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Sequential doxycycline and colchicine combination therapy in Covid-19: The salutary effects

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ABSTRACT

Coronavirus virus disease 2019 (COVID-19) is a viral infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), actually considered as a global pandemic. The entry-point for SARS-CoV-2 is angiotensin converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4), which are highly expressed in the lung. Among other complications, COVID-19leads to fatal pneumonia, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) due to development of cytokine storm (CS). The pathogenesis of SARS-CoV-2 infection depends on the viral load and human innate/adaptive immune response that are required for viral elimination in the first phase of COVID-19. However, an exaggerated immune response in the second phase of COVID-19 results in immune overreaction and CS-induced ALI and ARDS. Thus, in view of these considerations, we report here a series of five patients with COVID-19 pneumonia who developed ALI. In addition to the supportive therapy, the patients received doxycycline in the first week and doxycycline plus colchicine in the second week. Following sequential therapy with doxycycline and/or colchicine in patients with COVID-19 pneumonia, the patients had reduction of disease severity and symptoms with better clinical and radiological outcomes. However, it is tough to confirm the link between this therapeutic combination and recovery from COVID-19 pneumonia, as it is a small case-series report. Nevertheless, this study gives a rational for large-scale prospective studies to evaluate the dual sequential effect of doxycycline and colchicine on the COVID-19 severity. This case-series illustrated that use of colchicine: doxycycline combination is linked with marked improvements in the clinical, laboratory and radiological outcomes in patients with COVID-19 pneumonia. However, we cannot sketch any definitive conclusion from our observation, despite we hypothesize that this combination therapeutic regimen may attenuate and treat COVID-19. Further, namely prospective, randomized, and controlled clinical studies are recommended in this regard.

1. Background

Considered a global pandemic, coronavirus virus disease 2019 (COVID-19) is a viral infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. Previously, in 2003 and 2012, the severe acute respiratory syndrome coronavirus type 1 (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), respectively, also led to fatal pneumonia, acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) [2]. SARS-CoV, MERS-CoV and SARS-CoV-2 are positive-sense, enveloped single-strand RNA *betacoronaviruses* with some phylogenetic similarities: SARS-CoV-2 has 79% similarity with SARS-CoV and 96% with bat coronavirus (CoV) [3]. It has been shown that SARS-CoV-2 has a higher ability of transmission and lower fatality rate compared to SARS-CoV

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and MERS-CoV [4]. For instance, on January 10, 2021, there were 92, 493,946 confirmed cases officially reported in more than 200 countries with 1,980,825 deaths globally [5].

The entry-point for SARS-CoV-2 is angiotensin converting enzyme 2 (ACE2) and dipeptidyl peptidase 4 (DPP4) receptors, which are highly expressed in different tissues, mainly in the lung, heart, kidney, testis, pancreas and endothelium [6]. The interaction of SARS-CoV-2 with lung alveolar type II cells, ACE2 and DPP4 lead to macrophages activation with subsequent exaggerated immune response, hypercytokinemia, and cytokine storm (CS) due to release of pro-inflammatory cytokines, such as interleukin (IL)-6, IL-1β and tumor necrosis factor (TNF)-α with activation of nod-like receptor pyrine 3 (NLRP3) inflammasome [7]. This interaction is dependent on viral load and human innate/adaptive immune response, required for viral elimination in the first phase of COVID-19. However, exaggerated immune responses in the second phase of COVID-19 result in immune overreaction and CS-induced ALI and ARDS [8]. Regarding clinical impact, most of SARS-CoV-2 infections (80%) are mild due to normal immune response and low viral load, being 15% considered severe and only 5% are established as critical, needing mechanical ventilation [9].

Different drug modalities have been adopted for management of COVID-19, including antiviral and immune-modulating drugs and complementary medicine to overcome the viral pathogenesis and the overstated the release of pro-inflammatory cytokines [10].

