



TNF- α Blockers Showed Prophylactic Effects in Preventing COVID-19 in Patients with Rheumatoid Arthritis and Seronegative Spondyloarthropathies: A Case–Control Study

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ABSTRACT

Introduction: The interaction between angiotensin-converting enzyme 2 (ACE2) and SARS-CoV-2 is a crucial factor in the viral infections leading to the release of inflammatory proteins, such as TNF- α . Thus, it is hypothesized that TNF- α blockers can prevent either COVID-19 incidence or its serious symptoms. TNF- α blockers are prescribed to treat various autoimmune disorders, including rheumatoid arthritis (RA) and seronegative spondyloarthropathies (SpA). Therefore, the objective of this work was to examine this hypothesis that TNF- α blockers can prevent COVID-19 incidence in patients with RA or SpA.

Methods: A case–control study was conducted through interviews based on a structured questionnaire to investigate the frequency of COVID-19 incidence in 254 eligible patients with RA or SpA about whom 45% were under treatment with one type of TNF- α blockers including infliximab, adalimumab, and etanercept at least for 3 months during the COVID-19 pandemic. Interviews were carried out twice, at the beginning and the end of the study (June–December 2020). Patients with COVID-19 during the study or before that were considered as cases. The control group was patients without COVID-19 experience. Data were analyzed using descriptive statistics, and logistic regression was used to determine the relationships between COVID-19 incidence and independent variables.

Results: A small percentage of patients treated with TNF- α blockers (5.22%, 6/115) experienced COVID-19, while a large percentage of patients with COVID-19 did not receive TNF- α blockers (27.34%, 38/139). According to odds ratio, adalimumab, infliximab, and etanercept decreased significantly the risk of developing COVID-19 up to 96.8, 95, and 80.3% ($p < 0.05$), respectively. Therefore, TNF- α blockers could probably decrease the chances of the COVID-19 incidence in patients with RA or SpA.

Conclusions: A direct and positive correlation between the use of TNF- α blockers and a reduction in the incidence of COVID-19 could suggest the prophylactic role of these drugs in

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preventing COVID-19 in patients with RA and SpA.

Keywords: COVID-19; Rheumatoid arthritis; Seronegative spondyloarthropathies; TNF- α blockers

Key Summary Points

Why carry out this study?

The release of inflammatory proteins, such as TNF- α , is a main part of infection with SARS-CoV-2 leading to a cytokine storm (hypercytokinemia).

Assessing the chances of developing COVID-19 in patients receiving TNF- α blockers to treat their autoimmune diseases could provide a valuable research opportunity to test the hypothesis that TNF- α blockers are able to prevent either COVID-19 incidence or its serious symptoms.

What was learned from the study?

TNF- α blockers including adalimumab, infliximab, and etanercept decreased significantly the risk of developing COVID-19 in patients with rheumatoid arthritis (RA) and seronegative spondyloarthropathies (SpA).

The low incidence of COVID-19 in patients treated with TNF- α blockers compared with the high percentage of COVID-19 in patients who did not receive any TNF- α blockers could confirm the prophylactic role of these drugs in preventing COVID-19 in patients with rheumatoid arthritis (RA) and seronegative spondyloarthropathies (SpA).

INTRODUCTION

Coronaviruses (CoVs) are a group of pathogenic enveloped positive-sense RNA viruses classified into four genera, including the alpha, beta, gamma, and delta coronaviruses, which belong to the *Nidovirales* order and Coronaviridae family [1]. They infect mammals and birds and cause some respiratory and intestinal diseases in humans [2, 3]. So far, seven human coronaviruses with different degrees of pathogenesis from mild to severe, including HCoV-229E, HCoV-OC43, HCoV-NL63, HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2, have been identified [3]. Since December 2019, SARS-CoV-2 has been introduced as the main factor of a severe acute respiratory syndrome called COVID-19 with a high rate of mortality. This disease is spreading with high prevalence in the human population, and there is currently no definitive and preventative treatment for it [4].

Angiotensin-converting enzyme 2 (ACE2) has been introduced as a specific receptor for SARS-CoVs, which leads to the virus' entrance into the host cells that express ACE2 [5, 6]. ACE2/SARS-CoV 2 interaction causes the activation of a sheddase protein, ADAM17, also named tumor necrosis factor- α -converting enzyme (TACE). Shedding activity of ADAM17 also targets the extracellular domains of other transmembrane proteins, including IL-6 receptor (IL-6Ra) and pro-TNF- α , which causes the formation of inflammations by releasing the soluble form of IL-6 receptor and TNF- α as a consequence of the SARS-CoV-2 infection [7, 8]. Since this process is highly dependent on the production of soluble TNF- α and IL-6R, the use of TNF- α blockers and IL-6R inhibitors may reduce virus activity and tissue damage.

