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Prophylactic effects of colchicine against COVID-19 in patients with Behcet's disease

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Abstract

Background: Behçet's disease (BD) is a multisystemic vasculitis that is treated with colchicine. Based on observations, the number of patients with COVID-19 in the BD patient population is low, and the severity of COVID-19 is also very low. Such observations led to the hypothesis that colchicine can reduce the occurrence and severity of COVID-19 in patients with BD.

Objectives: This study aimed to investigate the effects of colchicine in preventing COVID-19 or reducing the severity of the disease in patients with BD, emphasizing the identification of the target proteins of colchicine.

Methods: According to a retrospective case-control study, the frequency of patients with COVID-19 was determined in two groups of BD patients treated with colchicine or not during the COVID-19 pandemic. Patient information was collected through telephone interviews and then subjected to statistical analysis. Also, based on in-silico studies and molecular docking, the target proteins of colchicine, which probably play a role in preventing the COVID-19, were identified.

Results: 65 qualified patients with BD participated in this study. Among them, 44 were women and 21 were men. In this population, only 40% of patients, including 20 women and 6 men, had experienced COVID-19. Also, only 27.7 percent of this population (11 women and 7 men) were treated with colchicine, and the majority of the population (72.3 %) was not under treatment with colchicine at the start of the COVID-19 pandemic. Based on the in-silico results, one hundred proteins were introduced as colchicine targets and it was shown that colchicine can effectively interact with some proteins such as Tubulin beta-1 chain, Histone deacetylases 6,1,3, ADAM17, E-selectin and angiotensin-converting enzyme 2.

Conclusion: Inhibiting the activity of Tubulin beta-1 chain proteins, Histone deacetylases 6,1,3, ADAM17, E-selectin, and angiotensin-converting enzyme 2 following the interaction of colchicine with these proteins can be the reason for the prophylactic effects of this drug in preventing COVID-19 or reducing the severity of the disease.

Keywords: Behcet's disease, Colchicine, COVID-19

1. Introduction

The global outbreak of COVID-19, caused by the SARS-CoV-2 virus, has led to an unprecedented and challenging situation. The disease manifests itself in a variety of ways, ranging from asymptomatic or mild forms to severe pneumonia, multiple organ failure, and even death (1-3). Inflammation is a significant factor in severe cases of COVID-19. Inflammatory markers such as C-reactive protein (CRP), ferritin, interleukin-6 (IL-6), interleukin-1 (IL-1), and other cytokines are seen to increase in severe cases (4, 5). Finding effective drugs to control and treat the inflammation caused by the new coronavirus, which leads to COVID-19, is a significant challenge. Currently, various medicines are used at different stages of the disease. Despite the many proposed drugs for treating COVID-19, none of them have proven to be completely effective (6). However, colchicine is one of the possible drugs that could be useful (7).

Colchicine is a medication primarily used to treat acute gout, certain auto-inflammatory syndromes like Behçet's disease (BD), Steele's disease in adults, and familial Mediterranean fever. It can also be used to treat pericarditis (8). Colchicine has been found to inhibit inflammation in the blood vessels, which can be beneficial for patients with coronary artery disease (9, 10).

Colchicine can reduce the production of free radicals such as superoxide in neutrophils (11). By disrupting the inflammatory activity, it leads to the suppression of caspase-1 activation and subsequently prevents the release of IL-1 and IL-18 (12). Colchicine, as an available drug, inhibits the NLRP3 part of inflammasomes. Due to NLRP3 activation and vascular endothelial inflammation during COVID-19, colchicine has been suggested as a possible treatment for SARS-CoV-2-associated inflammatory disease (12, 13). A limited number of small clinical trials have evaluated the effects of colchicine in hospitalized patients with COVID-19. However, none of the studies had sufficient evidence to identify the drug's effect on preventing mortality (14, 15). In one study, Yasmin et al. showed that the use of colchicine in COVID-19 patients reduced inflammation, including CRP levels in patients (16). While other studies showed that colchicine reduced the production of inflammatory mediators through inhibition of NLRP3 (17).

Behçet's disease (BD) is a systemic vasculitis that manifests in a variety of clinical symptoms, such as skin lesions, mucosal ulcers, ocular inflammation, vascular, neurological, and gastrointestinal problems (18, 19). Since BD shares characteristics with autoimmune-autoinflammatory diseases (20, 21), it is sometimes treated with colchicine and immunosuppressive drugs like corticosteroids, azathioprine, cyclosporine A, cyclophosphamide, and tumor necrosis factor alpha inhibitors (22).

Given the COVID-19 pandemic, there were concerns that patients with Behçet's disease (BD) who receive immunosuppressants would be at a higher risk of contracting the virus. However, based on our observations, the frequency of COVID-19 among these patients is not as high as expected. This led to the hypothesis that drugs used by BD patients, including colchicine, may reduce the infection rate or severity of COVID-19. Conducting research on BD patients who also receive colchicine could offer a valuable opportunity to test this hypothesis under real conditions. Thus, this study aimed to investigate the effects of colchicine in preventing COVID-19 or reducing its severity in patients with BD.

2. Methods

This project was done in two levels. The first level included a retrospective case-control study conducted to investigate the frequency of COVID-19 patients in two groups of BD patients who, during the COVID-19 pandemic, either received colchicine or were not treated with colchicine. The second level consisted of in-silico studies conducted to find the target proteins of colchicine that are likely to play a role in preventing the infection of COVID-19.

Patients with BD in hospitals and rheumatology clinics affiliated with the Isfahan University of Medical Sciences, participated in our study. Based on the medical records available for the patients, each patient was contacted by phone and interviewed after obtaining their consent. The cases were BD patients who experienced Covid-19 before or during our study and their disease was confirmed by RT-PCR, CT scan, or treatment in medical centers. Patients with BD who did not have any experience of COVID-19 until the end of the study were considered as controls for this study. In this study, colchicine was the exposure, and COVID-19 was the outcome. Inclusion criteria included suffering from BD for more than six months and having mental health to participate in the study. In addition, those BD patients who were treated with colchicine must have received this drug for at least three months during the pandemic period. The exclusion criteria from this study were failure to answer the phone up to three times, smoking, having cancer, or having malignancy were exclusion criteria. The data

was analyzed using descriptive statistics (absolute frequency, relative frequency).

To identify the target proteins of colchicine that played a role in preventing the infection of COVID-19, the target proteins of colchicine were predicted with the help of in silico methods and using the Swiss Target Prediction website. Then, based on the prediction result, the interaction of colchicine with those proteins with a higher probability of being a target for colchicine was investigated by molecular docking with Molecular Operating Environment (MOE) software.

2.1. Statistical Analysis

Data analysis was performed using standard epidemiological and biomedical statistical frameworks:

Primary Analysis: A two-tailed Fisher's Exact Test was utilized instead of a standard Pearson Chi-Square test. This selection was required because the baseline assumption for Chi-Square was violated (the cell count for patients treated with colchicine who experienced COVID-19 was (n=2), which falls below the minimum expected frequency threshold of 5.

Confidence Intervals: The 95% confidence intervals (CI) for all group percentages were calculated using the Wilson Score method, which provides the most accurate intervals for small sample frequencies.

