





BRIEF REPORT

# Serological response to SARS-CoV-2 is attenuated in patients with inflammatory bowel disease and can affect immunization

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly contagious respiratory virus transmitted through oral/respiratory droplets, has recently caused the devastating COVID-19 pandemic.<sup>1</sup> The COVID-19 infection has spread rapidly around the world and become a major global health problem. It has been hypothesized that neutralizing humoral immune response is required for protection against the pandemic and that the level of anti-SARS-CoV-2 antibodies plays a critical role in the immunity. Moreover, the duration of antibody persistence is directly related to the duration of immunization against the COVID-19 infection.<sup>2</sup>

Inflammatory bowel disease (IBD) is a group of chronic inflammation-mediated conditions of the gastrointestinal tract, including Crohn's disease (CD) and ulcerative colitis (UC).<sup>2</sup> The prevalence of IBD is estimated to be between 0.5% and 1% of the population. Patients with IBD are at a high risk of

malnutrition, which can impair immune response and increase the risk of opportunistic infections.<sup>3</sup> In addition, patients suffering from IBD are commonly treated with immunosuppressive or biological drugs, including corticosteroids, thiopurines (e.g. azathioprine), methotrexate (MTX), anti-tumor necrosis factor (anti-TNF) agents (e.g. infliximab and adalimumab), and other biologics (e.g. vedolizumab and ustekinumab), which can affect immune responses. Therefore, there is some concern that IBD patients might exhibit significantly impaired serological responses and subsequently reduced immunity to COVID-19, especially those treated with immunomodulators.

Recent studies have found no difference between the general population and IBD patients with regard to SARS-CoV-2 infections, except that the latter are mainly asymptomatic.<sup>4</sup> Accordingly, it has been shown that the level of antibodies is low in asymptomatic patients. Concerns increase when the immune system is weakened, which means a decrease in the

anti-SARS-CoV-2 antibody IgG (immunoglobulin G) titer, thus leading to the COVID-19 infection.<sup>5</sup>

Immunosuppressive medications prescribed for patients with IBD could result in limiting the cytokine storm, which is a characteristic of severe COVID-19.<sup>6</sup> This means that these therapeutic approaches might downregulate the immune system of patients who take this type of medication.<sup>7</sup> Kumar *et al.* have found that in patients with IBD who were vaccinated against influenza, an immune response was induced but the use of immunomodulatory therapy was associated with insufficient rates of seroconversion.<sup>8</sup> Therefore, low efficacy of COVID-19 vaccination may be observed among IBD patients compared to other individuals.

The aim of this study was to investigate anti-SARS-CoV-2 IgG and IgM titers in IBD patients diagnosed with COVID-19 compared to a non-IBD cohort with COVID-19 infection.

## Materials and Method

We conducted a multicenter observational study to investigate the seroprevalence of anti-SARS-CoV-2 antibodies between the two cohorts of IBD and non-IBD patients with recently confirmed COVID-19 infection (defined as a recent “positive” result from a reverse transcriptase-polymerase chain reaction test or RT-PCR). Participants were recruited in multiple gastroenterology clinics in Tehran, Mashhad, Qazvin, Karaj, Isfahan, and Bandar Abbass from June to November 2020, during the second and third waves of the COVID-19 outbreak in Iran.

The inclusion criterion for the IBD cohort was all clinically and pathologically confirmed IBD cases that were diagnosed by an expert gastroenterologist in those selected hospitals and with a positive anti-SARS-CoV-2 antibody test, which was confirmed by a PCR test for those who had infection in at least the previous 4 weeks. The exclusion criteria included all patients who had been diagnosed with cancer or other gastrointestinal disorders at the time of the visit and all patients with negative IgM and IgG COVID-19 enzyme-linked immunosorbent assay (ELISA) test results. In addition, the control group was comprised of the general population infected by SARS-CoV-2 and not diagnosed with IBD, other autoimmune diseases, or cancers.

Written informed consent was obtained from all the patients, who then completed a questionnaire. Moreover, a detailed assessment of gastrointestinal symptoms was performed together with the evaluation of IBD activity using either the Crohn’s disease activity index (CDAI) or the partial Mayo score.