Tumor necrosis factor alpha (TNF- α) blockers are biologic disease-modifying anti-rheumatic drugs (bDMARDs) used to treat inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, psoriasis, and inflammatory bowel disease [9–11]. Approved TNF- α blockers/inhibitors for clinical use include a chimeric monoclonal antibody (infliximab), two human monoclonal antibodies (adalimumab and golimumab), a fragment of a human monoclonal

antibody (certolizumab pegol), and a human fusion protein (etanercept) [10]. In addition to the therapeutic benefits of TNF- α blockers, a higher risk of serious infections with various bacteria (e.g., *Mycobacteria* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus pneumoniae*) viruses (e.g., *Varicella zoster*, Herpes virus, hepatitis B/C virus) and fungi/protozoa (e.g., *Candida*, *Leishmania* spp.) has been reported in patients taking these drugs [12]. However, previous studies have shown that the use of TNF- α blockers probably has not been associated with poor prognosis or mortality caused by influenza [13].

According to the proposed mechanism for the role of TNF- α in the infection caused by the coronavirus and also the possible effects of TNF- α blockers in reducing virus activity, we decided to evaluate the prophylactic effects of these drugs in getting COVID-19 in autoimmune patients with rheumatoid arthritis (RA) and seronegative spondyloarthropathies (SpA) treated with TNF- α blockers for at least 3 months. Therefore, the objective of this study was to examine the hypothesis that TNF- α blockers can prevent COVID-19 incidence.

METHODS

Two hundred and sixty patients with RA or SpA whose medical status had been recorded in hospitals and rheumatology clinics of the Isfahan University of Medical Sciences were considered for this case-control study. Data for this study were collected from June 2020 to December 2020. Based on the available medical records of the patients, telephone contact was made with each patient, and interviews were carried out with them (Fig. 1). Oral and written consent was obtained from all patients. According to the items in the questionnaire designed for this study (Table 1), all patients ($n = 254$) who agreed to participate in this study were interviewed twice and their answers were recorded, once at the beginning of the study (June 2020) and after 6 months of follow-up at the end of the study (December 2020). Patients were aged from 9 to 96 years old and comprised 64 men and 190 women. The cases in this study

were the confirmed patients with RA or SpA who either developed COVID-19 before interviews or had COVID-19 at interview time, and their disease was confirmed by RT-PCR, CT scan of the lung, and treatment in medical centers ($n = 44$). Patients who did not experience COVID-19 at all until the end of the study were included in the control group ($n = 210$). Consuming TNF- α blockers was considered as exposure and getting COVID-19 as an outcome. Inclusion criteria were having RA or SpA for more than 6 months, not taking ACEI (ACE inhibitors), and having sufficient mental and physical ability to participate in this study. In addition to the inclusion criteria listed for all patients, patients who received TNF- α blockers should have been treated with these inhibitors for at least 3 months during the COVID-19 pandemic. No answering the phone call after three follow-ups, smoking, and having malignancy were considered as exclusion criteria. Finally, 254 patients were included in this study, and six patients were excluded. The data source for both cases and controls were hospitals, outpatient clinics records, and interviews based on a structured questionnaire. All registered RA and SpA patients were included as the study subjects without sampling (census). All patients included in this study continued to receive their drugs (DMARDs) when they were infected with SARS-CoV-2 virus. Data were analyzed using descriptive statistics (absolute frequency, relative frequency, mean and standard deviation). Variables including gender, age, type of autoimmune diseases, course of autoimmune diseases, type of DMARDs (prednisolone, hydroxychloroquine, azathioprine, methotrexate, sulfasalazine), type of TNF- α blockers (infliximab, etanercept, adalimumab), background diseases (cardiovascular diseases, hypertension, pulmonary diseases, diabetes), travel and close contact with COVID-19 patients were used as independent variables for comparison of frequencies between case (patients with COVID-19) and control (patient without COVID-19) groups by the Chi-square test (Table 1).

To determine the relationships between the dependent variable (COVID-19 incidence) and independent variables listed above, logistic

