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# Subclinical amyloid deposition in inflammatory bowel diseases: A two hospital study

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ABSTRACT

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), predominantly affects young patients and leads to intestinal complications. Amyloidosis, which involves abnormal protein deposition, is a serious complication of IBD, with a low incidence. Early detection of subclinical amyloid deposits is crucial for preventing fatal outcomes; however, routine investigations are lacking. We aimed to retrospectively examine subclinical amyloid deposition in adult patients with IBD. Surgical specimens from 249 patients with IBD were collected from the databases of two hospitals. The specimens were subjected to staining and immunohistochemistry, and clinical information was collected simultaneously. The amyloid positivity rate was 0.8 % in CD (1/131) and 0 % in UC (0/118) based on Congo red staining. The patient with amyloid deposits was a female in her 80 s who lacked a family history of amyloidosis. The subtype was amyloid A. Clinical history revealed intestinal resection in her 30 s and subsequent abdominal symptoms. To the best of our knowledge, this is the first study to collect >100 surgically examined specimens from adults with CD or UC. In older patients with a long and complex clinical course, aggressive analysis of amyloids would be better.

## 1. Introduction

Inflammatory bowel disease (IBD), which is a group of diseases of unknown etiology and includes diseases such as Crohn's disease (CD) and ulcerative colitis (UC), is more common in young patients. CD is a granulomatous inflammatory disease that causes fibrosis and intestinal ulcerations. CD is often accompanied by perianal lesions and other systemic complications. UC is a diffuse inflammatory disease primarily affecting the colonic mucosa. Patients with UC with a long-term course of ≥ 10 years are often affected by cancer.

Amyloid is an abnormal fibrous protein, and the organ damage caused by amyloid deposition is called amyloidosis. The main amyloid proteins deposited in the intestine are AA, AL, Aβ2 M, and ATTR. In chronic inflammatory diseases, such as CD and rheumatoid arthritis, AA is frequently deposited around blood vessels and may be accompanied by circulatory disorders. Confirmation of amyloid deposition by Congo red (CR) staining is necessary for the pathological diagnosis of amyloidosis.

Amyloidosis is a serious complication of IBD. Although the incidence of amyloidosis in IBD is low (< 3 %) [1–3], it can be fatal if it causes renal failure. Amyloidosis, a complication of IBD, occurs infrequently and may be a pitfall in clinical practice. If subclinical amyloid deposits can be detected before they develop into amyloidosis, early management can be performed; however, amyloid deposits are not routinely investigated in the treatment of IBD. Therefore, the frequency of subclinical amyloid deposition prior to amyloidosis in patients with IBD

*Abbreviations:* IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; CR, Congo red; IHC, immunohistochemistry; HE, hematoxylin and eosin; SAA, serum amyloid A.

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remains largely unknown. Furthermore, no consensus regarding which patients are at high risk of developing amyloidosis has been established.

In this study, we retrospectively investigated subclinical amyloid deposition in adult patients with IBD (including a small number of pediatric patients) at two hospitals. Simultaneously, clinical information was closely scrutinized in cases where amyloid deposition was observed.

## 2. Materials and methods

### 2.1. Patients

We collected jejunal, ileal, and colorectal surgical specimens from 249 patients using the pathological database (2010–2023) of Osaka University Hospital and Osaka Police Hospital. This study included 131 and 118 patients clinically diagnosed with CD and UC, respectively. The former group included 101 males and 30 females (age: 11–80 years; mean age: 40.4 years). The latter group included 78 males and 40 females (age: 12–81 years; mean age: 48.5 years). In each case, one section with significant macroscopic findings, such as erosion, ulceration, or stenosis, was targeted. If macroscopic findings were poor, an arbitrary section was used.