Effect Size: A post-hoc power calculation was conducted based on the final sample size of 65 patients. The strength of the association was measured using the Phi (Φ)-Coefficient.

Odds Ratios (OR): Relative odds and their corresponding 95% Wald Confidence Intervals were derived to quantify the strength of the clinical effect.

Confounding Variable Analysis: Baseline demographic distributions (Sex vs. COVID-19 and Sex vs. colchicine) were analyzed using two-tailed Fisher's Exact Tests to rule out biological sex as a confounding variable.

3. Results

3.1. Analytical data

3.1.1. Primary Outcome: Colchicine Efficacy vs. COVID-19 Infection

The core objective was to observe if colchicine prevented COVID-19 infection in BD patients. The cross-tabulation matrix of the 65 patients is structured as follows:

Group of BD patients treated with colchicine during the COVID-19 pandemic (n = 18): 2 patients infected with COVID-19; 16 remained uninfected.

Group of BD patients untreated with colchicine during the COVID-19 pandemic (n = 47): 24 patients infected with COVID-19 COVID-19; 23 remained uninfected.

Statistical Metrics: Medicated Infection Rate: 11.1% (95% CI: [3.1%, 32.8%]). Non-Medicated Infection Rate: 51.1% (95% CI: [37.2%, 64.7%]). Fisher's Exact Test Significance: $p = 0.0041$ (Statistically highly significant, ($p < 0.01$)). Effect Size (Phi Coefficient): (Phi (Φ) = 0.36) (Indicates a solid medium-to-large clinical effect). Direct Odds Ratio (OR): 0.12 (95% CI: [0.02, 0.58]) — Taking the colchicine reduces the absolute odds of catching COVID-19 by 88%. Inverse Odds Ratio (OR): 8.35 (95% CI: [1.72, 40.42]) — Patients who did not take the colchicine were 8.35 times more likely to contract COVID-19.

3.1.2. Baseline Demographic and Confounding Variable Analysis (Sex)

To prove the validity of the primary outcome, sex was evaluated across both infection status and medicine distribution.

A. Patient Sex vs. COVID-19 Status: Women (n = 44): 20 developed COVID-19 (45.5% infection rate; 95% CI: [31.7%, 60.1%]). Men (n = 21): 6 developed COVID-19 (28.6% infection rate; 95% CI: [13.8%,

50.0%]). Fisher's Exact Test: p = 0.280 (Not statistically significant, (p > 0.05)). Odds Ratio (OR): 2.08 (95% CI: [0.68, 6.37]) — The confidence interval crosses 1.0, proving sex is not a valid baseline predictor for infection in this cohort.

B. Patient Sex vs. colchicine Distribution: Women (n = 44): 11 received the colchicine (25.0% medication rate). Men (n = 21): 7 received the medicine (33.3% medication rate). Fisher's Exact Test: p = 0.558 (Not statistically significant, (p > 0.05)). Odds Ratio (OR): 0.67 (95% CI: [0.21, 2.07]) — The confidence interval crosses 1.0, confirming that colchicine distribution was statistically uniform and free of gender bias.

65 qualified patients with BD participated in this study. Among them, 44 were women and 21 were men. In this population, only 40% of patients, including 20 women and 6 men, had experienced COVID-19. Also, only 27.7 percent of this population (11 women and 7 men) were treated with colchicine, and the majority of the population (72.3 %) was not under treatment with colchicine at the start of the COVID-19 pandemic (Figure 1).

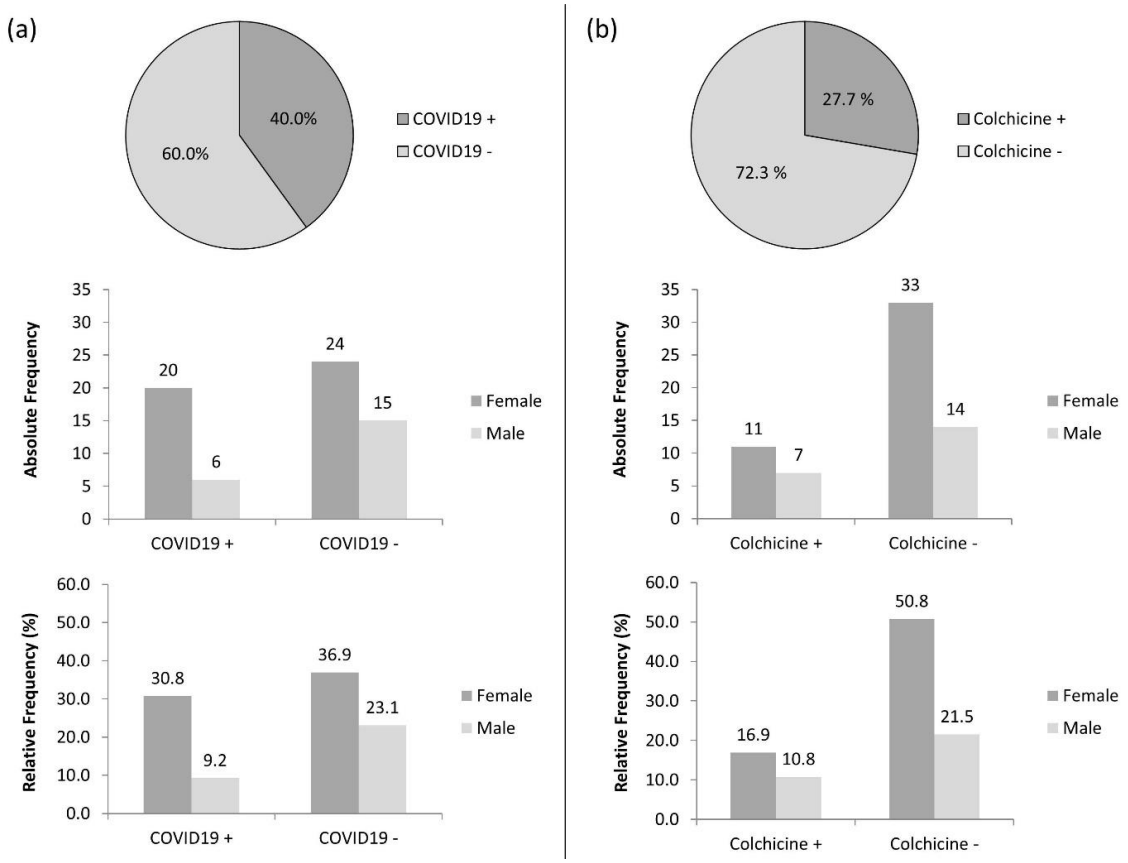

Figure 1. Absolute and relative frequency of patients with Behçet's disease in terms of COVID-19 (a) and colchicine use (b). in COVID-19 (a), 26 patients had Covid and 39 did not. Of the 26, 20 were female and 6 were male. Of the 39, 24 were female and 15 were male. In colchicine use (b), 18 patients (including 11 females and 7 males) had received colchicine, while 47 patients (33 females and 14 males) had not received colchicine.