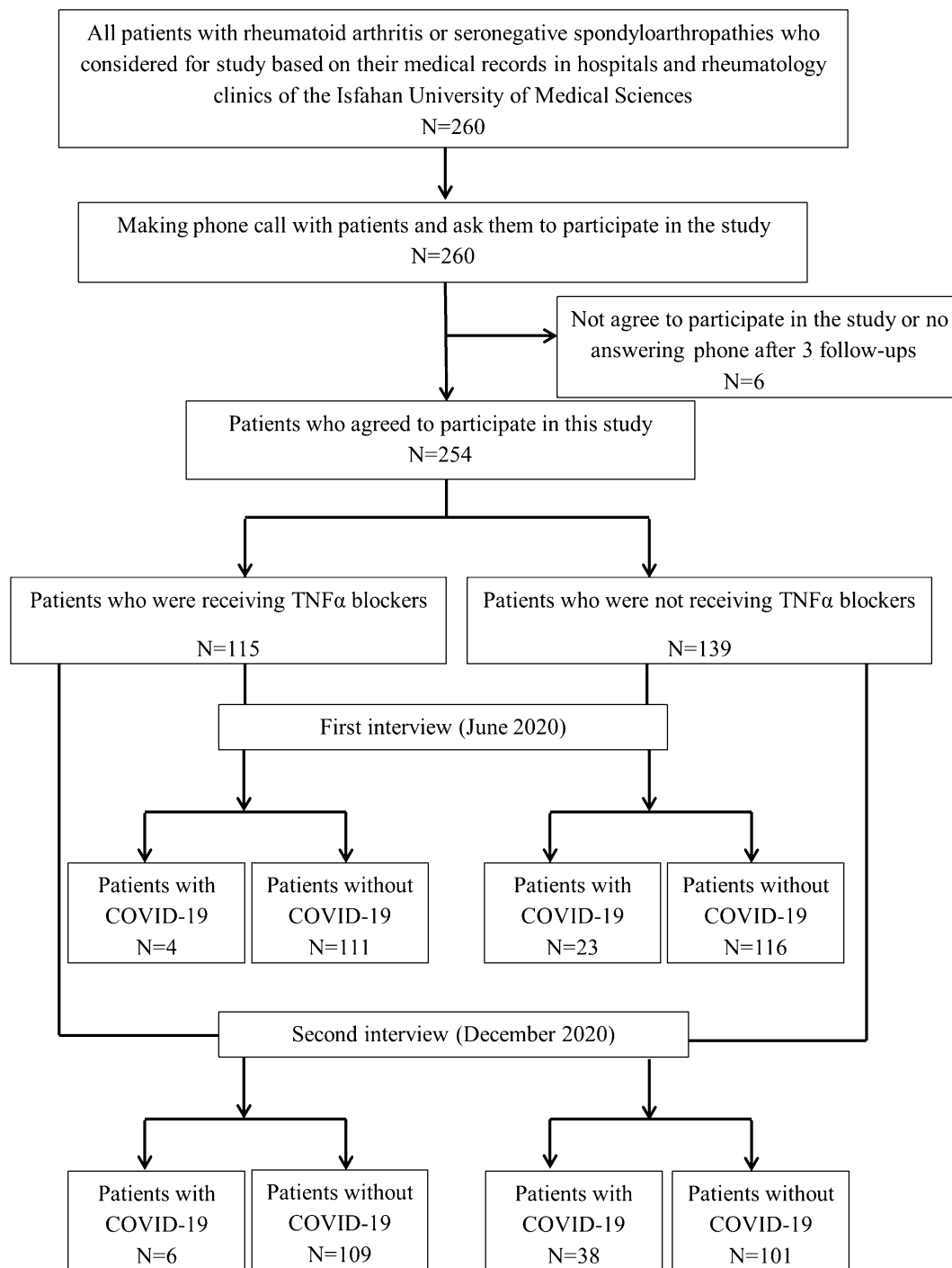


Fig. 1 Flowchart showing the different steps of data collection

regression was used. Based on the results of the Chi-square test, those variables whose different levels showed statistically significant differences between the case and control groups were used

to establish a logistic regression model. However, two variables, including travel and close contact with COVID-19 patients, were excluded from the model because of the intervention of

Table 1 Demographic characteristics of 254 patients with rheumatoid arthritis or seronegative spondyloarthropathies, absolute and relative frequencies of patients responding to questionnaire items in control and case groups

Case (<i>n</i> = 44)		Control (<i>n</i> = 210)		Case vs. control <i>p</i> value
Characteristics	Frequency <i>n</i> , (%)	Characteristics	Frequency <i>n</i> , (%)	
Age in years, mean (49.27) ± SD (12.5)		Age in years, mean (50.38) ± SD (14.7)		0.641
Age ranking		Age ranking		
Age ≤ 20	0	Age ≤ 20	4, (1.9)	–
20 < age ≤ 40	9, (20.5)	20 < age ≤ 40	49, (23.3)	0.763
40 < age ≤ 60	25, (56.8)	40 < age ≤ 60	105, (50.0)	0.499
60 < age ≤ 80	10, (22.7)	60 < age ≤ 80	50, (23.8)	0.884
80 < age ≤ 100	0	80 < age ≤ 100	2, (1)	–
Gender		Gender		
Female	40, (90.9)	Female	150, (71.4)	0.116
Male	4, (9.1)	Male	60, (28.6)	0.001*
Type of autoimmune disease		Type of autoimmune disease		
Seropositive rheumatoid arthritis (RA) +	33, (75)	Seropositive rheumatoid arthritis (RA) +	137, (65.2)	0.398
Seronegative rheumatoid arthritis (RA) -	1, (2.3)	Seronegative rheumatoid arthritis (RA) -	31, (14.8)	0.002*
Ankylosing spondylitis (AS)	7, (15.9)	Ankylosing spondylitis (AS)	31, (14.8)	0.857
Psoriatic arthritis (PsA)	2, (4.5)	Psoriatic arthritis (PsA)	7, (3.3)	0.480
Behçet's disease (BD)	1, (2.3)	Behçet's disease (BD)	4, (1.9)	1.000
Course of autoimmune diseases		Course of autoimmune diseases		
1–5 years	35, (79.5)	1–5 years	113, (53.8)	0.025*
6–10	6, (13.6)	6–10	43, (20.5)	0.237
11–15	1, (2.3)	11–15	27, (12.9)	0.005*
16–20	1, (2.3)	16–20	14, (6.7)	0.096
21–25	0	21–25	2, (1)	–
26–30	1, (2.3)	26–30	7, (3.3)	0.655
31–35	0	31–35	2, (1)	–
36–40	0	36–40	1, (0.5)	–
41–45	0	41–45	0	–
46–50	0	46–50	1, (0.5)	–

