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The prevalence of IgG4-positive plasma cell infiltrates in inflammatory bowel disease patients without autoimmune pancreatitis

PANCREAS

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Original Article

ABSTRACT

Background/Aims: IgG4-related autoimmune disease can exist in other organs even when there is no evidence of autoimmune pancreatitis. The aim of our study was to determine the prevalence of IgG4-positive plasma cells in the histopathological evaluations of colon biopsy specimens in IBD patients.

Materials and Methods: The number of IgG4-positive plasma cells with strong cytoplasmic immunoreactivity was counted in each colon biopsy from inflammatory bowel disease patients who had no evidence of autoimmune pancreatitis. Five high power fields (HPFs) in the highest density plasma cell infiltration area were counted and were then averaged. An average >10 cells/HPF was considered significant for IgG4-related disease.

Results: We detected IgG4-positive plasma cell staining in the colon of 21 of 119 patients (17.6%). Of these 21 patients, 5 had elevated serum IgG4 levels (>140 mg/dL). Of the total, 4.2% (5/119) had both IgG4-immunstaining and elevated IgG4 serum levels. The demographic features, disease type and activity, and response to treatment (especially to steroid treatment) were similar between the IgG4-negative and IgG4-positive groups.

Conclusion: In our study, 4.2% of patients with the diagnosis of IBD had elevated IgG4 serum levels and significant IGg4 immunostaining. Together, these two parameters indicate the possible diagnosis of an IgG4-related systemic disease.

Keywords: IgG4-related disease, inflammatory bowel disease, autoimmune pancreatitis

INTRODUCTION

IgG4-related systemic disease (IgG4-RSD) has been recognized recently as a unique classification for several previously described organ-specific inflammatory conditions, characterized by elevated serum levels of IgG4 and the occurrence of a sclerosing lymphoplasmacytic inflammatory reaction rich in IgG4-positive plasma cells in one or more organs (1).

High levels of IgG4-positive plasma cells are commonly seen in autoimmune pancreatitis (AIP). It has recently become evident that AIP is one component of a larger multi-system disease, and IgG4-positive plasma cells have been identified in many extrapancreatic tissues, including the colon, biliary tract, liver, and lungs. Thus, the term "IgG4-related autoimmune disease" has been proposed.

Awareness of IgG4-related disease is important, as it has been shown to mimic other conditions, such as malignancy (2). Affected organs have a lymphoplasmacytic infiltrate and fibrosis (3-5). The clinical presentation differs from patient to patient, ranging from completely asymptomatic patients to those with jaundice, nausea, vomiting, and weight loss. Radiographic findings are similarly nonspecific. However, tissue immunostaining of affected organs reveals abundant infiltration with IgG4-positive plasma cells (6,7).

A relationship between pancreatitis and inflammatory bowel disease (IBD) was first proposed over five decades ago. In a postmortem study of patients with ulcerative colitis (UC), Ball et al. found that 46/86 (53%) patients had chronic interstitial pancreatitis. Another autopsy study in patients with Crohn disease (CD) revealed

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evidence of pancreatic fibrosis in 15/39 (38%) patients (2). The colitis cases with IgG4-related disease are described as being aggressive in clinical onset and resistant to traditional treatments (8).

Previous studies have suggested an association between AIP and other disorders, such as primary sclerosing cholangitis, Sjögrens syndrome, and retroperitoneal fibrosis. More recent studies have suggested that sclerosing inflammation mimicking these conditions may represent a systemic manifestation of AIP. This idea is supported by the fact that the infiltrate tends to be rich in IgG4-positive plasma cells, regardless of the organ site involved (9). Barthet et al. (10) proposed that pancreatitis is an extraintestinal manifestation of IBD. Their case series found that 11 of 427 IBD patients in southern France had been clinically diagnosed with pancreatitis. In 3 patients, medications were identified as the cause of acute pancreatitis. The remaining eight patients had unexplained chronic pancreatitis, based on pancreatic symptoms and a pancreatogram or biopsy consistent with chronic pancreatitis. From this cohort and a review of the published literature, the incidence of chronic pancreatitis in patients with IBD has been estimated to be 1.2%, which is 250-fold higher than that seen in the general population (10).