The study proposal and protocol were approved by the Ethics Committee of the Research Institute of Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences (Ethical No. IR.SBMU.RIGLD.REC.1399.002).

At the time of visiting, 5 ml of venous blood was collected into a tube containing a clot activator and gel separator. The levels of anti-SARS-CoV-2 IgG and IgM antibodies were measured through ELISA targeting nucleocapsid (N) antigens. Iran’s Food and Drug Administration approved the ELISA kit (Pishtaz Teb, Tehran, Iran; catalog numbers PT-SARS-COV-2. IgM-96 and PT-SARS-COV-2. IgG-96) used to assess antibodies in both IBD patients and controls.

All statistical analyses were performed by SPSS version 26 (IBM Corp, Armonk, NY, USA). Numeric clinical and demographic variables were analyzed for mean and standard deviation (SD) or as the median and confidence interval (CI), and nominal variables are presented as frequencies. The Wilcoxon test and *t*-test were used to compare qualitative and quantitative variables, respectively. Additionally, plots were illustrated via R “ggplot2” and “ggpubr” packages.

## Results

In total, 232 COVID-19-infected persons were enrolled in this study, including 121 patients with IBD and 111 non-IBD controls. In the IBD cohort, 81 (34.4%) patients were diagnosed with UC based on colonoscopic, histological, and clinical requirements, and 40 (17.2%) patients were diagnosed with CD. The median age was  $38.94 \pm 12.51$ ,  $40.48 \pm 13.68$ , and  $44.65 \pm 17.25$  in the UC, CD, and non-IBD groups, respectively. The demographic and clinical characteristics of patients are summarized in Table 1. In our study, the common COVID-19 symptoms included fever (12.9%), cough (9.1%), headache (11.6%), smell and taste loss (12.1%), and myalgia (11.2%). Moreover, the most common gastrointestinal symptoms were abdominal pain (2.2%), nausea (2.6%), and diarrhea (6.9%).

As shown in Table 1, specific anti-SARS-CoV-2 IgG and IgM antibody titers were significantly lower in patients with IBD compared with controls ( $P < 0.001$ ) but no significant difference was observed between CD and UC patients in the IBD group. The average IgG titer was found to be  $2.55 \pm 2.36$  in UC,  $2.89 \pm 4.17$  in CD, and  $5.56 \pm 4.78$  in non-IBD groups ( $P < 0.001$ ). Moreover, the average IgM titer was  $0.67 \pm 0.69$ ,  $0.78 \pm 0.92$ , and  $2.18 \pm 2.40$  among the UC, CD, and control groups, respectively ( $P < 0.001$ ) (Fig. 1). Statistically, no significant association was found between the disease activity indices (i.e. CDAI and Mayo score) and the level of antibodies.

In addition, the correlation between the type of therapy and anti-SARS-CoV-2 antibodies revealed an association between IBD-related therapy and the lower levels of IgM and IgG antibodies compared to the non-IBD group. The results indicated that the proportion of patients with a positive anti-SARS-CoV-2 antibody test was lower in 5 ASA + MTX/AZA as conventional therapy (IgM = 0.61, IgG = 3.06,  $P < 0.00$ ) and anti-TNF/MTX/azathioprine (IgM = 0.81, IgG = 2.67,  $P < 0.00$ ) than in non-IBD control subjects (IgM = 2.09, IgG = 5.38).

## Discussion

It has been hypothesized that there is a decline in antibody levels among immunocompromised patients with asymptomatic or mild COVID-19 infection. We aimed to investigate whether the rate of antibody production in IBD patients with mild COVID-19 infection is the same as that in healthy individuals with positive COVID-19 and mild symptoms. The results of our study show that specific anti-SARS-CoV-2 IgG and IgM titers were significantly lower in IBD patients compared to the controls ( $P < 0.001$ ). Moreover, these findings indicate that there is no statistically significant difference in anti-SARS-CoV-2 antibody titer between UC and CD patients. Furthermore, no significant correlation is found between the CDAI or partial Mayo score and anti-SARS-CoV-2 antibody levels. However, our results need to