Regarding the effect of colchicine, the results showed that 25% of the patients who received colchicine did not get COVID-19, while only 3% of the patients treated with colchicine (two people) experienced mild COVID-19. In addition, fewer BD patients treated with colchicine experienced COVID-19 compared to patients who did not receive colchicine. However, there was no significant difference in the incidence of COVID-19 among patients who were not treated with colchicine (Figure 2).

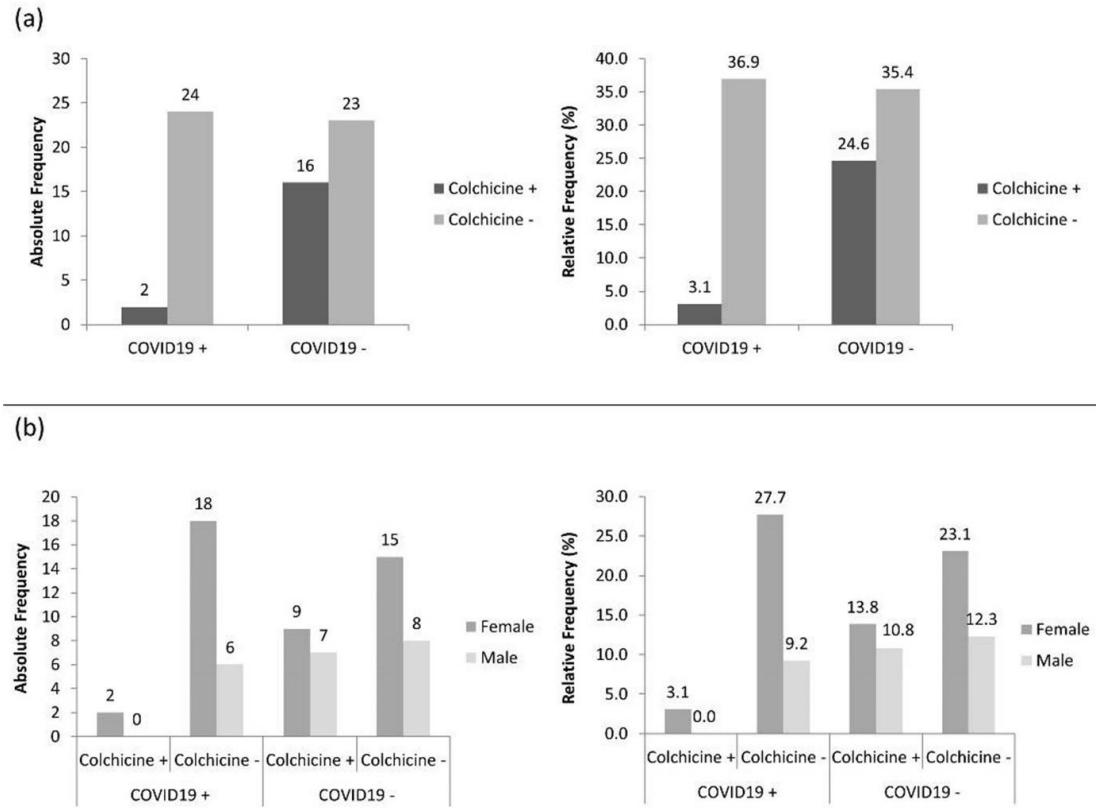

Figure 2. Absolute and relative frequency of patients with Behçet's disease in terms of the effect of colchicine in preventing COVID-19 regardless of colchicine (a) and based on gender (b). a: Of the 26 patients with COVID-19, 2 had received colchicine. In contrast, only 16 patients without COVID-19 had received colchicine. b: Patients with or without COVID-19 were stratified by gender.

Based on Swiss Target Prediction analysis, one hundred proteins were identified as possible targets of colchicine in humans (Table 1), and these proteins were classified into 5 protein groups including enzymes, kinases, scavengers, structural proteins, and phosphodiesterases (Figure 3).

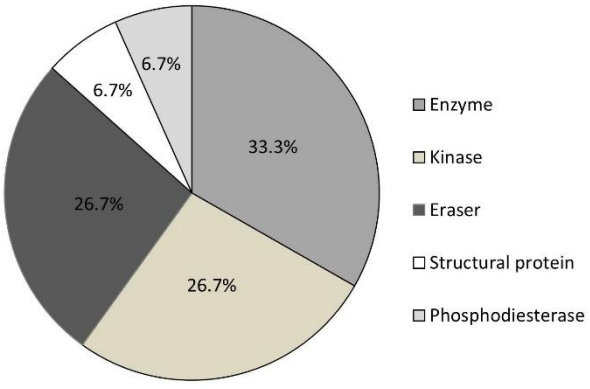

Figure 3. Relative abundance of major protein groups as colchicine targets

Although 100 proteins were introduced as targets, four proteins were identified with greater probability to be biological targets for colchicine based on the prediction algorithms of Swiss Target Prediction. They are beta-tubulin 1, histone deacetylase 6, histone deacetylase 1, and histone deacetylase 3.

The interaction of colchicine with these 4 proteins and two other proteins from Table 1, including Selectin-E and ADAM17, whose

role in development of COVID-19 has been proved in previous studies, was investigated by molecular docking. In addition, the interaction of colchicine with the ACE2 protein, which is known as the SARS-CoV-2 virus receptor but was not identified as a colchicine target based on Swiss Target Prediction analysis, was studied through molecular docking with the MOE software (Figure 4).