Table 1 continued

Case (<i>n</i> = 44)		Control (<i>n</i> = 210)		Case vs. control <i>p</i> value
Characteristics	Frequency <i>n</i> , (%)	Characteristics	Frequency <i>n</i> , (%)	
DMARDs		DMARDs		
Yes	41, (93.2)	Yes	210, (100)	0.614
No	3, (6.8)	No	0	–
Type of DMARDs		Type of DMARDs		
Prednisolone	31, (70.5)	Prednisolone	135, (64.3)	0.547
Hydroxychloroquine	28, (63.6)	Hydroxychloroquine	92, (43.8)	0.054
Azathioprine	1, (2.3)	Azathioprine	5, (2.4)	1.000
Methotrexate	29, (65.9)	Methotrexate	122, (58.1)	0.472
Sulfasalazine	4, (9.1)	Sulfasalazine	51, (24.3)	0.009*
Infliximab	1, (2.3)	Infliximab	29, (13.8)	0.003*
Etanercept	4, (9.1)	Etanercept	39, (18.6)	0.059
Adalimumab	1, (2.3)	Adalimumab	41, (19.5)	0.000*
TNF- α blockers		TNF- α blockers		
Yes	6, (13.64)	Yes	109, (51.90)	0.000*
No	38, (86.36)	No	79, (37.62)	0.000*
Background diseases		Background diseases		
Yes	17, (38.64)	Yes	62, (29.52)	0.279
No	27, (61.36)	No	148, (70.48)	0.432
Type of background diseases		Type of background diseases		
Cardiovascular disease	3, (6.8)	Cardiovascular disease	15, (7.1)	1.000
Hypertension	11, (25)	Hypertension	44, (21.0)	0.555
Pulmonary disease	6, (13.6)	Pulmonary disease	9, (4.3)	0.018*
Diabetes	5, (11.4)	Diabetes	14, (6.7)	0.346
Close contact with COVID-19 patients		Close contact with COVID-19 patients		
Yes	25, (56.8)	Yes	3, (1.43)	0.000*
No	19, (43.2)	No	207, (98.57)	0.000*
Travel		Travel		
Yes	5, (11.4)	Yes	3, (1.43)	0.004*
No	39, (88.6)	No	207, (98.57)	0.379

Table 1 continued

Case (<i>n</i> = 44)		Control (<i>n</i> = 210)		Case vs. control <i>p</i> value
Characteristics	Frequency <i>n</i> , (%)	Characteristics	Frequency <i>n</i> , (%)	
Symptoms for COVID-19%		Symptoms for COVID-19 (%)		
Yes	42, (95.45)	Yes	1, (0.48)	–
No	2, (4.55)	No	209, (99.52)	0.000*
Type of COVID-19 symptoms (%)		Type of COVID-19 symptoms%		
Sneeze	1, (2.3)	Sneeze	1, (0.48)	–
Nasal discharge	0	Nasal discharge	1, (0.48)	–
Dry cough	22, (50)	Dry cough	0	–
Productive cough	2, (4.5)	Productive cough	1, (0.48)	–
Headache	20, (45.5)	Headache	0	–
Muscle pain	28, (63.6)	Muscle pain	0	–
Abdominal pain	5, (11.4)	Abdominal pain	0	–
Nausea	15, (34.1)	Nausea	0	–
Vomiting	5, (11.4)	Vomiting	0	–
Diarrhea	6, (13.6)	Diarrhea	1, (0.48)	–
Weakness	28, (63.6)	Weakness	0	–
Dyspnea	19, (43.2)	Dyspnea	0	–
Fever	20, (45.5)	Fever	0	–
Anosmia	10, (22.7)	Anosmia	0	–
Severity of COVID-19				
Low	26, (59.1)			
Moderate	14, (31.8)			
High	4, (9.1)			
COVID-19 remission				
No	0			
Partial	0			
Complete	44, (100)			
Death caused by COVID-19				
Yes	0			
No	44, (100)			

The control group includes patients who did not experience COVID-19 (*n* = 210). The case group is patients with COVID-19 (*n* = 44)

**p* < 0.05

patients in the conscious prevention of COVID-19 by following approved health protocols, which leads to a deliberate low incidence of COVID-19 in these patients and nullifying the odds ratio. Logistic regression was also used to control confounders, including gender, the type of DMARDs except for TNF- α blockers and the course of autoimmune disease. Hosmer and Lemeshow test, classification table, and variables in the equation were used to show the goodness of fit of the established model, categorization results, and model parameters, respectively. Odds ratio ($\text{Exp}(B)$), p value, and 95% confidence interval for $\text{Exp}(B)$ were used for analysis and inference. According to the results obtained from the table of variables in the equation, we did further calculation and analysis to determine the impact factor of variables with significant ($p < 0.05$) odds ratio (OR) on the percentages of COVID-19 incidence and COVID-19 prevention and included their results in Table 2. For each variable, the inverse of the odds ratio of COVID-19 incidence was considered as odds ratio of COVID-19 prevention. The impact factor of variables could indicate the chance value of each variable to develop and prevent COVID-19.