**Table 1** Demographic, clinical, and serological characteristics of patients

Clinical characteristics	Overall	UC	CD	Control
Age, year	41.9 ± 15.28	38.94 ± 12.51	40.48 ± 13.68	44.65 ± 17.25
Gender				
Male	116 (50)	34 (42.5)	23 (57.5)	59 (53.2)
Female	116 (50)	47 (57.5)	17 (42.5)	52 (46.8)
Clinical disease activity				
CDAI				
Remission	31 (77.5)	—	31 (77.5)	—
Mild	2 (5)	—	2 (5)	—
Moderate	2 (5)	—	2 (5)	—
Severe	0 (0)	—	0 (0)	—
NA	5 (12.5)	—	5 (12.5)	—
Partial mayo score				
Remission	40 (50)	40 (50)	—	—
Mild	22 (27.5)	22 (27.5)	—	—
Moderate	9 (11.3)	9 (11.3)	—	—
Severe	4 (5)	4 (5)	—	—
IBD-related therapy				
Biological therapy	58 (24.9)	33 (40.2)	25 (64.1)	—
Conventional therapy	63 (27)	49 (58)	14 (35.9)	—
None	112 (48.1)	0	0	112 (100)
COVID-19 serology test				
(IgM)				
Positive	114 (49.5)	26 (31.7)	12 (30.8)	76 (69.7)
Negative	118 (50.5)	56 (68.3)	27 (69.2)	35 (31.3)
Titer	1.41 ± 1.90	0.67 ± 0.69	0.78 ± 0.92	2.18 ± 2.40
(IgG)				
Positive	170 (73.3)	64 (78)	33 (84.6)	76 (67.9)
Negative	62 (26.7)	18 (22)	6 (15.4)	36 (32.1)
Titer	3.99 ± 4.29	2.36 ± 2.55	2.89 ± 4.17	5.56 ± 4.78
IBD-related therapy antibody titer				
(IgM)				
Biological therapy	0.81 ± 0.88	0.69 ± 0.72	0.97 ± 1.05	—
Conventional therapy	0.6 ± 0.64	0.65 ± 0.68	0.43 ± 0.5	—
None	2.18 ± 2.04	—	—	2.18 ± 2.04
(IgG)				
Biological therapy	2.67 ± 3.86	2.06 ± 3.24	2.76 ± 4.62	—
Conventional therapy	2.96 ± 3.84	2.92 ± 3.98	3.11 ± 3.36	—
None	5.56 ± 4.78	—	—	5.56 ± 4.78

Data are expressed as mean ± SD and *N* (%).

