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SIGNIFICANCE OF TISSUE IgG4 POSITIVE PLASMA CELLS IN ULCERATIVE COLITIS

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Abstract

Inflammatory bowel disease (IBD) is a series of intestinal nonspecific inflammatory conditions, comprising of predominantly ulcerative colitis (UC) and Crohn's disease (CD). Although the pathogenesis considered are multifactorial involving genetic predisposition, epithelial barrier defects, dysregulated immune responses and environmental factors but still it is not clear. There is evidence that T lymphocytes are involved in UC, but role of B cells remains unclear. The roles of IgG4-expressing plasma cells in the pathogenesis of UC is obscure. However, IgG4 was earlier regarded as a noninflammatory antibody. We hypothesized that colonic biopsies with increased numbers of IgG4-positive plasma cells might be restricted to ulcerative colitis.

AIM: To study the significance of IgG4-positive plasma cell infiltration in colonic mucosa in patients with Ulcerative Colitis (UC).

MATERIAL AND METHODS: The present prospective study included 44 cases of UC diagnosed by colonoscopy. Mayo score was done in all the cases. Biopsies from the lesions were taken, fixed in 10% formalin and routinely processed. Paraffin tissue blocks were prepared, sections were cut at 3-4 µm and the slides were stained with Hematoxylin and Eosin [H & E] stain and Immunohistochemistry for IgG4 in all the cases.

RESULTS:

Among the 44cases, characteristic histopathology findings of UC were observed in 30 cases and 14 cases showed overlapping features of both UC and CD and were termed as Indeterminate Colitis (IC). IgG4 was positive in disease activity index [M3 & M2] [40/44 (91%)] and negative in M1 and MO [4/44 (9%)].

CONCLUSION:

IgG4 is positive in high disease activity index and negative in low disease activity index. Thus, IgG4 can be helpful in assessing the severity of UC cases.

Keywords*: IBD, UC, Immunohistochemistry, IgG4*

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I. INTRODUCTION

UC is a chronic inflammatory disease limited to the colon with superficial mucosal inflammation that extends proximally in a contiguous manner leading to ulcerations, severe bleeding, toxic megacolon, and fulminant colitis. Although the pathogenesis of UC is not clear, it is acknowledged to the dysfunction of intestinal

immune homeostasis, characterized by continuous or remittent inflammation. There is evidence that T lymphocytes are involved in UC, but role of B cells remains unclear. It has been suggested that excessive B cell derived plasmacytic infiltration in the intestinal lamina propria is characteristic of UC. [(1)] Though IgG4 accounts for the smallest proportion of all IgG isotypes, it has been commonly ignored. However, its role in various autoimmune disorders has now become a research focus along with the identification and prevalence of IgG4-related disease. Hence the present study was conducted. (1)

AIM:

To study the significance of IgG4-positive plasma cell infiltration in colonic mucosa in patients with UC.

MATERIALS AND METHODS:

A descriptive prospective study was conducted at a tertiary health care center between January 2018 and June 2022. Biopsy received from patients with a diagnosis of UC on colonoscopy were included in the study. Patients with autoimmune pancreatitis or primary sclerosing cholangitis, as well as those who have had biological therapies for IBD, were not included in the study. In all these cases, the disease activity index [Mayo score] was done.

The Mayo Score for ulcerative colitis assesses disease severity and are used to monitor patients during treatment which involves the following parameters:

- stool frequency,
- rectal bleeding,
- mucosal appearance at endoscopy and
- physician rating of disease activity.

Mayo scores ranges between 0 and 12, with higher scores indicating greater severity. [(2)]

All the colonoscopic biopsies were routinely fixed, processed as per the department histopathology processing protocol. The paraffin blocks prepared were cut at 3-4μm and two unstained slides were prepared. One slide was stained with H & E stain and other slide was used for immunohistochemistry [IgG4].

Histology criteria for diagnosis of UC was Epithelial neutrophil infiltration, cryptitis, crypt abscesses, basal plasmacytosis, ulceration, and erosion were examined on histopathology. [(3)]

Immunohistochemistry was done by antigen retrieval which was accomplished by protease digestion after monoclonal anti-human IgG4 antibody and later applied to 3-4μm thick sections.