All statistical analyses were performed with SPSS 21 software. Microsoft Excel 2010 was used to draw the bar graphs and pie charts. Demographic characteristics and relative frequency of patients responding to questionnaire items are summarized in Table 1. This study was carried out based on the ethical principles of the Declaration of Helsinki, and all study procedures were approved by the Isfahan University of Medical Science Ethics Committee (Code No. IR.MUI.MED.REC.1399.380). STROBE checklist was considered to write this manuscript.

RESULTS

The analysis of data obtained from questionnaires showed that among 254 patients (190 women and 64 men) participating in this study with different autoimmune diseases (RA or SpA), 44 patients (17.32%), including four men and 40 women experienced COVID-19 (Fig. 2). Although 115 patients were under treatment

with one type of TNF- α blockers (infliximab, etanercept, adalimumab), a large percentage of them (94.78%), equivalent to 42.91% of total patients (254 patients), did not develop COVID-19 (Fig. 3).

All patients (100%) with a disease period of more than 15 years were treated with one type of TNF- α inhibitors. In addition, from 148 patients with a disease course of 1–5 years, 35 got COVID-19, which was the highest percentage of COVID-19 incidence (23.64%) among different categories of disease course. Meanwhile, 41 patients in the group of disease course of 1–5 years were under treatment with TNF- α inhibitors. In terms of gender distribution, a higher percentage of men received the inhibitor [67.18% (43/64) men vs. 37.89% (72/190) women]. A lower incidence of COVID-19 was observed in men in comparison with women [6.25% (4/64) men vs. 21.05% (40/190) women].

Our data could also confirm that TNF- α inhibitors may mitigate or ameliorate the COVID-19 disease course. Our results have shown that in the group of patients receiving TNF- α inhibitors ($n = 115$), only six patients experienced COVID-19, and the number of patients with high severity of COVID-19 ($n = 2$) was half the number of patients with low severity of COVID-19 ($n = 4$). All these patients suffering COVID-19 recovered completely, and there were no deaths caused by COVID-19.

According to the Chi-square test, there was a statistically significant difference ($p = 0.000$) in the frequency of COVID-19 incidence among patients with autoimmune diseases. The Chi-square analysis showed no significant relationship between COVID-19 and age, type of autoimmune diseases, and background diseases. However, the Chi-square test of independence confirmed that four parameters including gender ($p = 0.002$), course of autoimmune diseases ($p = 0.02$), taking DMARDs ($p = 0.000$), and type of TNF- α blockers ($p = 0.000$) are not independent of COVID-19.

Based on the logistic regression analysis in this study, the logistic model could predict the effects of independent variables (gender, course of autoimmune diseases, and type of DMARDs) on the dependent variable (COVID-19) with

Table 2 Regression coefficients, standard errors, Wald's test, odds ratio with 95% CI and variable impact factor on incidence and prevention of COVID-19 in patients with rheumatoid arthritis or seronegative spondyloarthropathies

Variables ^a	B	SE	Wald	p value	Exp(B) (Odds ratio)	95% CI for EXP(B)		Variable impact factor for variables with significant OR	
						Lower	Upper	COVID-19 incidence (%) Exp(B) × 100	COVID-19 prevention 1/Exp(B) (times) or 100 – [Exp(B) × 100]
Gender	0.763	0.588	1.681	0.195	2.144 ns	0.677	6.793		
Course of autoimmune diseases	– 0.208	0.243	0.732	0.392	0.813 ns	0.505	1.307		
Infliximab	– 2.986	1.156	6.668	0.010	0.050*	0.005	0.487	5%	20 times or 95%
Etanercept	– 1.624	.750	4.688	0.030	0.197*	0.045	0.857	19.7%	5.08 times or 80.3%
Adalimumab	– 3.450	1.274	7.337	0.007	0.032*	0.003	0.385	3.2%	31.2 times or 96.8%
Sulfasalazine	– 1.345	0.651	4.272	0.039	0.261*	0.073	0.933	26.1%	3.8 times or 73.9%
Methotrexate	– 1.022	0.482	4.501	0.034	0.360*	0.140	0.925	36%	2.8 times or 64%
Azathioprine	1.841	1.491	1.524	0.217	6.300 ns	0.339	116.993		
Hydroxychloroquine	– 0.023	0.459	0.002	0.960	0.977 ns	0.397	2.404		
Prednisolone	– 0.277	0.445	0.387	0.534	0.758 ns	0.317	1.814		
Constant	– 0.884	1.274	0.482	0.488	0.413 ns				

ns Not significant

* < 0.05

^a Variable(s) entered on step 1: gender, course of autoimmune diseases, infliximab, etanercept, adalimumab, sulfasalazine, methotrexate, azathioprine, hydroxychloroquine, prednisolone

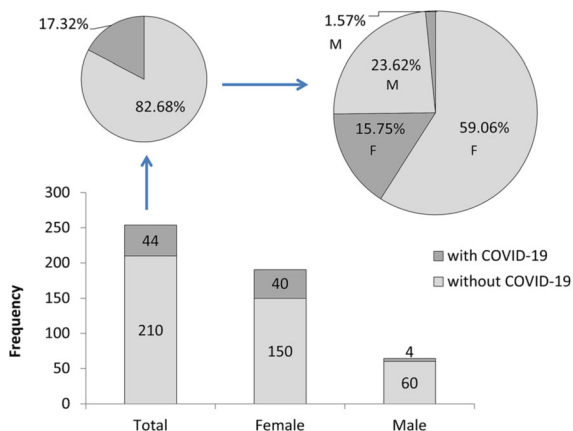


Fig. 2 The absolute frequency and relative frequency of COVID-19 in women and men with rheumatoid arthritis or seronegative spondyloarthropathies. The letters F and M stand for female and male, respectively