Overall, previous studies do not provide clear answers as to the relationship between AİP and IBD. Specifically, the frequency of concomitant symptomatic patients with AIP is unknown. In those with AIP and IBD, it is unclear if IBD is merely an associated immune-mediated disease or a true extrapancreatic manifestation of AIP (9,11). Almost 6% of patients with proven AIP had a diagnosis of IBD, compared with a prevalence of 0.4%-0.5% in the general population, potentially implying a 12-15-fold increase in risk. Patients with both AIP and IBD may have an increased extent and severity of IBD and the finding of IgG4-positive cells on colon biopsy suggests that IBD represents an extrapancreatic manifestation of AIP (9). From case reports and case-control studies, it has been estimated that the incidence of IBD among patients with a prior diagnosis of AIP is approximately 7.6%. However, the incidence of IgG4-positive plasma cell-mediated colitis in the absence of AIP is unclear. As noted above, IgG4-related sclerosing disease is a systemic disease that can occur without pancreatic involvement (2).

Taking into consideration the fact that IgG4-related autoimmune disease can exist in other organs, even when there is no evidence of AIP, our aim in this study was to determine the prevalence of IgG4-positive plasma cells in the histopathological evaluations of colon biopsy specimens in IBD patients.

MATERIALS AND METHODS

Patient evaluation

Patients who underwent ileocolonoscopy for the detection of activation were included in this study. This study was approved by the instutional review board. All patients' demographic features were collected (age and gender).. The simplified endoscopic scoring of CD (SES-CD) scoring system was used for the CD patients and Mayo endoscopic subscoring was used for UC patients. Scores between 0-2 were counted as remission in SES-CD while 0 or 1 was counted as remission in Mayo endoscopic subscoring. None of the patients had radiologic or biochemical signs of pancreatitis. Pancreatitis was excluded with normal serum amylase and lipase levels, as well as abdominal computerized tomography and endoultrasonography. The IgG4 serum levels were determined with the nephelometric assay in patients with marked IgG4 immuno-staining in their colon biopsy specimens. Only those with positive staining had the serological testing done.

Ileocolonoscopic evaluation

The patients were placed on a liquid diet for three days prior to the procedure. On the day before the colonoscopy, the patient was given a laxative preparation (2×200 mL phospho soda) and large quantities of fluid. The patients were sedated with midazolam and propofol at individualized dosages. The ileocecal valve was intubated in all of the patients. The biopsies were obtained from inflamed areas of the colon.

Histopathological evaluation

Colorectal biopsies were formalin-fixed and paraffin-embedded. Hematoxylin and eosin (H&E) stained sections were reviewed by a single pathologist (NE). Subsequently, tissue immuno-staining utilizing monoclonal antihuman IgG4 antibody (Sigma-Aldrich, United Kingdom) was performed using standard immunohistochemical techniques. The number of IgG4-positive plasma cells with strong cytoplasmic immunoreactivity was counted in 5 high power fields (HPFs) in each biopsy specimen within the section with the highest density of plasma cell infiltration. The average number of IgG4-positive plasma cells/HPF was calculated. An average of >10 cells/HPF was considered significant for the presence of IgG4-RSD (Figures 1, 2).

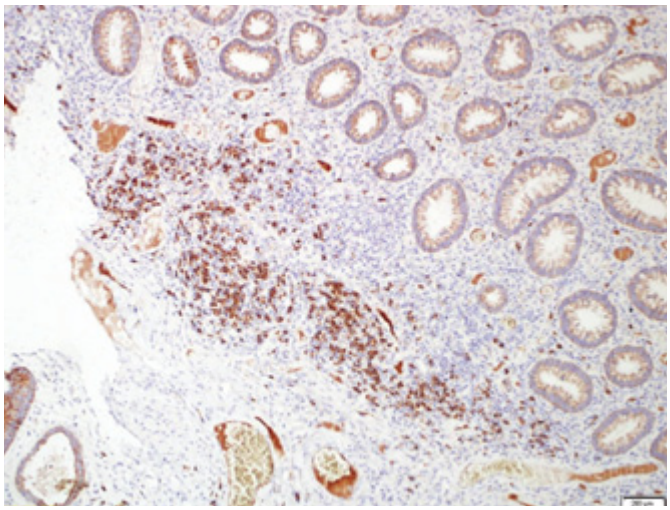

Figure 1. Aggregation of IgG4 stained plasma cells.

