na+/K+-ATPase". *The Journal of Clinical Investigation* 110.11 (2002): 1739-1747.
7. Thadepalli H., *et al.* "Microflora of the human small intestine". *American Journal of Surgery* 138.6 (1979): 845-850.