84.3% efficiency. In other words, the model could predict correctly by 84.3% the relationship between COVID-19 incidence and gender, course of autoimmune diseases, and type of DMARDs. In addition, Hosmer and Lemeshow test indicated the goodness of fit of the established model (Chi-square = 3.614 $p = 0.823$). Table 2 shows that all three TNF- α blockers, methotrexate, and sulfasalazine significantly decreased the odds of COVID-19 incidence in patients with RA or SpA. According to odds ratio (OR) data, the risk of developing COVID-19 was significantly decreased up to 31.25, 20, 5.076, 3.83, and 2.78 times ($p < 0.05$) with the use of adalimumab, infliximab, etanercept, sulfasalazine, and methotrexate, respectively. Among the drugs that significantly decreased the odds of COVID-19, the highest percentage of impact on the prevention of COVID-19 was related to adalimumab (96.8%) while methotrexate had the lowest effect (64%) on preventing COVID-19. Although the OR for COVID-19 incidence in women is approximately two times in comparison with men, the effect of gender on developing COVID-19 in combination with other independent variables is not statistically significant. However, if gender was the only independent variable included in this model, its effect would be statistically significant.

DISCUSSION

The COVID-19 pandemic has led to world-wide investigations to characterize the molecular mechanisms of cellular infection and inflammation by SARS-CoV-2. The release of TNF- α is one of the pro-inflammatory proteins caused by the interaction between angiotensin-converting enzyme 2 (ACE2) and SARS-CoV-2 [7]. There is a hypothesis that TNF- α blockers play a critical role in preventing either COVID-19 incidence or its serious symptoms. We investigated the effects of three TNF- α blockers, including infliximab, adalimumab, and etanercept, on the frequency of COVID-19 incidence in patients with RA or SpA. In fact, our study on patients with these inflammatory diseases who were also receiving TNF- α blockers provided valuable results to confirm this hypothesis in humans under real conditions.

Our results indicated that a small percentage of patients taking TNF- α blockers (about 5%, which is equal to six out of 115 patients) showed COVID-19 symptoms; whereas, about 27% of patients (38 out of 139 patients) who were not under treatment with TNF- α blockers developed COVID-19. The comparison of these percentages implies that TNF- α blockers probably decreased the chances of COVID-19 incidence. This significant result may be related to the function of TNF- α blockers in the inhibition of TNF- α activity. TNF- α mediates different biological activities through binding to its receptors TNFR1 or TNFR2. The level of TNF- α increases in response to pathogens, such as bacteria and viruses, and this pro-inflammatory cytokine is responsible for inflammations [14]. As mentioned above, the release of TNF- α occurs during infection with SARS-CoV-2, which along with an excessive release of other pro-inflammatory cytokines causes a cytokine storm (hypercytokinemia) [15]. According to previous studies, the rapid progression of COVID-19, multiple organ failure, and mortality will happen following the cytokine storm in patients with COVID-19. Currently, an effective strategy in the treatment and rescue of patients with COVID-19 is the control of the cytokine storm [16]. It was also reported that treatment of