Fields with the highest perceived density of IgG4-positive plasma cells, were taken with a 40x objective lens. Cells having plasmacytoid morphology and cytoplasmic immunostaining were counted. A minimum of 5 high power fields (HPFs) in each specimen were counted to determine the number of immunohistochemically detected IgG4-positive plasma cells in the lamina propria, and the mean value was determined as the average point. IgG4-positive plasma cell infiltration was divided into two groups based on average points: IgG4-positive and IgG4-negative. IgG4 positivity was defined as > 10 IgG4-positive PCs/HPF,[(4)] while IgG4 negativity was defined as < 10 IgG4-positive PCs/HPF.

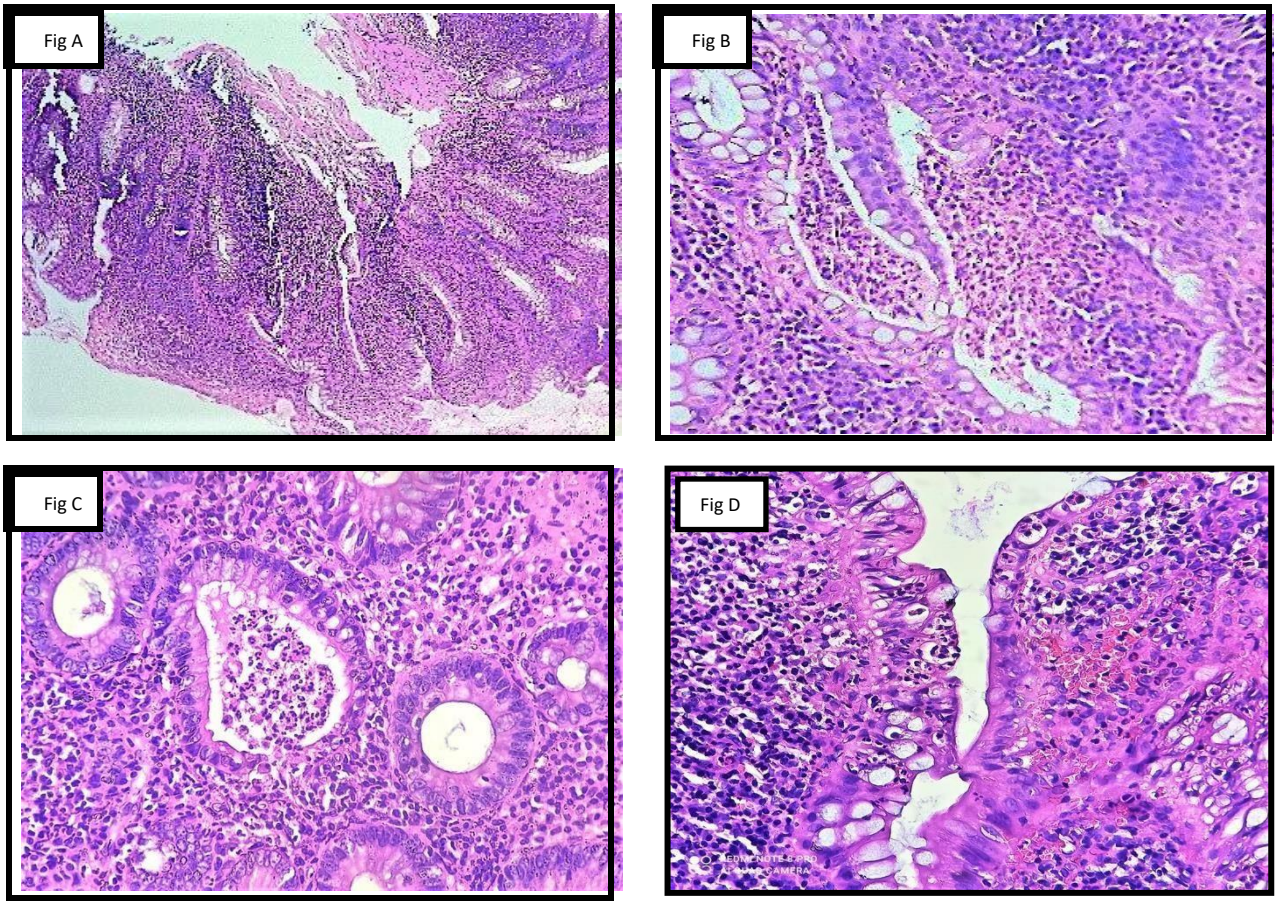
RESULTS:

This study included 44 cases of UC diagnosed on colonoscopy; histopathological examination confirmed 30 cases; remaining 14 cases showed overlapping features of UC and CD and were termed as Indeterminate Colitis (IC). The mean age of the UC was 31.1years (range 15-57years). M:F ratio was 1:0.5

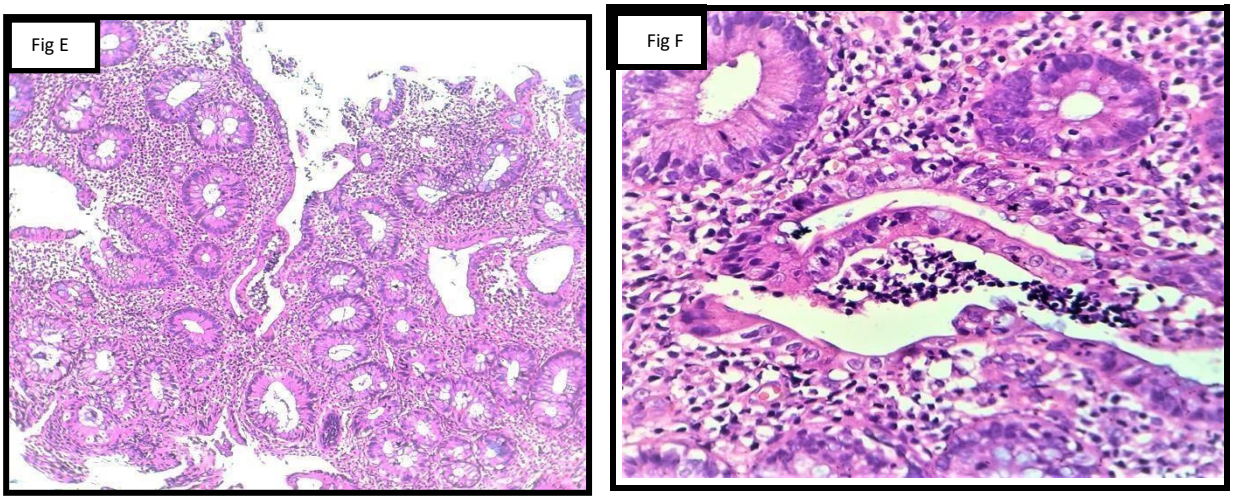
According to Mayo score/ Disease Activity Index, 29 cases (66%) scored as M3, 11 cases (25%) scored as M2, 3 cases (7%) scored as M1 and 1 case (2%) as M0. A total of 30 cases (68%) showed features of UC by routine histopathology [H & E stain] based on above mentioned criteria. The remaining 14 cases (32%) showed overlapping features of UC and CD and hence termed as IC.

Table 1: Shows distribution of number of cases according to Disease Activity Index/Mayo scoring system.

Disease Activity Index	No. of cases
M0	1 (2%)
M1	3 (7%)
M2	10 (23%)
M3	30(68%)



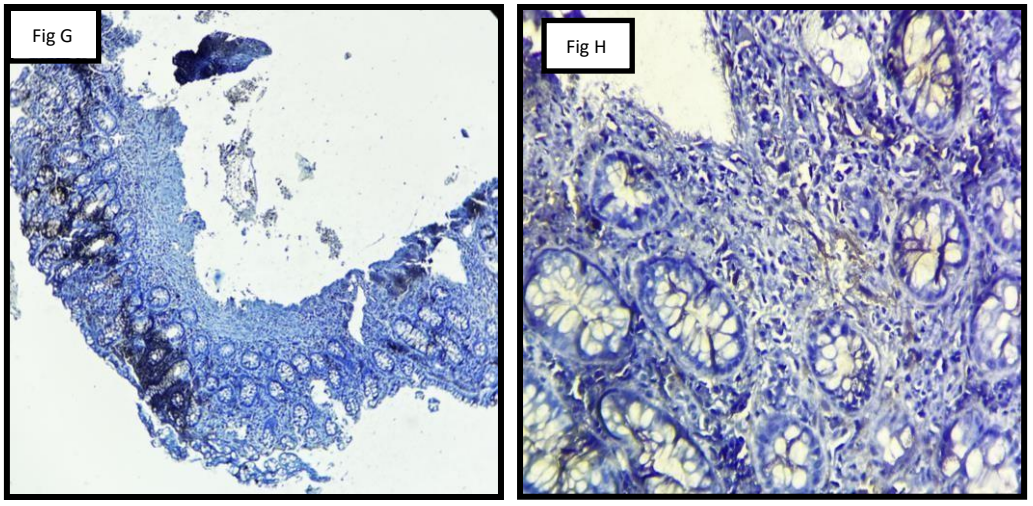
FIGURES (A) 100X,(B) ,(C) & (D) 400x, H&E , shows features of UC with crypt distortion, crypt abscess, mucus depletion and lymphoplasmacytic infiltration.