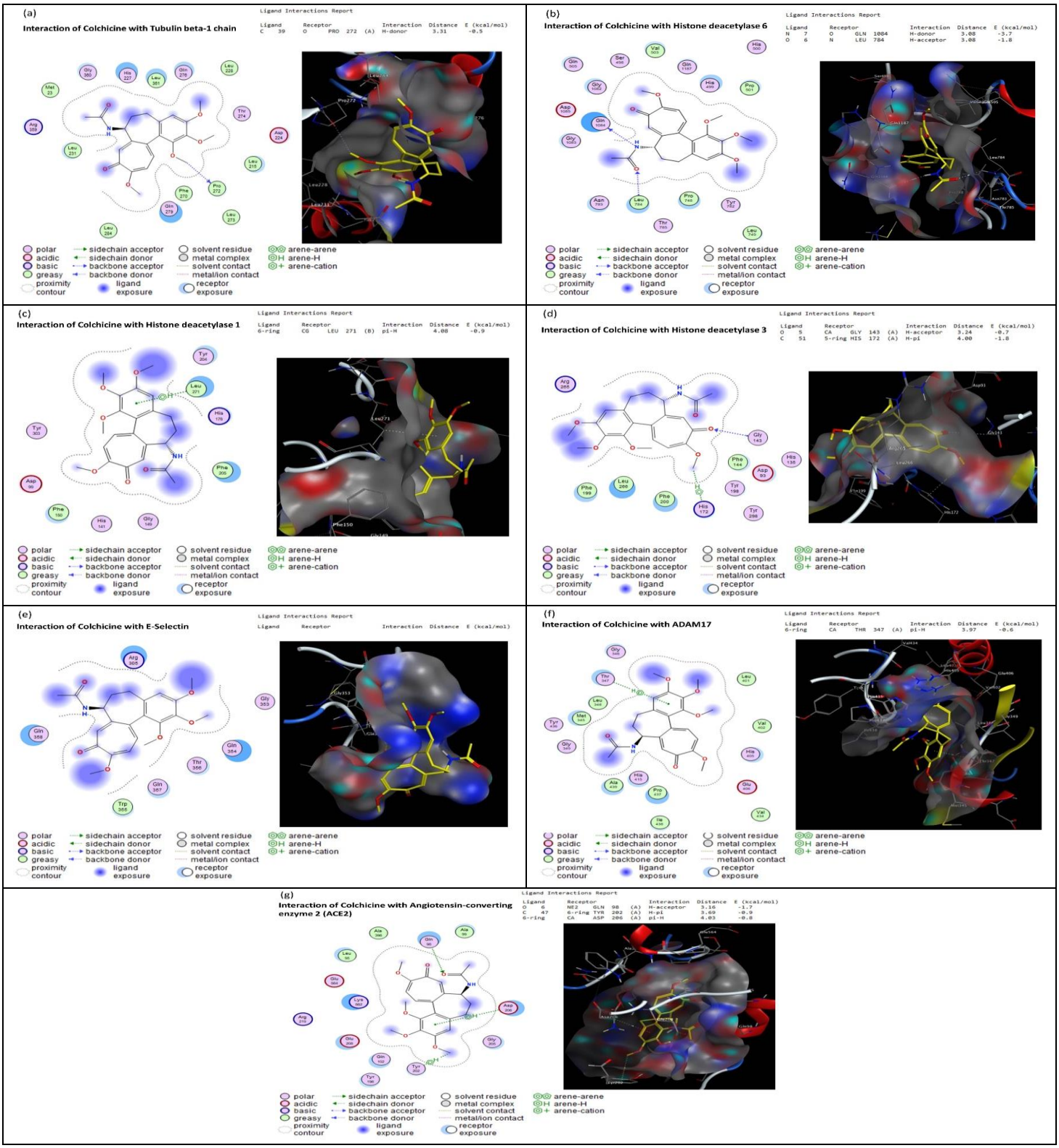

Figure 4. Two-dimensional and three-dimensional representation of the interaction of colchicine with Tubulin beta-1 chain, Histone deacetylases 6,1,3, Selectin-E, ADAM17 and ACE2 proteins (a-g).

Table 1. Predicted protein targets for colchicine based on SwissTargetPrediction

	Target	Common name	Uniprot ID	ChEMBL ID	Target Class	Probability/ Known actives (3D/2D)
1	Tubulin beta-1 chain	TUBB1	Q9H4B7	CHEMBL1915	Structural protein	28 / 5
2	Histone deacetylase 6	HDAC6	Q9UBN7	CHEMBL1865	Eraser	118 / 5
3	Histone deacetylase 1	HDAC1	Q13547	CHEMBL325	Eraser	318 / 8
4	Histone deacetylase 3	HDAC3	O15379	CHEMBL1829	Eraser	65 / 7
5	Histone deacetylase 2	HDAC2	Q92769	CHEMBL1937	Eraser	109 / 6
6	Tyrosine-protein kinase ABL	ABL1	P00519	CHEMBL1862	Kinase	227 / 0
7	Tyrosine-protein kinase SRC	SRC	P12931	CHEMBL267	Kinase	279 / 0
8	PI3-kinase p110-delta subunit	PIK3CD	O00329	CHEMBL3130	Enzyme	344 / 0
9	DNA-dependent protein kinase	PRKDC	P78527	CHEMBL3142	Kinase	147 / 0
10	PI3-kinase p110-beta subunit	PIK3CB	P42338	CHEMBL3145	Enzyme	233 / 0
11	Tyrosine-protein kinase HCK	HCK	P08631	CHEMBL3234	Kinase	104 / 0
12	PI3-kinase p110-gamma subunit	PIK3CG	P48736	CHEMBL3267	Kinase	288 / 0
13	PI4-kinase beta subunit	PI4KB	Q9UBF8	CHEMBL3268	Enzyme	55 / 0
14	PI3-kinase p110-alpha subunit	PIK3CA	P42336	CHEMBL4005	Enzyme	645 / 0
15	Phosphodiesterase 10A	PDE10A	Q9Y233	CHEMBL4409	Phosphodiesterase	902 / 0
16	Tyrosine-protein kinase SYK	SYK	P43405	CHEMBL2599	Kinase	330 / 0
17	Serine/threonine-protein kinase mTOR	MTOR	P42345	CHEMBL2842	Kinase	420 / 0
18	MAP kinase p38 alpha	MAPK14	Q16539	CHEMBL260	Kinase	708 / 0
19	Phosphodiesterase 2A	PDE2A	O00408	CHEMBL2652	Phosphodiesterase	69 / 0
20	Lymphocyte differentiation antigen CD38	CD38	P28907	CHEMBL4660	Enzyme	26 / 0
21	Prostanoid EP2 receptor	PTGER2	P43116	CHEMBL1881	Family A G protein-coupled receptor	33 / 0
22	Epidermal growth factor receptor erbB1	EGFR	P00533	CHEMBL203	Kinase	603 / 0
23	Chymase	CMA1	P23946	CHEMBL4068	Protease	77 / 0
24	Insulin-like growth factor I receptor	IGF1R	P08069	CHEMBL1957	Kinase	249 / 0
25	Sodium channel protein type X alpha subunit (by homology)	SCN10A	Q9Y5Y9	CHEMBL5451	Voltage-gated ion channel	42 / 0
26	Legumain	LGMN	Q99538	CHEMBL4244	Protease	26 / 0
27	Rho-associated protein kinase 2	ROCK2	O75116	CHEMBL2973	Kinase	126 / 0
28	c-Jun N-terminal kinase 1	MAPK8	P45983	CHEMBL2276	Kinase	337 / 0
29	MAP kinase signal-integrating kinase 2	MKNK2	Q9HBH9	CHEMBL4204	Kinase	36 / 0
30	MAP kinase-interacting serine/threonine-protein kinase MNK1	MKNK1	Q9BUB5	CHEMBL4718	Kinase	14 / 0
31	Phosphodiesterase 5A	PDE5A	O76074	CHEMBL1827	Phosphodiesterase	262 / 0
32	Androgen Receptor	AR	P10275	CHEMBL1871	Nuclear receptor	90 / 0
33	Progesterone receptor	PGR	P06401	CHEMBL208	Nuclear receptor	145 / 0
34	Serine/threonine-protein kinase Aurora-B	AURKB	Q96GD4	CHEMBL2185	Kinase	231 / 0