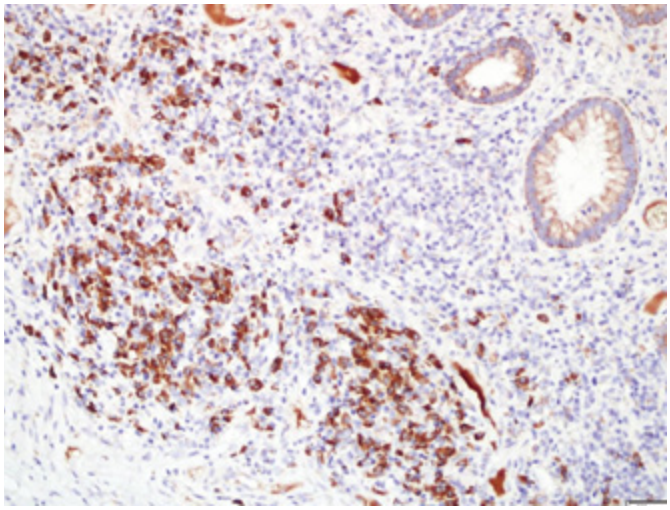

Figure 2. A high power field showing more than 10 IgG4 stained plasma cells.

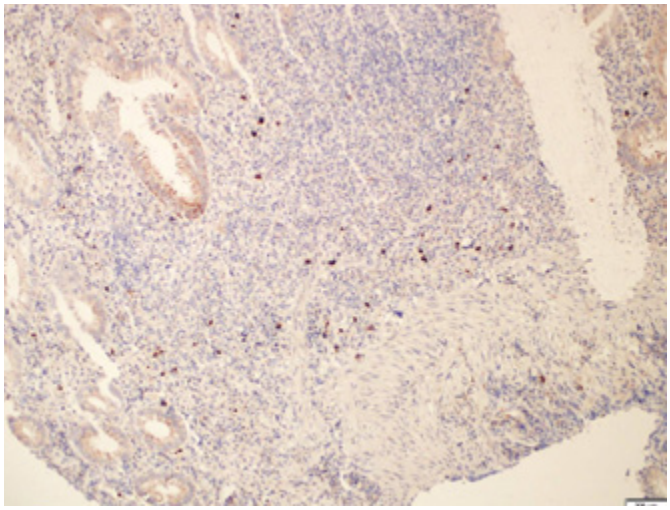

Figure 3. Aggregation of IgG4 stained plasma cells.

Statistical evaluation

Statistical Package for Social Science (SPSS) (Windows 11.5) (IBM, Chicago, IL) software was used for the analysis. Descriptive analysis was shown by the mean±standard deviation, and nominal variables were shown as numbers (n) or percentages (%). The Student t-test, Pearson chi square test, and the likelihood ratio test were used. The results were accepted as significant at $p<0.05$.

RESULTS

A total of 119 IBD patients were included; 106 had UC, 6 had CD, and 7 had indeterminate colitis. We detected IgG4-positive plasma cells in 21 of the 119 patients (17.6%). In 5 of these 21 patients, elevated serum IgG4 levels were detected (>140 mg/dL). Of the total, 4.2% (5/119) of patients had both IgG4-immunostaining and elevated IgG4 serum levels. The demographic features, disease type and activity, and response to treatment (especially to steroid treatment) were similar between the serologically and histologically IgG4-negative and serologically and histologically IgG4-positive groups.

Figures 1-4 illustrate the IgG4 staining of the intestinal mucosa. The demographic and clinical findings are summarized in Table 1. The features of the two groups, histopathologically IgG4-positive and histopathologically IgG4-negative are summarized in Table 2. The patients are categorized as A) histopathologically IgG4-negative, B) histopathologically IgG4-positive but with serum IgG4 levels <140 mg/dL, and C) histopathologically IgG4-positive but with serum IgG4 levels ≥140 mg/dL. The features of these three groups are summarized in Table 3.