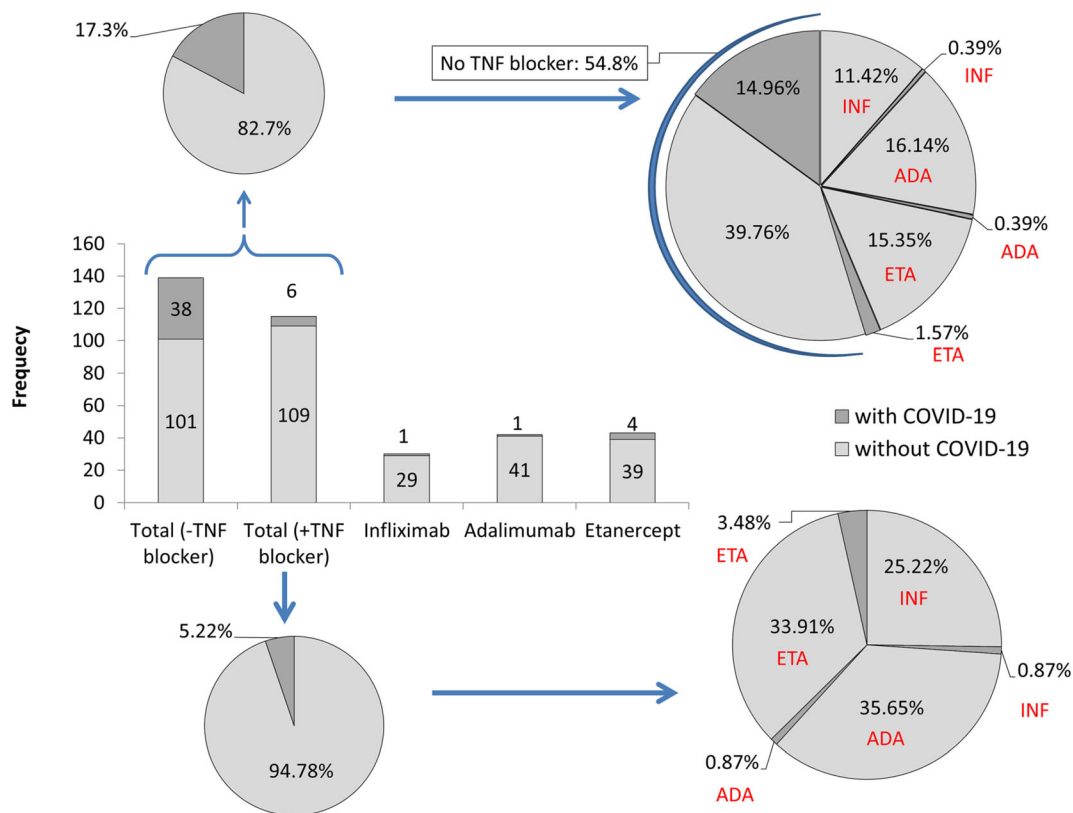


Fig. 3 The absolute frequency and relative frequency of COVID-19 in patients with rheumatoid arthritis or seronegative spondyloarthropathies who received either

TNF- α blockers (+ TNF- α blockers) including infliximab (INF), etanercept (ETA) and adalimumab (ADA) or not (-TNF- α blockers)

patients with immune-mediated inflammatory diseases with cytokine inhibitors could reduce the risk of SARS-CoV-2 infection [17]. Therefore, TNF- α blockers could be considered as a potential candidate for this treatment strategy. For example, it was reported that the severity of COVID-19 symptoms and the need for hospitalization were lower in patients with inflammatory bowel disease (IBD) who were receiving anti-TNF treatments [18]. In another study, no correlation between consumption of TNF- α blockers and increased severity of COVID-19 in patients with rheumatic diseases has been observed [19]. Moreover, the results obtained from data of the COVID-19 Global Rheumatology Alliance registry indicated that anti-TNF decreased the chance of hospitalization caused by COVID-19 in patients suffering from rheumatic disease [20] and TNF inhibitors are not

associated with death caused by COVID-19[21]. These mentioned reports are also in consist with our results that only two patients receiving TNF- α blocker (etanercept) experienced a high severe form of COVID-19, and there was no death caused by COVID-19. Currently, drugs blocking inflammatory cytokines, such as TNF- α inhibitors, are used in the treatment of severe cases of COVID-19 [22].

Although TNF- α contributes to antibody production, an excessive increase in TNF- α and its resulting cytokine storm, in addition to inducing sepsis, leads to damage to the lymphoid germinal centers and disruption of the production of antibodies produced by B cells. As a result, it could be the main reason for the weak and transient antibody response in COVID-19. However, the restoration of the immune system and antibody production in

malaria-infected mice by removing excess TNF [23] can draw attention to the use of anti-TNF drugs to rebuild the immune system destroyed by COVID-19. Moreover, TNF- α inhibitors can probably decrease the level of other pro-inflammatory cytokines, including IL-6, in patients with COVID-19 as the effect of anti-TNF therapy on down regulation of IL-6 has been shown in both serum and synovial tissue cultures from patients with rheumatoid arthritis [24].

The results of previous studies [18–20, 25] showed the positive effects of anti-TNF drugs on reducing the severity of COVID-19 and hospitalization percentage. Moreover, our results indicated the effect of TNF- α inhibitors in decreasing the incidence of COVID-19. According to the molecular pathways of cell infection with SARS-CoVs [7, 26], a negative feedback mechanism can be supposed for the low rate of COVID-19 in patients treated with TNF- α blockers. In fact, TNF- α inhibitors, by preventing the release of the active form of TNF, probably lead to the inhibition of shedding activity of ADAM17 and cleavage of the extracellular domain of ACE2 protein, a specific receptor for SARS-CoV-2, and consequently can block the interaction of ACE2 with SARS-CoV-2 and viral infections.

Moreover, the results of our study showed that the percentage of COVID-19 was significantly higher in patients treated with etanercept than in patients who received infliximab and adalimumab [9.03% (4/43) for ETA vs. 3.3% (1/30) and 2.4% (1/42) for INF and ADA, respectively]. Similarly, in a study on patients with RA or spondyloarthritis, it has been shown that 50% of these patients taking etanercept developed COVID-19 while no case of COVID-19 was observed among patients receiving adalimumab [27]. In addition, according to a case report, a 60-year-old man with spondyloarthritis developed COVID-19 just 2 days after the weekly subcutaneous injection of 50 mg etanercept [28]. The variations in molecular structure, pharmacokinetic nature, and action mechanism of these TNF- α blockers may be a reason for their effectiveness in preventing COVID-19. TNF- α blockers prevent the binding and reacting of TNF- α with its receptors in a

variety of ways depending on the type of inhibitor. For example, infliximab and adalimumab are monoclonal antibodies that can specifically bind to both active soluble and membrane-bound forms of TNF- α with high affinity, and consequently inhibit the reaction of TNF- α with its receptors. In contrast, etanercept is a fusion protein that competes with soluble TNF- α to bind the cell surface receptors of TNF [29].