Colchicine is a lipophilic tricyclic alkaloid derived from meadow saffron named *Cochicum autumnale*, has been used over 2000 years as the main remedy for gout and painful conditions. Colchicine acts as an inhibitor of cellular microtubule assembly, and binds to the tubulin to form tubulin-colchicine complex that interfere with microtubule formation and polymerization, mainly in cells with high proliferative rate, like neutrophils [11]. Colchicine also has anti-inflammatory effects through different mechanisms, including inhibition of neutrophil functions and chemotaxis, expression of adhesion molecules, suppression of pro-inflammatory cytokines release and inhibition of NLRP3 inflammasomes [12]. Therefore, colchicine is used in the treatment and prophylaxis of gout flares, crystal arthropathy and familial Mediterranean fever (FMF) as well as inflammatory disorders, like pericarditis [13]. Thus, as colchicine has a potent anti-inflammatory potential, different clinical trials have been done to explore its therapeutic benefits in the management of COVID-19. A Greek clinical trial study (GRECCO-19), by Deftereos et al. [14], recommended the use of colchicine in the management of COVID-19.

On the other hand, doxycycline is a bacteriostatic antibiotic from the tetracycline class; inhibits bacterial protein synthesis through blocking ribosomal 30 subunit. Doxycycline like other tetracycline exerts anti-inflammatory effect in different inflammatory disorders, including periodontitis, gingivitis, osteoarthritis, rheumatoid arthritis and ARDS [15]. The anti-inflammatory effect of doxycycline is linked with inhibition of nuclear factor kappa-B (NF-κB), p38, mitogen-activated protein kinase (MAPK) signaling pathway and inhibition the release of pro-inflammatory cytokines [16]. In addition, doxycycline therapy is effective against viral and chemotherapy-induced ALI through down-streaming of matrix metalloproteinase (MMP-2, MMP-9) [17]. For example, Fredeking et al. [18] illustrated that doxycycline reduces pro-inflammatory cytokines in patients with dengue hemorrhagic fever. Furthermore, different reports disclosed the potential benefit of doxycycline therapy in the management of COVID-19 due to its anti-inflammatory and anti-SARS-CoV-2 activities [19].

Taken together, since COVID-19 patients are in an enormous need for both anti-inflammatory and antiviral treatment as well as protection against ALI, studies addressing the effect of a planned combination therapy are warranted. Thus, in view of these considerations, we report a series of five patients with COVID-19 pneumonia who developed ALI. The patients in addition to the supportive therapy were placed on doxycycline (100 mg/day) in the first week and doxycycline (100 mg/day) plus colchicine (1 mg/day) in the second week. In this case-series study, there was no any concomitant use of other antibiotics or any drugs proposed to be beneficial in COVID-19 pneumonia. The main goal of this study was to illustrate the potential impact of a combination therapy with doxycycline-colchicine on the clinical outcomes of patients with Covid-19 pneumonia.

This study was approved by the Ethical Committee and Scientific Board in College of Medicine, Al-Mustansiyriah University, Baghdad, Iraq in October 2020 according to the ethical reference number 33NTE in July 1, 2020. Informed verbal and written consent was taken from the recruited patients for their participation in this study.

2. Case one

On 10, October 2020, a 36-years-old employment man, presented with fever, headache, profuse sweating, nausea, anorexia, vomiting, dry cough and anosmia for 1-week duration without history of any chronic diseases. On examination, the patient presented with body temperature 39.7 °C, heart rate 112 beat/min, blood pressure 110/60 mmHg, and oxygen saturation 92% at room air. Laboratory findings illustrated positive anti- COVID-19 test for IgM (3.07 mIu/ml) and negative for IgG (cut-off value of 0.00–0.04 mIu/ml for both IgM and IgG), real-time polymerase chain reaction (RT-PCR) was positive, and leukocytosis with lymphopenia, C-reactive protein (CRP) 23 mg/L (0.0–5), serum ferritin 453 ng/mL (20–250), lactate dehydrogenase (LDH) 300 U/L (100–190), and D-dimer 473 ng/mL (<230). Radiological findings by chest X-ray and computed tomography (CT) scan revealed bilateral ground glass opacity (GGO). The patient declined hospitalization and was instructed for general health guidelines and prescribed supportive therapy and oxygen on need. From the first day, he received a doxycycline capsule (100 mg) twice daily for one week and doxycycline (100 mg) twice daily plus colchicine (0.5 mg/day) in the next week. Following a 2-week of therapy, the patient showed clinical, radiological and laboratory improvements, with negative RT-PCR for SARS-CoV-2 and returned to his work normally.