### 2.3. Staining and immunohistochemistry (IHC)

All tissue specimens were fixed in 10 % formalin and neutral buffered solution, embedded in paraffin in Osaka University Hospital and Osaka Police Hospital. They were cut into 4-μm-thick serial sections in Kinki Central Hospital and Osaka Police Hospital. They were used at Kinki Central Hospital for 1) hematoxylin and eosin (HE) staining and 2) CR staining and 3) IHC. CR staining was performed according to the manufacturer's instructions (MUTO PURE CHEMICALS). The IHC assay was performed using the Roche BenchMark ULTRA IHC/ISH Staining Module (Ventana Medical Systems) according to the manufacturer's instructions and a previous study [4]. The primary antibodies used in this study and their dilution ratios are listed in Table 1. Positive controls were selected from previous amyloidosis samples. Determination of amyloid deposition was based on the consensus of two pathologists (Y.H. and K.K.).

## 3. Results

The amyloid-positivity rate was 0.8 % (1/131) and 0 % (0/118) in patients with CD and UC, respectively, based on CR staining. The patient was in her 80 s and had amyloid deposits. This case is described in detail as follows. She had no family history of amyloidosis.

### 3.1. Clinical summary

The patient had undergone two intestinal resections for intestinal obstruction in her 30 s; however, the details are unknown. After surgery, her abdominal symptoms improved, and she stopped visiting the hospital. In year X-8 (approximately 40 years after surgery), she visited another hospital because of anorexia and abdominal pain. She was followed up with oral administration of mesalazine; however, mesalazine was discontinued in year X-1 because of mesalazine-induced kidney injury. However, computed tomography images showed a right ovarian cyst. The patient underwent bilateral salpingo-oophorectomy in year X-1, and the pathological diagnosis was serous cystadenoma. Subsequently, the abdominal discomfort and distension worsened and remitted. Colonoscopy revealed stenosis at the postoperative anastomotic site. She visited another hospital in year X, was treated with adalimumab for 1 month, and underwent right hemicolectomy. The laboratory findings at admission are shown in Table 2. The gross findings of the right hemicolectomy specimen are shown in Fig. 1.

**Table 1**
Primary antibodies used in this analysis.

| Antibody | Clone | Dilution | Manufacturer |
|---|---|---|---|
| Amyloid A | mc1 | 1:20 | Dako |
| Prealbumin | EPR3219 | 1:1000 | abcam |

### 3.2. Histological findings with HE staining

Extensive ulcers with inflammatory exudates were observed. A small crypt abscess was observed in the ulcer. Crypt distortion and pyloric gland metaplasia were observed in the mucosa surrounding the ulcers. Severe inflammatory cell infiltration by neutrophils was observed in the lamina propria and subserosa, and the muscularis propria were partially obscured. The walls of many arterioles and venules in the submucosa were eosinophilic and structureless (Fig. 2A). No apparent inflammation was observed around the blood vessels. Neural hyperplasia was observed in the submucosa. Fibrosis was observed in the submucosa and subserosa. No evidence of granuloma or malignancy was observed.

### 3.3. CR staining

Orange-red deposits were observed on the walls of the submucosal arterioles and venules (Fig. 2B). When observed under simple polarized light, the deposits on the blood vessel walls appeared apple-green.

### 3.4. IHC

The deposits on the vessel walls of the submucosal arterioles and venules were positive for amyloid A (Fig. 2C) and negative for prealbumin (Fig. 2D).

## 4. Discussion

In this study, we collected > 100 surgically examined specimens from adult patients with CD and UC, and reported subclinical amyloid deposition for the first time. Previous reports showed that 0.9–3 % of CD cases and 0–0.07 % of UC cases were complicated by amyloidosis [1–3]. All cases examined for subtypes showed amyloid A, and a male predilection was also reported for CD cases with amyloidosis. Previous reports have shown that all patients with IBD with amyloidosis have amyloidotic renal dysfunction [1,5]. In a study examining subclinical amyloid deposition in pediatric cases, the amyloid deposition rate was 3.3 % and 0 % in CD and UC, respectively [6].