Our study showed that a higher percentage of people with a long course of the disease received TNF- α inhibitors. So that all patients in groups of disease course more than 15 years were treated with one type of TNF- α inhibitors. Moreover, by comparing the population of men and women treated with TNF- α inhibitors, a higher percentage of men received the inhibitor [67.18% (43/64) men vs. 37.89% (72/190) women]. These results imply that gender and the duration of the disease might be determinant factors that lead to a shift of treatment strategy for patients with rheumatic diseases from prescribing cDMARDs or tsDMARD to the use of biological drugs (bDMARDs) such as TNF- α inhibitors due to inadequate response to csDMARDs or tsDMARD. For example, there is a lower persistence of anti-TNF- α therapy in females than males because of more side effects in women. In addition, studies showed that men and women respond differently to biological treatments. In fact, in women, immune-stimulating therapies (e.g., vaccines) are more effective, while the effectiveness of immunosuppressive therapies (e.g., TNF- α inhibitors) is higher in men than in women [30, 31].

The highest percentage of COVID-19 incidence was also observed among patients with a disease course of 1–5 years (23.64%, 35/148). By comparing disease courses, the ratio of patients with COVID-19 in a specific disease course to the total of patients in the same disease course decreased significantly by increasing the course of the disease. So that in disease courses more than 25 years, the patients with COVID 19 were not observed, except for one case for a disease course of 26–30 years. It seems that passing time and taking DMARDs for a long time leads to recovery of the immune system and a decrease in the chance of getting COVID-19. In addition, the incidence of COVID-19 was lower in men

than in women [6.25% (4/64) men vs. 21.05% (40/190) women]. This result contradicts previous studies on the effect of gender on the incidence of COVID-19, which stated that men are more likely to be infected with SARS-CoV-2 than women, which has been attributed to X-linked heredity of angiotensin-converting enzyme 2 (ACE2), higher expression of ACE2 in men, and the effect of female sex hormones on activity and expression of ACE2 [32, 33].

In addition to the results related to prophylactic effects of TNF- α blockers on preventing COVID-19, our results also showed that methotrexate and sulfasalazine significantly reduced the chances of developing COVID-19. Inhibition of nucleoside biosynthesis by methotrexate leads to impaired DNA/RNA synthesis and cell division, which may also destroy SARS-CoV-2 genome replication and prevent viral infection. Furthermore, methotrexate significantly decreases the level of inflammatory cytokines, such as IL-6 and TNF- α [34, 35]. The inhibitory effect of sulfasalazine on TNF- α expression can be the main reason for preventing SARS-CoV-2 infection [36]. Indeed, sulfasalazine might mimic the role of TNF- α blockers with an almost similar mechanism in decreasing COVID-19 incidence. In a previous study, it was already reported that no patients with RA or spondyloarthritis receiving sulfasalazine experienced COVID-19 [27]. Furthermore, a low percentage of patients receiving different DMARDs showed the severe form of COVID-19 and were hospitalized, implying that taking DMARDs did not increase the severity of COVID-19 in patients with RA and SpA. Similarly, the study of effective factors in hospitalization for COVID-19 demonstrated that DMARDs were not associated with a higher odds ratio of hospitalization risk for COVID-19 [37]. Although previous studies suggested that hydroxychloroquine may inhibit cell entry of SARS-CoV-2 [38], our results showed that the OR for COVID-19 incidence in patients receiving hydroxychloroquine was not statistically significant. Therefore, it is impossible to conclude that hydroxychloroquine can prevent COVID-19 incidence. Despite a report on the effective role of glucocorticosteroids in increasing the chances of hospitalization due to

COVID-19 in patients with RA and spondyloarthritis [39], our results showed that there was no significant difference in the frequency of people taking this drug between the control and case groups, and a very small percentage of these patients experienced severe COVID-19 and were hospitalized.

However, this study also had its restrictions. One of the main limitations in our study was the intervention of patients with autoimmune diseases in the conscious prevention of COVID-19 by following approved health protocols, resulting in a low incidence of COVID-19 in these patients. Another limitation was related to receiving different combinations of DMARDs by patients and the interaction between TNF- α blockers and other DMARDs that makes difficult the determination of more effective DMARD combinations in preventing COVID-19.

CONCLUSIONS

In conclusion, our study indicated that TNF- α blockers, including infliximab, adalimumab, and etanercept, not only overcame inflammatory reactions but also decreased odds of COVID-19 incidence in patients with autoimmune disorders. Moreover, TNF- α blockers might help to prevent the cytokine storm caused by SARS-CoV-2 infection and consequently decreased the severity of COVID-19 and the need for hospitalization. Therefore, the use of TNF- α blockers probably had a direct and positive correlation with the decreased COVID-19 incidence, suggesting the prophylactic role of these drugs in preventing COVID-19 in autoimmune patients suffering from RA and SpA.

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Compliance with Ethics Guidelines. This study was performed in line with the principles of the Declaration of Helsinki 1964. Approval was granted by the Ethics Committee of Isfahan University of Medical Science (Date: 2020, Code No. IR.MUI.MED.REC.1399.380). Informed consent was obtained from all individual participants included in the study. No identifying information of patients was included in the manuscript.

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

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