3. Case two

On October 23, 2020, a 47-year-old man with history of type 2 diabetes mellitus (T2DM) on regular insulin therapy presented with low-grade fever, headache, malaise and fatigue, dry cough for 4 days duration. On examination, the patient presented with body temperature 37.2 °C, heart rate 83 beat/min, blood pressure 130/75 mmHg, oxygen saturation 94% on room air. Laboratory findings illustrated positive anti-COVID-19 test for IgM (1.02 mIu/ml), negative for IgG (cut-off value of 0.00–0.04 mIu/ml for both IgM and IgG), RT-PCR positive, leukocytosis with lymphopenia, CRP 10 mg/L (0.0–5), serum ferritin 280 ng/mL (20–250), LDH 231 U/L (100–190), D-dimer 254 ng/mL (<230), fasting blood glucose (FBG) 145 mg/dL, and glycated hemoglobin (HbA1c) 6.7%. Radiological findings, chest X-ray and CT scan revealed mild bilateral GGO. The patient was informed for general health guidelines and prescribed supportive therapy, being given doxycycline (100 mg) twice daily for the first one week and doxycycline (100 mg)/twice daily plus colchicine (0.5 mg/day) in the next second week. During the first week of doxycycline therapy, the FBG was reduced. In the second week, the patient reported myalgia and diarrhea, and in the subsequent two weeks of therapy the patient showed clinical, radiological and laboratory improvements with negative RT-PCR for SARS-CoV-2 and repaid to his work routinely.

4. Case three

A 68-year-old retired man, a known case of essential hypertension on telmisartan treatment (160 mg/day) since 2012, on December 10, 2020, presented with low-grade fever, headache, malaise and fatigue, dry cough and anosmia for 1-week duration. On examination, the patient presented with a body temperature 37.6 °C, heart rate 80 beat/min,

blood pressure 140/95 mmHg, oxygen saturation 97% on room air. Laboratory findings illustrated positive anti-COVID-19 test for IgM (1.02 mIu/ml) and negative for IgG (cut-off value of 0.00–0.04 mIu/ml for both IgM and IgG), leukocytosis with lymphopenia, CRP 34 mg/L (0.0–5), serum ferritin 278 ng/mL (20–250), LDH 290 U/L (100–190), and D-dimer 300 ng/mL (<230). Radiological findings, chest X-ray and CT scan illustrated mild unilateral GGO. The patient received supportive therapy with doxycycline (100 mg)/twice daily for one week and doxycycline (100 mg) twice daily plus colchicine (0.5 mg/day) in the next week. In the second week, the patient reported recurrent hypoglycemic events. In the subsequent two weeks of therapy, the fever resolved with negative RT-PCR for SARS-CoV-2 and the patient returned to his home with good health.

5. Case four

A 74-year-old housewife woman a known case of hypertension on candesartan therapy (16 mg/day) since 2010. On November 5, 2020 presented with dyspnea, low-grade fever, headache, dry cough and anosmia for 10 days duration. She was misdiagnosed as having a typhoid fever and received ciprofloxacin therapy for seven days without any response. On examination, the patient presented with body temperature 38.6 °C, heart rate 83 beat/min, blood pressure 140/95 mmHg, oxygen saturation 94% on room air. The investigations illustrated a positive anti-COVID-19 test for IgM, RT-PCR positive, leukocytosis with lymphopenia, CRP 11 mg/L (0.0–5), serum ferritin 233 ng/mL (20–250), LDH 180 U/L (100–190), and D-dimer 100 ng/mL (<230). Radiological findings, chest X-ray and CT scan showed mild bilateral GGO. She was treated by symptomatic therapy and doxycycline 100mg/twice daily for one week and doxycycline100mg/twice daily plus colchicine 0.5 mg/day in the next week. Following two weeks of therapy, the fever resolved with negative RT-PCR for SARS-CoV-2 and the patients returned to his home with good health.