35	Leucine-rich repeat serine/threonine-protein kinase 2	LRRK2	Q55007	CHEMBL10751	Kinase	110 / 0
36	Heat shock protein HSP 90-alpha	HSP90AA1	P07900	CHEMBL3880	Other cytosolic protein	135 / 0
37	DNA topoisomerase II alpha	TOP2A	P11388	CHEMBL1806	Isomerase	3 / 0
38	Thyrotropin-releasing hormone receptor (by homology)	TRHR	P34981	CHEMBL1810	Family A G protein-coupled receptor	32 / 0
39	Cyclin-dependent kinase 2/cyclin A	CDK2 CCNA1	P24941 P78396	CHEMBL20941	Other cytosolic protein	172 / 0
40	Thrombin and coagulation factor X	F10	P00742	CHEMBL244	Protease	263 / 0
41	Serine/threonine-protein kinase PLK1	PLK1	P53350	CHEMBL3024	Kinase	126 / 0
42	Matrix metalloproteinase 1	MMP1	P03956	CHEMBL332	Protease	342 / 0
43	ALK tyrosine kinase receptor	ALK	Q9UM73	CHEMBL4247	Kinase	110 / 0
44	Cytochrome P450 19A1	CYP19A1	P11511	CHEMBL1978	Cytochrome P450	304 / 0
45	Phosphodiesterase 7A	PDE7A	Q13946	CHEMBL3012	Phosphodiesterase	108 / 0
46	Ribosomal protein S6 kinase 1	RPS6KB1	P23443	CHEMBL4501	Kinase	162 / 0
47	Serine/threonine-protein kinase Aurora-A	AURKA	O14965	CHEMBL4722	Kinase	434 / 0
48	Sonic hedgehog protein (by homology)	SHH	Q15465	CHEMBL5602	Unclassified protein	10 / 0
49	Complement factor D	CFD	P00746	CHEMBL21767	Protease	250 / 0
50	Intercellular adhesion molecule-1	ICAM1	P05362	CHEMBL3070	Adhesion	29 / 0
51	Selectin E	SELE	P16581	CHEMBL3890	Adhesion	29 / 0
52	Tyrosine-protein kinase ZAP-70	ZAP70	P43403	CHEMBL2803	Kinase	34 / 0
53	Ephrin receptor	EPHB4	P54760	CHEMBL5147	Kinase	104 / 0
54	Carnitine O-palmitoyltransferase 1, liver isoform	CPT1A	P50416	CHEMBL12931	Enzyme	75 / 0
55	Nuclear receptor ROR-gamma	RORC	P51449	CHEMBL17411	Nuclear receptor	60 / 0
56	Cyclin-dependent kinase 9	CDK9	P50750	CHEMBL3116	Kinase	42 / 0
57	Tyrosine-protein kinase TYK2	TYK2	P29597	CHEMBL3553	Kinase	85 / 0
58	ATP-binding cassette sub-family G member 2	ABCG2	Q9UNQ0	CHEMBL5393	Primary active transporter	75 / 0
59	MAP kinase ERK2	MAPK1	P28482	CHEMBL4040	Kinase	461 / 0
60	ADAM17	ADAM17	P78536	CHEMBL3706	Protease	183 / 0
61	Hepatocyte growth factor receptor	MET	P08581	CHEMBL3717	Kinase	517 / 0
62	Sodium channel protein type IX alpha subunit	SCN9A	Q15858	CHEMBL4296	Voltage-gated ion channel	100 / 0
63	Bromodomain-containing protein 4	BRD4	O60885	CHEMBL11631	Reader	217 / 0
64	15-hydroxyprostaglandin dehydrogenase [NAD+]	HPGD	P15428	CHEMBL12932	Enzyme	13 / 0
65	Tyrosine-protein kinase JAK3	JAK3	P52333	CHEMBL2148	Kinase	284 / 0
66	Tyrosine-protein kinase JAK1	JAK1	P23458	CHEMBL2835	Kinase	264 / 0
67	Cyclin-dependent kinase 2	CDK2	P24941	CHEMBL301	Kinase	254 / 0

68	MAP kinase ERK1	MAPK3	P27361	CHEMBL3385	Kinase	52 / 0
69	MAP kinase p38 beta	MAPK11	Q15759	CHEMBL3961	Kinase	34 / 0
70	Macrophage colony stimulating factor receptor	CSF1R	P07333	CHEMBL1844	Kinase	191 / 0
71	Platelet-derived growth factor receptor beta	PDGFRB	P09619	CHEMBL1913	Kinase	129 / 0
72	Tyrosine-protein kinase receptor FLT3	FLT3	P36888	CHEMBL1974	Kinase	199 / 0
73	Vanilloid receptor	TRPV1	Q8NER1	CHEMBL4794	Voltage-gated ion channel	358 / 0
74	Serine/threonine-protein kinase PIM1	PIM1	P11309	CHEMBL2147	Kinase	196 / 0
75	MAP kinase-activated protein kinase 2	MAPKAPK2	P49137	CHEMBL2208	Kinase	124 / 0
76	Serine/threonine-protein kinase Chk2	CHEK2	O96017	CHEMBL2527	Kinase	28 / 0
78	CDK8/Cyclin C	CCNC CDK8	P24863 P49336	CHEMBL30384	Kinase	43 / 0
79	Bcl2-antagonist of cell death (BAD)	BAD	Q92934	CHEMBL3817	Other cytosolic protein	11 / 0
80	Serine/threonine-protein kinase PIM2	PIM2	Q9P1W9	CHEMBL4523	Kinase	111 / 0
81	Serine/threonine-protein kinase Chk1	CHEK1	O14757	CHEMBL4630	Kinase	165 / 0
82	Corticotropin releasing factor receptor 1	CRHR1	P34998	CHEMBL1800	Family B G protein-coupled receptor	195 / 0
83	Carbonic anhydrase VII	CA7	P43166	CHEMBL2326	Lyase	80 / 0
84	Ribosomal protein S6 kinase alpha 3	RPS6KA3	P51812	CHEMBL2345	Kinase	104 / 0
85	Phosphodiesterase 3	PDE3A	Q14432	CHEMBL241	Phosphodiesterase	63 / 0
86	Carbonic anhydrase I	CA1	P00915	CHEMBL261	Lyase	387 / 0
87	Phosphodiesterase 3B	PDE3B	Q13370	CHEMBL290	Phosphodiesterase	47 / 0
88	Carbonic anhydrase VI	CA6	P23280	CHEMBL3025	Lyase	31 / 0
89	Carbonic anhydrase XII	CA12	O43570	CHEMBL3242	Lyase	252 / 0
90	Formyl peptide receptor 1	FPR1	P21462	CHEMBL3359	Family A G protein-coupled receptor	13 / 0
91	Carbonic anhydrase XIII	CA13	Q8N1Q1	CHEMBL3912	Lyase	51 / 0
92	Lipoxin A4 receptor	FPR2	P25090	CHEMBL4227	Family A G protein-coupled receptor	22 / 0
93	Receptor protein-tyrosine kinase erbB-2	ERBB2	P04626	CHEMBL1824	Kinase	123 / 0
94	Kinesin-1 heavy chain/ Tyrosine-protein kinase receptor RET	RET	P07949	CHEMBL2041	Kinase	102 / 0
95	Glycogen synthase kinase-3 beta	GSK3B	P49841	CHEMBL262	Kinase	465 / 0
96	c-Jun N-terminal kinase 3	MAPK10	P53779	CHEMBL2637	Kinase	190 / 0
97	Receptor protein-tyrosine kinase erbB-4	ERBB4	Q15303	CHEMBL3009	Kinase	10 / 0
98	Cyclooxygenase-2	PTGS2	P35354	CHEMBL230	Oxidoreductase	274 / 0
99	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3	PFKFB3	Q16875	CHEMBL23310	Enzyme	123 / 0
100	Matrix metalloproteinase 13	MMP13	P45452	CHEMBL280	Protease	249 / 0

Based on MOE, the interaction between protein and ligand is determined by a parameter called S. More negative values of S indicate stronger binding affinity between ligand and target protein, which implies that less free energy is required to bind the ligand to the protein. As a result, there is a greater possibility for the interaction between the ligand and the target protein. In the present study, the most negative affinity value of protein and ligand (S) belongs to the interaction between histone deacetylase6 and colchicine, which indicates a stronger binding affinity between colchicine (ligand) and the histone deacetylase6. As a result, the possibility of interaction between colchicine molecule and histone deacetylase6 will be very high. On the other hand, the highest value of S, the highest free energy, and the weakest interaction is related to Selectin-E (Table 2).