**Table 2**
Laboratory findings at admission.

| | |
|---|---|
| Hematology | |
| White Blood Cell count | 11860 /μL |
| Red Blood Cell count | 328×10^4 /μL |
| Hemoglobin | 9.3 g/dL |
| Platelet count | 44.6×10^4 /μL |
| Biochemistry | |
| Total protein | 8.0 g/dL |
| Albumin | 3.9 g/dL |
| Creatine kinase | 38 U/L |
| Asparate aminotransferase | 32 U/L |
| Alanine aminotransferase | 33 U/L |
| Lactate dehydrogenase | 196 U/L |
| Alkaline phosphatase | 132 U/L |
| Total bilirubin | 0.5 mg/dL |
| Direct bilirubin | 0.2 mg/dL |
| γ-Glutamyl transpeptidase | 112 U/L |
| Creatinine | 1.36 mg/dL |
| Serology | |
| C-reactive protein | 0.40 mg/dL |
| Leucine-rich alpha2 glycoprotein | 41.2 μg/mL |

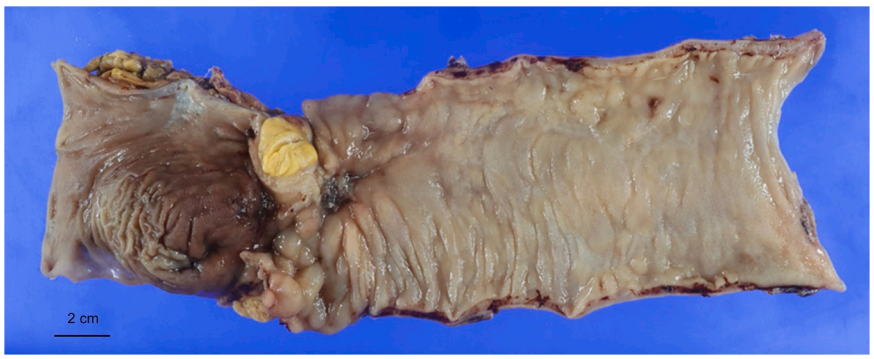


**Fig. 1.** Right hemicolectomy specimen. Postoperative anastomosis site was seen 290 cm from the Treitz ligament. The oral side of the specimen consisted of the ileum (right), and the anal side consisted of the ileocecal region and right colon (left). Near the anastomosis site, a stenosis with uneven mucosa and an irregularly shaped ulcer measuring 2 cm were observed.