DISCUSSION

In our study, 4.2% of patients with IBD had elevated IgG4 serum levels and significant IgG4 immunostaining. These two parameters together represent the possible diagnosis of IgG4-related systemic disease (IgG4-RSD). None of these patients demonstrated radiological or biochemical signs of pancreatitis. This is the first study evaluating the incidence of IgG4-RSD in a large population of IBD patients.

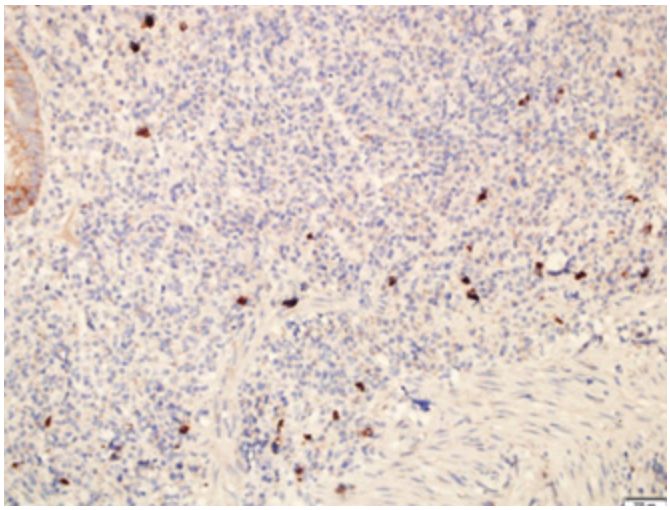

Figure 4. A high power field showing more than 10 IgG4 stained plasma cells.

IgG4-RSD is a systemic inflammatory disease characterized by tissue infiltrates of IgG4-postive plasma cells and elevated serum IgG4 levels. While IgG4-RSD often involves the pancreas, extrapancreatic organs are also frequently affected (12). The pathogenesis of IgG4-RSD is unknown. IgG4-RSD may remain isolated in a single organ or progress over time to involve multiple sites (13). Pathological findings similar to AIP have been identified in a number of extra-pancreatic organs such as the liver, lymph nodes, and aorta, as well in lesions of previously unexplained chronic inflammatory diseases, such as retroperitoneal fibrosis, Riedel thyroiditis, Mikulicz chronic sclerosing dacryoadenitis, and Kuttner chronic sclerosing sialadenitis. Regardless of the location, IgG4-RSD is characterized by tissue infiltration with polyclonal IgG4-positive plasma cells and lymphocytes, with associated fibrosis (14-16).

As mentioned previously, extra-pancreatic IgG4-RSD can occur without pancreatic disease. A lack of awareness regarding this entity may delay diagnosis (8,12). Kamaisawa et al. (4) stated that elevated serum IgG4 levels and dense IgG4 immunostaining of the affected tissue are the two necessary criteria for the

Table 1. Features of the patients, results of the IgG4-stained plasma cells, serologic testing and endoscopic activity

Variables	n=119
Age	47.4±14.5 (18-80)*
Gender	
Male	73 (61.3%)
Female	46 (38.7%)
Diagnosis	
Crohn disease	6 (5.0%)
Undetermined colitis	7 (5.9%)
Ulcerative colitis	105 (88.2%)
Not-specified	1 (0.8%)
Number of IgG4-stained plasma cells in HPF	
First HPF	9.5±14.2 (0-110)*
Second HPF	7.5±12.7 (0-120)*
Third HPF	6.9±11.4 (0-80)*
Forth HPF	6.9±12.5 (0-115)*
Fifth HPF	6.8±12.8 (0-120)*
Mean	7.5±12.2 (0-109)*
IgG4 plasma cell infiltration	21 (17.6%)
Serum IgG4 ≥140 mg/dL	5 (4.2%)
Endoscopic activity	74 (62.2%)