Table 2. S value (binding affinity) for the interaction between colchicine and selected target proteins.

Interaction of Colchicine with protein targets	
Protein name	**Binding Affinity (S)**
Tubulin beta-1 chain	-6.18
Histone deacetylase 6	-7.16
Histone deacetylase 1	-5.98
Histone deacetylase 3	-5.74
Selectin-E	-5.67
ADAM17	-6.93
Angiotensin-converting enzyme 2 (ACE2)	-6.79

4. Discussion

In the study population, which consisted of 65 patients with Behçet's disease, 26 patients experienced COVID-19, and only two of them received colchicine at the time of the COVID-19 pandemic, while 16 people under colchicine treatment did not experience this disease. Therefore, this can indicate the possible effect of colchicine in preventing COVID-19.

How colchicine prevents COVID-19 can be related to the interaction of this molecule with proteins involved in virus infection and immune system responses. Based on SwissTargetPrediction, 100 target proteins were predicted for colchicine. Most of them are enzymes that control various cell activities and cell signaling. By using these proteins in the cell, viruses, including the SARS-CoV-2 virus, can probably produce several copies, disrupt the cell's function, and eventually cause disease (23). If the function of these proteins is already inhibited by some molecules, viruses probably cannot use them to penetrate and reproduce, as a result, the viral disease is prevented.

According to Table 2, one of the target proteins of colchicine is Tubulin beta-1 chain (TUBB1). Previous studies have shown that colchicine prevents the polymerization of tubulin by binding to the beta-tubulin subunit, leading to the inhibition of microtubule function (24-26). As one of the main components of the cytoskeleton, microtubules are essential for various processes in eukaryotic cells, including mitosis, morphogenesis, axonal transport, ciliary movement, intracellular transport, and secretion. They are also involved in the integrity of the Golgi apparatus (27). Colchicine can disrupt the aforementioned activities by preventing tubulin polymerization.

The SARS-CoV-2 virus attacks host cells

and reproduces by hijacking cellular molecular machinery. After infecting the host cell, this virus takes control of the microtubules of the cell skeleton and the microtubule-organizing centers of the host, which leads to the entry of more viruses into the cell. The proliferation of the virus due to intracellular transfers and cell-to-cell spread of viruses with the formation of filopodia causes disturbances in the functioning of the immune system, which include a wide range of reactions from no response or ineffective responses to cytokine storm (28, 29). Therefore, it can be expected that compounds such as colchicine, which inhibit tubulin polymerization and microtubule activity, can play an important role in preventing SARS-CoV-2 infection and regulating immune system responses against COVID-19.

Our results showed that colchicine can also interact with histone deacetylase 6, 1, and 3 proteins. Histone deacetylases (HDACs) remove acetyl functional groups from lysine amino acids of histone and non-histone proteins. Removal of the acetyl group from histone proteins causes changes in gene expression and chromatin structure. In addition, the removal of acetyl groups from non-histone proteins causes changes in their function and as a result, changes in cellular processes (30). Studies have shown that histone deacetylase inhibitors, such as sodium butyrate and panobinostat, can prevent the expression of the ACE2 gene, which encodes the angiotensin-converting enzyme 2 protein. This protein is the receptor of some viruses, including SARS-CoV-2, and causes the virus to enter the host cell. It has also been shown that the clinical use of panobinostat reduces the amount of ACE2 protein, so it has been suggested that this drug can prevent COVID-19 (31). Since the mortality caused by COVID-19 is mainly caused by the imbalance of immune responses and the uncontrolled release of pro-inflammatory cytokines, immune system-modulating drugs can prevent this. It has been found that histone deacetylases also regulate the innate and acquired immune response at different levels and the inhibition of these enzymes can affect the immune responses. For example, histone deacetylase inhibitors reduce the secretion of cytokines by epithelial cells of the respiratory tract, monocytes, and macrophages. This anti-inflammatory effect occurs along with the reduction of monocyte activation, exhaustion of T cells, and increased differentiation of T cells towards central memory T cells. In addition, histone deacetylase inhibitors prevent the expression of IFN-I and downstream effects in respiratory tract epithelial cells and immune cells, as a result, it has been suggested that the therapeutic approach based on histone deacetylase inhibitors can be a promising drug treatment for patients with severe COVID-19 (32). According to the results of the present study on the in-silico interaction of colchicine with histone deacetylases, this drug probably acts as an inhibitor of histone deacetylases in physiological conditions, due to the highest negative affinity (S) value among the investigated proteins is related to histone deacetylase 6, and this protein can be one of the main targets of colchicine. In another in-silico study, colchicine was also identified as a potential inhibitor of histone deacetylases (33). Moreover, in-vitro studies have shown that some colchicine derivatives act as dual inhibitors of tubulin-histone deacetylase (34). Therefore, the role of colchicine as a histone deacetylase inhibitor can indicate a possible mechanism for the effect of colchicine in preventing COVID-19 or its mitigating effect in the severity of this disease.

ADAM17 is a protein that plays a significant role in the entry of the SARS-CoV-2 virus into the host cell and the production of inflammatory cytokines TNFα and IL6 (35, 36). Swiss Target Prediction identified this protein as a target for colchicine. According to the MOE result, the S value of the

interaction of ADAM17 with colchicine was more negative than the interaction of colchicine with beta-tubulin as its known target. This indicates that colchicine can probably act as an ADAM17 inhibitor. Therefore, the interaction of ADAM17 with colchicine can be one of the other possible mechanisms in preventing COVID-19 or reducing the severity of this disease by colchicine.

E-selectin is another target of colchicine introduced by Swiss Target Prediction. Previous studies have shown that colchicine inhibits the expression of this protein. Since this protein is a key molecule for connecting leukocytes to endothelial cells and attracting monocytes and neutrophils to the inflamed tissues, its inhibition reduces inflammation (37). Although, based on the results of molecular docking, this protein showed a weaker interaction with colchicine than other proteins, in physiological conditions, its activity may be inhibited by colchicine. Therefore, colchicine probably reduces tissue inflammation by inhibiting both the expression of this protein and its function and, as a result, reduces the severity of the COVID-19 disease.