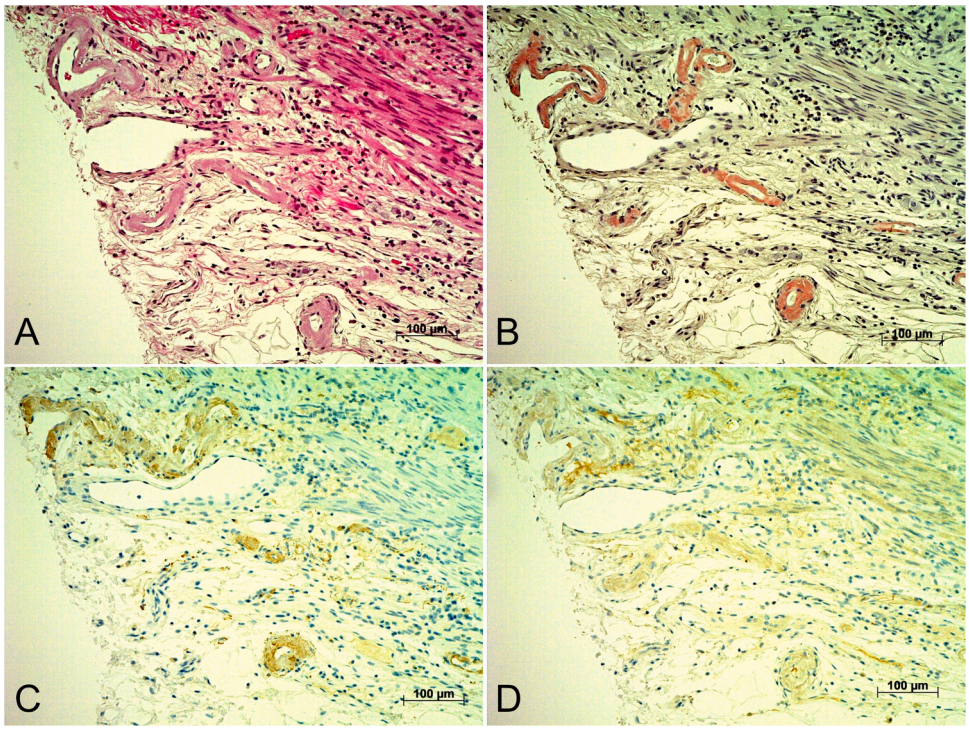


**Fig. 2.** Arterioles and venules in the submucosal layer. A) HE staining, B) CR staining, C) IHC for Amyloid A, D) IHC for prealbumin.Original magnification 100 ×, scale bar = 100 μm. HE, hematoxylin and eosin; CR, Congo red; IHC, immunohistochemistry.

In addition, many individual case reports of amyloidosis with IBD have been published. Several cases of CD associated amyloidosis were reported. The subtype was amyloid A, and amyloid deposition was detected in various organs such as the kidney, thyroid, urinary bladder, or pituitary gland [7–9]. Many cases of remission with anti-tumor necrosis factor agents such as infliximab have been reported [1,10–14]. On the other hand, only a few reports of amyloidosis with UC have been reported [15–17]. The subtype was AA, and amyloid deposition was detected in the gastrointestinal tract, thyroid, or kidney. In some cases, the disease was treated with colchicine, salazopyrin, or prednisolone.

The subclinical amyloid deposition rate (0.8 %) in CD confirmed in this study was lower than that reported by Kahn E et al. [6] and the complication rate of amyloidosis in CD (0.9–3 %) reported in the previous studies [1–3]. This result might be caused by an increase in treatment methods, such as the current spread of molecular target therapeutics. Furthermore, although the populations of both CD and UC in this study showed a male predilection, the patients with subclinical amyloid deposition were female, and the male predilection was not apparent. In this case, analysis for amyloid nephropathy was not performed sufficiently, and the presence or absence of amyloid nephropathy was unknown. Amyloidosis is more likely to occur in CD than in UC [3], which is consistent with the results of the present study.

In this study, in the case of CD, subclinical amyloid deposition was observed in the vascular wall of the submucosa. Amyloid A was deposited as previously described. Serum amyloid A (SAA) could be produced in the liver followed by intestinal inflammation and deposited in the form of amyloid A on the walls of intestinal blood vessels via the bloodstream. SAA is a precursor of amyloid A and was reported as a biomarker for CD and UC [18–20]. A study using model animals indicated that SAA might protect the intestinal epithelium [21]. Although the case was an older patient, senile systemic amyloidosis was ruled out because prealbumin was immunohistochemically negative. In addition, decreased renal function was observed. If renal function would continue to decline, ruling out amyloid nephropathy might be necessary. In older patients with a long and complex clinical course, aggressive amyloid analysis is preferred. She may have developed Crohn's disease in her

30 s when intestinal obstruction was observed.

This study has a few limitations. First, only a portion of the surgical specimens was used to analyze the amyloids, and the entire surgical specimen was not sectioned. Secondly, this study included a limited number of patients from both hospitals.

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## Ethical statement

This study was approved by the Ethical Review Boards of Osaka University Hospital (Proposal No.23334), Osaka Police Hospital (Proposal No.1864), and Kinki Central Hospital (Proposal Nos.466 and 480). All procedures were performed in accordance with committee guidelines and regulations.

## CRediT authorship contribution statement

**Yuichiro Hamamoto:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Validation, Writing – original draft. **Michihiro Kawamura:** Investigation, Resources. **Kansuke Kido:** Investigation, Resources, Validation, Writing – review & editing. **Takayuki Ogino:** Investigation, Resources. **Yuki Sekido:** Resources. **Hideki Iijima:** Resources. **Hironao Yasuoka:** Investigation, Resources. **Tsunekazu Mizushima:** Investigation, Project administration, Resources, Supervision, Writing – review & editing.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests. Yuichiro Hamamoto reports financial support was provided by Hyogo Medical Association. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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