6. Case five

On July 22, 2020, a 49-year-old man presented with dyspnea, severe headache, nausea, anorexia, vomiting, dry cough and anosmia for two weeks duration. Physical examination revealed high body temperature (40.7 °C), heart rate 123 beat/min, blood pressure 110/50 mmHg, and oxygen saturation 87% on room air. Laboratory investigations demonstrated positive anti-COVID-19 test for IgM (13.45 mIu/ml) and IgG (4.83 mIu/ml), positive RT-PCR, leukocytosis 27.96×10^3 with lymphopenia 16.07×10^3, CRP 44 mg/L (0.0–5), serum ferritin 906 ng/mL (20–250), LDH 731 U/L (100–190), and D-dimer 422 ng/mL (<230). Radiological findings by chest X-ray and CT scan revealed severe bilateral GGO affecting 50% of the lung. He was hospitalized and managed by supportive therapy, high pressure oxygen nasal cannula, and empirical antibiotic therapy including ceftriaxone and azithromycin, as well as dexamethasone injections for one week. On the beginning of the fourth week of disease, doxycycline (100 mg)/twice daily was given. On the fifth week of COVID-19 pneumonia, the RT-PCR test became negative, despite of that the radiological findings were still present, but to a lesser extent. On the sixth week of COVID-19 pneumonia, colchicine (0.5 mg/day) was added to the doxycycline therapy (100 mg, twice) for the next week. At the end of doxycycline-colchicine combination therapy, a dramatic improvement in all clinical, laboratory findings and clinical outcomes was stated. He was discharged without need for oxygen therapy following an additional one week with negative RT-PCR.

7. Clinical course summary

All reported cases were COVID-19 pneumonia with mild-to-moderate clinical presentation, except for case (5), who was severe and needs supportive therapy with non-invasive ventilation. Also, all cases were hospitalized for about two weeks, except for case (1), who was treated at home. The comorbidities have also been reported, in case 2 (T2DM) and in cases 3 and 4 (hypertension). Before doxycycline therapy, all patients were symptomatic with high levels of biomarkers of active disease, like leukocytosis, lymphopenia, CRP, serum ferritin, D-dimer, LDH and positive RT-PCR. The radiological findings were detectable by lung CT scan as bilateral or unilateral ground glass appearance (GGA). COVID-19 patients were treated sequentially with doxycycline capsule (100 mg/twice daily) in the first week, and doxycycline capsule (100 mg/twice daily) plus colchicine (0.5 mg/day) in the second week. Following doxycycline treatment in the first week, patients showed mild clinical and laboratory improvements, but lung GGA and positive RT-PCR were still evident. In the second week, colchicine was added to treatment with doxycycline in the form of doxycycline: colchicine combination at doses of 200 mg/day and 0.5 mg/day, respectively. At the end of the second week of combination therapy, dramatic clinical and laboratory improvements with negative RT-PCR and resolution of lung GGA were stated (Table 1, Fig. 1).

8. Discussion

The present study revealed the beneficial effect of doxycycline and colchicine sequential therapy in the management of COVID-19 pneumonia, through a pronounced reduction in the disease severity and symptoms with better clinical and radiological outcomes. However, it is tough to confirm the association between this combination therapy and recovery from COVID-19 pneumonia due to a small case-series report. Nevertheless, this study gives a rational for large-scale prospective study to evaluate the dual sequential effect of doxycycline and colchicine in COVID-19 therapy. The patients reported here were not critically ill and did not need intensive care and presented with various high-risk comorbidities, like T2DM (case 2), hypertension and old age (case 3 and 4). Indeed, it has been stated that T2DM and old age increase the COVID-19 risk and severity due to immunological disturbances and high level of pro-inflammatory cytokines production [20]. Zhao et al. [21], reported that high neutrophil: lymphocyte ratio (NLR), IgE, TNF-α, IL-6 and CRP in T2DM patients noticeably predispose for a higher COVID-19 severity.

In our case-series report, inflammatory biomarkers (ferritin, CRP), tissue injury biomarker (LDH), and coagulation biomarker (D-dimer) were increased in all cases with bilateral lung infiltration, with exception of case 3, in which unilateral lung infiltration was linked with low levels of inflammatory biomarkers. Francone et al. [22] disclosed that higher lung CT scan score is linked to the elevated pro-inflammatory/inflammatory cytokines and COVID-19 severity in symptomatic patients.

Furthermore, in this study, administration of doxycycline in the first week and combination of doxycycline with colchicine in the second week for COVID-19 management, led to a noteworthy recovery in clinical, laboratory and radiological findings with exception of case 5, in which lung damage was still present, despite of negative RT-PCR. Grillo et al. [23] illustrated that lung fibrotic pathological findings are unlikely to resolute in severe COVID-19 patients and the resident lung damage might be due to CS-induced lung injury, prolonged low-grade viral infection and secondary cytokine cascade. Of note, case 5 received dexamethasone which has noteworthy effect in the management of COVID-19 [24] and recovery of this patient might due to dexamethasone effect which could potentiates the anti-inflammatory effect of doxycycline and/or colchicine.