Angiotensin-converting enzyme 2 plays a key role in virus entering into host cells and causing disease by SARS-CoV-2 (38, 39). Although ACE2 was not identified as a colchicine target protein by Swiss Target Prediction, due to its proven role in COVID-19 infection, we investigated its interaction with colchicine by molecular docking. It was found that the S value of the interaction of ACE2 protein and colchicine is even more negative than the interaction of colchicine with beta-tubulin, which is a known target of colchicine. Therefore, by stronger interaction with ACE2, colchicine can disrupt the interaction between this protein and SARS-CoV-2, inhibit the entry of the virus into the host cell, and ultimately prevent the development of the disease. On the other hand, as mentioned earlier, the expression of ACE2 protein is inhibited by some histone deacetylase inhibitors. Since colchicine has also been introduced as an inhibitor of histone deacetylases, colchicine can probably also inhibit the expression and function of ACE2.

5. Conclusion

The findings of this study demonstrate that the prophylactic use of the evaluated medicine (colchicine) is associated with a highly significant reduction in COVID-19 infection rates among patients with Behçet's disease (BD) .

With a low infection rate of only 11.1% in the medicated cohort compared to 51.1% in the non-medicated cohort, p-value of 0.0041 ($p<0.01$), the data reveals a powerful protective benefit. Patients who did not receive the treatment faced more than eight times higher odds of contracting the virus.

Crucially, rigorous analysis confirmed that baseline demographic factors, such as biological sex, did not bias medication (colchicine) distribution or independently drive infection outcomes. Furthermore, despite a modest sample size of 65 patients, the study achieved a post-hoc statistical power well above the standard 80% benchmark. This mathematically confirms that the sample was fully sufficient to support these highly significant findings.

In conclusion, colchicine serves as an effective prophylactic measure against COVID-19 within this specific patient population. These results support further research and wider clinical trials to confirm these protective effects on a larger scale.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Authors' contributions: Behrokh Shojaie and Mansour Salesi contributed to the study conception and designed the questionnaire for this study. Behrokh Shojaie and Mansour Salesi collected data. Behrokh Shojaie analyzed data and interpreted results. Behrokh Shojaie prepared, wrote and edited the manuscript. Mansour Salesi and Behrokh Shojaie critically revised the manuscript. All authors read and approved the final manuscript.

Ethics approval: All the procedures performed in the studies involving human participants were in accordance with the ethical standards of the local ethics committee of Isfahan University of Medical Sciences (IR.ARI.MUI.REC.1400.069), as well as the 1964 Helsinki declaration. Written informed consent was obtained from all patients and healthy subjects.

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References

1. Parasher A. COVID-19: Current understanding of its Pathophysiology, Clinical presentation and Treatment. Postgrad Med J. 2021;97(1147):312-20. https://doi.org/10.1136/postgradmedj-2020-138577 PMid:32978337 PMCid:PMC10017004
2. Pourpashang P. Kawasaki-like disease associated with COVID-19 (Kawa-COVID-19); an overview of recent evident. Journal of Preventive Epidemiology. 2025;11(1):e39274-e. https://doi.org/10.34172/jpe.2025.39274
3. Nokhostin F, Dargahi MM, Tutunchi S, Rezaeeyan H. Evaluation of prognostic/diagnostic value of Hematological markers in the detection of inflammation in coronavirus disease: a review study. 2020. https://doi.org/10.30699/jambs.28.128.171
4. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the Inflammatory Response to Severe COVID-19 Illness. Am J Respir Crit Care Med. 2020;202(6):812-21. https://doi.org/10.1164/rccm.202005-1583OC PMid:32584597 PMCid:PMC7491404
5. Subkhan M, Malikul Mulki T, Ambar NS, Irfana L, Widyaningsih S, Permana PBD, et al. Low platelet counts and monocytosis were associated with reduced risk of severe COVID-19 manifestation: a single-center study from Indonesia. Immunopathol Persa. 2025;11(2):e40595-e. https://doi.org/10.34172/ipp.2025.40595
6. Rizk JG, Kalantar-Zadeh K, Mehra MR, Lavie CJ, Rizk Y, Forthal DN. Pharmaco-Immunomodulatory Therapy in COVID-19. Drugs. 2020;80(13):1267-92. https://doi.org/10.1007/s40265-020-01367-z PMid:32696108
7. Reyes AZ, Hu KA, Teperman J, Wampler Muskardin TL, Tardif JC, Shah B, et al. Anti-inflammatory therapy for COVID-19 infection: the case for colchicine. Ann Rheum Dis. 2021;80(5):550-7. https://doi.org/10.1136/annrheumdis-2020-219174 PMid:33293273 PMCid:PMC8491433
8. Slobodnick A, Shah B, Krasnokutsky S, Pillinger MH. Update on colchicine, 2017. Rheumatology (Oxford). 2018;57(suppl_1):i4-i11. https://doi.org/10.1093/rheumatology/kex453 PMid:29272515 PMCid:PMC5850858
9. Fiolet ATL, Opstal TSJ, Mosterd A, Eikelboom JW, Jolly SS, Keech AC, et al. Efficacy and safety of low-dose colchicine in patients with coronary disease: a systematic review and meta-analysis of randomized trials. Eur Heart J. 2021;42(28):2765-75. https://doi.org/10.1093/eurheartj/ehab115 PMid:33769515
10. Imazio M, Brucato A, Adler Y. A randomized trial of colchicine for acute pericarditis. N Engl J Med. 2014;370(8):781. https://doi.org/10.1056/NEJMc1315351
11. Nuki G. Colchicine: its mechanism of action and efficacy in crystal-induced inflammation. Curr Rheumatol Rep. 2008;10(3):218-27. https://doi.org/10.1007/s11926-008-0036-3 PMid:18638431
12. Martínez GJ, Celermajer DS, Patel S. The NLRP3 inflammasome and the emerging role of colchicine to inhibit atherosclerosis-associated inflammation. Atherosclerosis. 2018;269:262-71. https://doi.org/10.1016/j.atherosclerosis.2017.12.027 PMid:29352570
13. Freeman TL, Swartz TH. Targeting the NLRP3 Inflammasome in Severe COVID-19. Front Immunol. 2020;11:1518. https://doi.org/10.3389/fimmu.2020.01518 PMid:32655582
14. Lopes MI, Bonjorno LP, Giannini MC, Amaral NB, Menezes PI, Dib SM, et al. Beneficial effects of

colchicine for moderate to severe COVID-19: a randomised, double-blinded, placebo-controlled clinical trial. RMD Open. 2021;7(1). https://doi.org/10.1136/rmdopen-2020-001455 PMid:33542047 PMCid:PMC7868202