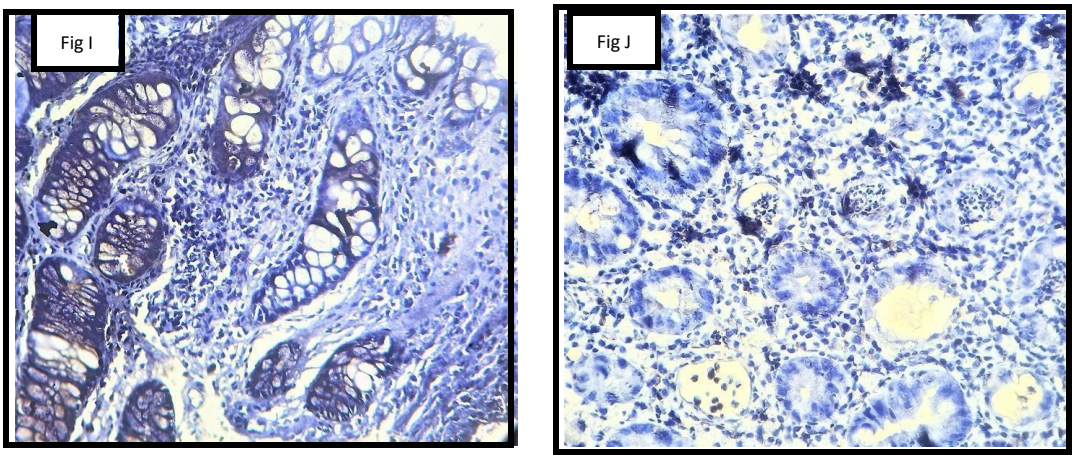
Figures (E) 100X& (F) 400X, H&E, Shows Indeterminate Colitis

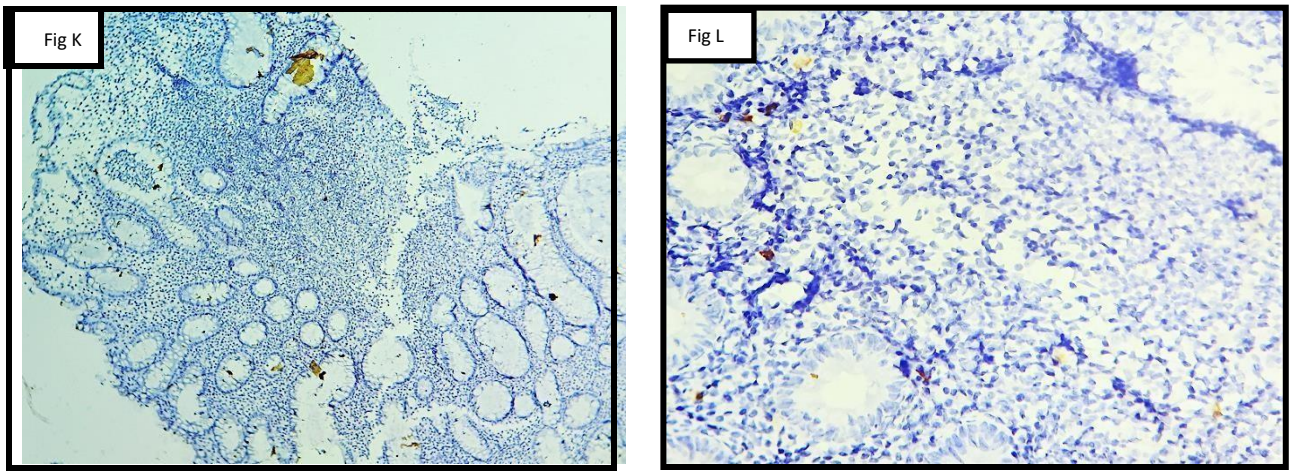
Tissue IgG4 immunohistochemistry:

In the present study of 44 cases of UC, a biopsy fragment with highest chronic inflammation was chosen for IgG4. Of the 44 cases, 30 cases which was confirmed as UC histologically showed IgG4 positivity and 10 cases of indeterminate colitis also showed IgG4 positivity. 4 cases of indeterminate colitis showed IgG4 negativity.