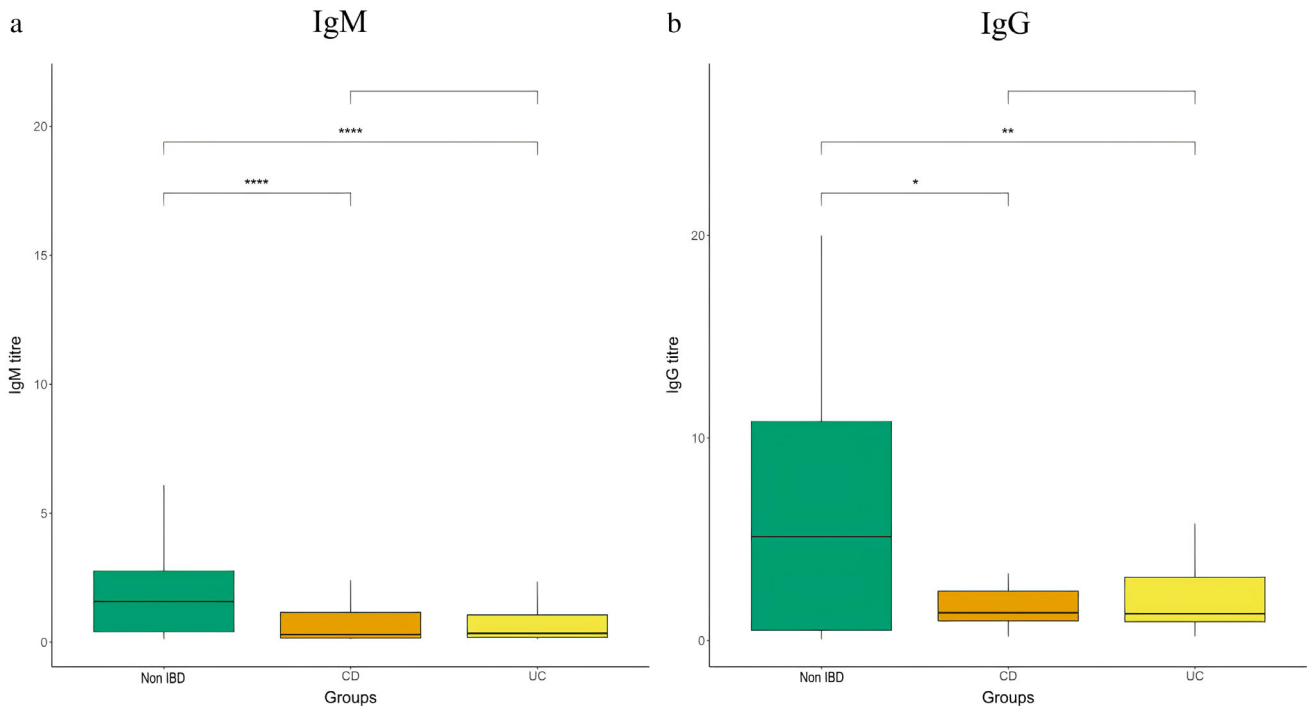
Biological therapy includes anti-TNF monoclonal antibodies (infliximab or adalimumab) and conventional therapy includes 5-ASA (5-aminosalicylic acid), MTX (methotrexate), and AZA (azathioprine).

CD, Crohn's disease; CDAI, Crohn's disease activity index; NA, not available; UC, ulcerative colitis.

be confirmed by further large-scale prospective cohort studies because most of our patients had CDAI and Mayo score between 0 to 1.

It has been reported that seropositivity is correlated with UC phenotype, recent steroid use, non-White ethnicity, higher income-deprivation score, living in the UK region, never being a smoker, no concomitant immunomodulator use, exposure to the COVID-19 patients, reported symptoms of suspected or probable COVID-19, and social distancing measures during the UK government's lockdown period.<sup>9</sup> Moreover, it has been previously shown that the seroprevalence of anti-SARS-CoV-2 antibodies was higher among younger patients with IBD<sup>9</sup>; however, we minimized this potential bias because of the similarity in the average age of IBD patients and the control cohort (41.9 ± 15.28).

In this study, we also investigated the association between a variety of medication options available for IBD patients and antibody status in response to SARS-CoV-2. Importantly, the conventional therapy (5 ASA + MTX/AZA) and biological therapy (anti-TNF) resulted in the lowest average anti-SARS-CoV-2 IgG titers among IBD patients (3.06 and 2.67) compared to the non-IBD controls (5.38) ( $P < 0.00$ ). These findings are consistent with those reported by Chanchlani *et al.* that anti-TNF-treated patients with IBD had attenuated seroconversion anti-SARS-CoV-2 antibody level.<sup>10</sup> Our results showed an association between IBD drugs and low IgG/IgM titer when compared to the non-IBD group with mild symptoms, which may finally increase their susceptibility to recurrent COVID-19 infection.



**Figure 1** (a) Load of IgM in UC, CD and non-IBD group. (b) Load of IgG in UC, CD and non-IBD group. (\**P* value < 0.01, \*\**P* value < 0.001, \*\*\**P* value < 0.0001)

The persistent functioning of antibodies against the COVID-19 has remained an important issue. It has been shown that humoral immunity against SARS-CoV-2 may not last long in people with mild illness.<sup>11</sup> These findings raise a concern about IBD patients' immunization. Their treatment with immunosuppressive medication may lead to a weakened immune system response, and, also, they have shown mild humoral immunity symptoms with a low titer of antibodies against SARS-CoV-2. Finally, these findings could contribute to understanding the efficacy of the COVID-19 vaccination in the IBD population.

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**Data Availability Statement.** All data generated or analyzed during this study are included in this published article.

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