15. Deftereos SG, Giannopoulos G, Vrachatis DA, Siasos GD, Giotaki SG, Gargalianos P, et al. Effect of Colchicine vs Standard Care on Cardiac and Inflammatory Biomarkers and Clinical Outcomes in Patients Hospitalized With Coronavirus Disease 2019: The GRECCO-19 Randomized Clinical Trial. JAMA Netw Open. 2020;3(6):e2013136. https://doi.org/10.1001/jamanetworkopen.2020.13136 PMid:32579195 PMCid:PMC7315286
16. Yasmin F, Najeeb H, Moeed A, Hassan W, Khatri M, Asghar MS, et al. Safety and efficacy of colchicine in COVID-19 patients: A systematic review and meta-analysis of randomized control trials. PLOS ONE. 2022;17(4):e0266245. https://doi.org/10.1371/journal.pone.0266245 PMid:35381033 PMCid:PMC8982874
17. Bonaventura A, Vecchié A, Dagna L, Tangianu F, Abbate A, Dentali F. Colchicine for COVID-19: targeting NLRP3 inflammasome to blunt hyperinflammation. Inflammation Research. 2022;71(3):293-307. https://doi.org/10.1007/s00011-022-01540-y PMid:35113170 PMCid:PMC8811745
18. Yazici H, Seyahi E, Hatemi G, Yazici Y. Behçet syndrome: a contemporary view. Nat Rev Rheumatol. 2018;14(2):107-19. https://doi.org/10.1038/nrrheum.2018.3 https://doi.org/10.1038/nrrheum.2017.208 PMid:29296024
19. Emmi G, Silvestri E, Squatrito D, D'Elios MM, Ciucciarelli L, Prisco D, et al. Behçet's syndrome pathophysiology and potential therapeutic targets. Intern Emerg Med. 2014;9(3):257-65. https://doi.org/10.1007/s11739-013-1036-5 PMid:24415351
20. Leccese P, Alpsoy E. Behçet's Disease: An Overview of Etiopathogenesis. Front Immunol. 2019;10:1067. https://doi.org/10.3389/fimmu.2019.01067 PMid:31134098 PMCid:PMC6523006
21. Tong B, Liu X, Xiao J, Su G. Immunopathogenesis of Behcet's Disease. Front Immunol. 2019;10:665. https://doi.org/10.3389/fimmu.2019.00665 PMid:30984205 PMCid:PMC6449449
22. Karadag O, Bolek EC. Management of Behcet's syndrome. Rheumatology (Oxford). 2020;59(Suppl 3):iii108-iii17. https://doi.org/10.1093/rheumatology/keaa086 PMid:32348509
23. Payne S. Virus Interactions With the Cell: Viruses. 2017:23-35. doi: 10.1016/B978-0-12-803109-4.00003-9. Epub 2017 Sep 1. https://doi.org/10.1016/B978-0-12-803109-4.00003-9 PMCid:PMC7173567
24. Sargsyan A, Sahakyan H, Nazaryan K. Effect of Colchicine Binding Site Inhibitors on the Tubulin Intersubunit Interaction. ACS Omega. 2023;8(32):29448-54. https://doi.org/10.1021/acsomega.3c02979 PMid:37599936 PMCid:PMC10433359
25. Hastie SB. Interactions of colchicine with tubulin. Pharmacol Ther. 1991;51(3):377-401. https://doi.org/10.1016/0163-7258(91)90067-V PMid:1792241
26. Little M, Ludueña RF. Structural differences between brain beta 1- and beta 2-tubulins: implications for microtubule assembly and colchicine binding. Embo j. 1985;4(1):51-6. https://doi.org/10.1002/j.1460-2075.1985.tb02316.x PMid:4018027 PMCid:PMC554150
27. Goodson HV, Jonasson EM. Microtubules and Microtubule-Associated Proteins. Cold Spring Harb Perspect Biol. 2018;10(6). https://doi.org/10.1101/cshperspect.a022608 PMid:29858272 PMCid:PMC5983186
28. Aminpour M, Hameroff S, Tuszynski JA. How COVID-19 Hijacks the Cytoskeleton: Therapeutic Implications. Life. 2022;12(6):814. https://doi.org/10.3390/life12060814 PMid:35743845 PMCid:PMC9225596
29. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022;23(1):3-20. https://doi.org/10.1038/s41580-021-00418-x PMid:34611326 PMCid:PMC8491763
30. Seto E, Yoshida M. Erasers of histone acetylation: the histone deacetylase enzymes. Cold Spring Harb Perspect Biol. 2014;6(4):a018713. https://doi.org/10.1101/cshperspect.a018713 PMid:24691964 PMCid:PMC3970420
31. Takahashi Y, Hayakawa A, Sano R, Fukuda H, Harada M, Kubo R, et al. Histone deacetylase inhibitors suppress ACE2 and ABO simultaneously, suggesting a preventive potential against COVID-19. Scientific Reports. 2021;11(1):3379. https://doi.org/10.1038/s41598-021-82970-2 PMid:33564039 PMCid:PMC7873266
32. Ripamonti C, Spadotto V, Pozzi P, Stevenazzi A, Vergani B, Marchini M, et al. HDAC Inhibition as Potential Therapeutic Strategy to Restore the Deregulated Immune Response in Severe COVID-19. Front Immunol. 2022;13:841716. https://doi.org/10.3389/fimmu.2022.841716 PMid:35592335 PMCid:PMC9111747
33. Buran K. Colchicine as a potential HDAC inhibitor: comparative binding energies and prospects for

cancer therapy repurposing. Journal of Research in Pharmacy. 2024;28(5):8. https://doi.org/10.29228/jrp.853
34. Zhang X, Kong Y, Zhang J, Su M, Zhou Y, Zang Y, et al. Design, synthesis and biological evaluation of colchicine derivatives as novel tubulin and histone deacetylase dual inhibitors. European Journal of Medicinal Chemistry. 2015;95:127-35. https://doi.org/10.1016/j.ejmech.2015.03.035 PMid:25805446
35. Jocher G, Grass V, Tschirner SK, Riepler L, Breimann S, Kaya T, et al. ADAM10 and ADAM17 promote SARS-CoV-2 cell entry and spike protein-mediated lung cell fusion. EMBO Rep. 2022;23(6):e54305. https://doi.org/10.15252/embr.202154305 PMid:35527514 PMCid:PMC9171409
36. Zipeto D, Palmeira JDF, Argañaraz GA, Argañaraz ER. ACE2/ADAM17/TMPRSS2 Interplay May Be the Main Risk Factor for COVID-19. Front Immunol. 2020;11:576745. https://doi.org/10.3389/fimmu.2020.576745 PMid:33117379 PMCid:PMC7575774
37. Parra-Medina R, Sarmiento-Monroy JC, Rojas-Villarraga A, Garavito E, Montealegre-Gómez G, Gómez-López A. Colchicine as a possible therapeutic option in COVID-19 infection. Clin Rheumatol. 2020;39(8):2485-6. https://doi.org/10.1007/s10067-020-05247-5 PMid:32556936 PMCid:PMC7301347
38. Beyerstedt S, Casaro EB, Rangel É B. COVID-19: angiotensin-converting enzyme 2 (ACE2) expression and tissue susceptibility to SARS-CoV-2 infection. Eur J Clin Microbiol Infect Dis. 2021;40(5):905-19. https://doi.org/10.1007/s10096-020-04138-6 PMid:33389262
39. Abd Al-Hameed JM, Ali BH. Evaluation of ERK-5 expression in type 2 diabetic patients following COVID-19 vaccination; a prospective case-control study. Journal of Renal Injury Prevention. 2026;15(3).