Figures (G) 100X & (H) 400X shows IgG4 positivity





Figures (K) &(L) 400X shows IgG4 negativity.

Correlation between IgG4 counts and Ulcerative Colitis:

In the present study we found a strong correlation between UC and IgG4 which showed a p value of 0.01. In our study we found that cases with Mayo score of M3 (30 cases) were diagnosed as UC on histopathology were IgG4 positive and cases with M0 and M1 (4 cases) were diagnosed as IC on histopathology and were negative for IgG4. We also found that cases with Mayo score of M2(10 cases) showed overlapping features of UC and CD and were diagnosed as IC. On immunohistochemistry IgG4 were positive in all these cases and thus we conclude that IgG4 is proportional to the severity of the disease.

Table 2: Mayo score with Histopathological diagnosis and IgG4 distribution

Mayo score	Histopathological diagnosis	IgG4 distribution
M0	IC (1 case)	Negative
M1	IC (3 cases)	Negative
M2	IC (10 cases)	Positive
M3	UC (30 cases)	Positive

DISCUSSION:

UC is an IBD manifesting flares frequently alternating with periods of remission. The present study investigated a neglected area of significance of tissue-based biomarkers in Ulcerative colitis. Previous studies have confirmed that T lymphocytes are a critical subset of immune cells in the pathogenesis of IBD. Every subset of T cells, including Th1, Th2, Th17, and regulatory T cells, has been reported to play a role in the pathogenesis of IBD. The role of B cells in the pathogenesis of IBD remains unclear. In the present study among 44 cases of UC diagnosed on colonoscopy, males were predominantly affected than the females in comparison with the study conducted by Simsek et al (5). According to Mayo score/ Disease Activity Index, 30 cases (68%) were scored as M3, 10 cases (23%) were scored as M2, 3 cases (7%) were scored as M1 and 1 case (2%) as M0.

All the 30 positive cases of UC on histopathology showed crypt architecture distortion, cryptitis, crypt abscess, basal plasmacytosis and mucus depletion in favor of ulcerative colitis. The remaining 14 cases showed overlapping features of Ulcerative colitis and Crohn's disease, so a definitive diagnosis was not possible, so such cases were termed as Indeterminate colitis.

Immunohistochemistry IgG4 were done for all cases which showed IgG4 positivity in 30 cases of UC cases and 10 cases of IC. 4 cases of IC showed IgG 4 negativity. In comparison to the study conducted by Chen X et al (5) and Kuwata et al (6), IgG4 plasma cell infiltration were seen to be significantly increased in the active case of colitis. In comparison with the study conducted by Simsek et al., (5) we found a strong correlation between UC and IgG4 positivity with a p- value of 0.01.

Many B lymphocytes differentiates into plasma cells with the production of various antibodies. Local signals from innate immune system determines T helper cell polarization. Differentiation into effector or memory T cells. Activated naive B cells migrates to germinal center where they undergo somatic hypermutation and affinity maturation and is differentiated into memory B cells or plasmablasts . IgG4/IgE class switch occurs under influence of cytokines. These antibodies are strongly involved in humoral immunity and may contribute to allergy and multiple autoimmune disorders. High levels of mucosal IgG4 are detected in patients with UC and has been linked to disease activity. Furthermore, these patients are more likely to require colectomy or azathioprine treatment. Tissue infiltration of IgG4+ plasma cells could decrease along with the decline in disease activity after treatment. The above observations imply that IgG4+ B cells may be involved in the immune response in UC.

CONCLUSION

In conclusion, patients with positive colonoscopy for UC and negative on histopathology can be categorized as IgG related colitis or IgG4 systemic related disease. The degree of tissue IgG4 infiltration in UC cases is directly proportional to the disease activity. These findings suggested that IgG4 infiltration appears to be a relevant marker of the inflammatory process caused by immune dysregulation in patients with UC. These patients were more likely to have severe and extensive lesions, and the high levels of mucosal IgG4. Thus, tissue IgG4 increases the diagnostic value of UC cases.

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