*mean +/- SD, HPF: hight power field

Table 2. Differentiation between IgG4 (-) and (+) groups

Variables	IgG4 (-) (n=98)	IgG4 (+) (n=21)	p value
Age	47.1±14.7	48.6±13.5	0.674
Gender			0.042
Male	56 (57.1%)	17 (81.0%)	
Female	42 (42.9%)	4 (19.0%)	
Endoscopic activity			0.641
(-)	38 (38.8%)	7 (33.3%)	
(+)	60 (61.2%)	14 (66.7%)	

Table 3. Differentiation between groups A*, B** and C***

Variables	Group A (n=98)	Group B (n=16)	Group C (n=5)	p value
Age	47.1±14.7	51.8±13.6	38.4±6.2	0.180
Gender				0.106
Male	56 (57.1%)	13 (81.3%)	4 (80.0%)	
Female	42 (42.9%)	3 (18.7%)	1 (20.0%)	
Endoscopic activity				0.328
(-)	38 (38.8%)	4 (25.0%)	3 (60.0%)	
(+)	60 (61.2%)	12 (75.0%)	2 (40.0%)	

(Group A)* histopathologically IgG4(-), (Group B)** histopathologically IgG4(+) but with serum IgG4 levels <140 mg/dL, and (Group C)*** histopathologically IgG4(+) but with serum IgG4 levels ≥140 mg/dL

diagnosis of extra-pancreatic IgG4-RSD. The sensitivity and specificity of serum IgG4 levels are 70% and 90% for the detection of IgG4 RSD, respectively. Additionally, serum IgG4 levels have been shown to correlate with disease severity (1). From case reports and case-control studies, it has been estimated that the incidence of IBD among patients with a prior diagnosis of AIP is approximately 7.6% (15). However, the incidence of IgG4-positive plasma cell-mediated colitis in the absence of AIP is unclear.

Patients with IBD are at an increased risk of developing both acute and chronic pancreatitis. Although pancreatitis can be seen in association with biliary lithiasis or administration of IBD-associated medications, such as 5-aminosalicylates (5-ASA), corticosteroids, azathioprine (AZA), or 6-mercaptopurine (6-MP), the contribution of these factors to the etiology of histopathologically proven pancreatitis appears to be low. Most cases of pancreatitis are clinically silent, and the frequency of clinical pancreatitis is markedly lower than that of either asymptomatic hyperamylasemia or evidence of exocrine pancreas insufficiency. It remains unclear whether pancreatitis is an extraintestinal manifestation of IBD (17).

Ravi et al. studied 71 patients with AIP and found that four had concurrent IBD (three UC and one CD) (14). All UC patients had pancolitis, and two required total colectomy for refractory disease. Immunostaining of biopsies from one of the patients with refractory UC revealed more than ten IgG4-positive cells/HPF, which is considered to be consistent with IgG4-related systemic disease (10). This suggests that additional patients with a clinical diagnosis of IBD, with or without a history of pancreatic disease, may have IgG4-related colitis.

Our study is unique in its investigation of the incidence of IgG4-RSD in the IBD population; however, it does have some limitations. Increased IgG4 immunostaining might be due to active inflammatory processes in IBD, and it was impossible to assess fibrosis as the biopsies were only mucosal. Additionally, our cases form a heterogeneous group clinically. However, our findings remain valuable, as it demonstrates the possible presence of IgG4-RSD in a population with IBD. Future studies should be designed to further define the extent of IgG4-RSD and its differentiation from other inflammatory conditions.

Ethics Committee Approval: Ethics committee approval was received for this study.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - E.S.Y., F.T., M.P.; Design - E.S.Y., F.T., M.P; Supervision - B.U.; Resource - E.S.Y., F.T., M.P, E.A., N.E., F.C.; Materials - E.S.Y., F.T., M.P.; Data Collection&/or Processing - E.S.Y., F.T., M.P.; Analy-

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sis&/or Interpretation - E.S.Y., F.T., M.P.; Literature Search - E.S.Y., F.T., M.P.; Writing - E.S.Y., F.T., M.P.; Critical Reviews - E.S.Y., F.T., M.P.

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