The rational protocol of the present study showed that administration doxycycline in the first week helped to overcomes SARS-CoV-2 infection and associated inflammatory reactions. Amide different recent literature studies, doxycycline inhibits the SARS-CoV-2 pathogenesis by blocking the DPP4/CD26/NF-κB axis. DPP4 is regarded as an entry-point for SARS-CoV-2 and site for NF-κB action, thus NF-κB inhibition by doxycycline reduces the DPP4 expression [25]. Conforti et al. [26] also showed that doxycycline in addition to its antibacterial

Table 1
Clinical outcome summary of COVID-19 cases at end of doxycycline or doxycycline in combination with colchicine treatments.

Cases	At time of admission	At end of doxycycline treatment	At end of doxycycline plus colchicine	Discharge
Case 1	Presented with fever, headache, profuse sweating, nausea, anorexia, vomiting, dry cough and anosmia. Positive RT-PCR, PaO2 92% leukocytosis and lymphopenia, bilateral GGO, CRP 23 mg/L	Mild clinical improvements, dry cough and anosmia were still present. RT-PCR positive, Pao2 90%, bilateral GGO, CRP 20 mg/L.	Dramatic clinical improvements, PaO2 97%, negative RT-PCR, clear lung, and CRP 8 mg/L.	Well[a]
Case 2	T2DM patient presented with low-grade fever, headache, malaise and fatigue, dry cough for 4 days duration. Positive RT-PCR, PaO2 94% leukocytosis and lymphopenia, bilateral GGO, CRP 10 mg/L, FBG 145 mg/dL, and HbA1c 6.7%.	Mild clinical improvements, dry cough and anosmia were still present. RT-PCR positive, Pao2 92%, bilateral GGO, CRP 8 mg/L, FBG 65 mg/dL, and HbA1c 6.7%.	Complete clinical recovery. RT-PCR negative, PaO2 98%, bilateral GGO, CRP 8 mg/L, FBG 90 mg/dL, and HbA1c 6.7%.	Well
Case 3	Hypertensive patient presented with low-grade fever, headache, malaise and fatigue, dry cough and anosmia for 1-week duration. Positive RT-PCR, PaO2 97% leukocytosis and lymphopenia, unilateral GGO, CRP 34 mg/L.	Mild clinical improvements, dry cough and anosmia were still present. Positive RT-PCR, PaO2 97%, unilateral GGO, CRP 20 mg/L.	Dramatic clinical improvement, PaO2 98%, negative RT-PCR, clear lung, and CRP 7 mg/L.	Well
Case 4	Hypertensive patient presented with low-grade fever, headache, malaise and fatigue, dry cough and anosmia for 1-week duration. Positive RT-PCR, PaO2 94% leukocytosis and lymphopenia, bilateral GGO, CRP 11 mg/L.	Mild clinical improvements, dry cough and anosmia were still present. Positive RT-PCR, PaO2 97%, bilateral GGO, CRP 8 mg/L.	Complete clinical improvement, negative RT-PCR, PaO2 99%, clear lungs, CRP 8 mg/L	Well
Case 5	Presented with dyspnea, severe headache, nausea, anorexia, vomiting, dry cough and anosmia for two weeks duration. Positive RT-PCR, PaO2 87% leukocytosis and lymphopenia, bilateral GGO, CRP 44 mg/L.	Mild clinical improvements, dry cough, anosmia and fever were still present. Negative RT-PCR, PaO2 90%, bilateral GGO, CRP 40 mg/L.	Dramatic clinical improvements, PaO2 96%, negative RT-PCR, clear lung, and CRP 12 mg/L.	Well

[a] Treated at home.

property is capable to treat secondary bacterial super-infections in COVID-19 patients, and exerts a marked anti-inflammatory effect through inhibition of pro-inflammatory cytokines and MMPs with attenuation the development of CS. As well, doxycycline blocks the core-receptor CD147 which is necessary for the SARS-CoV-2 entry into T lymphocytes [27]. Doxycycline may directly interrupt the replication and life cycle of SARS-CoV-2 through proteolysis induction of viral non-structural proteins and inhibition of RNA-dependent polymerase or indirectly through increasing the intracellular concentration of zinc which block the SARS-CoV-2 replication [28]. Moreover, doxycycline has anticoagulant effect [29] which could attenuate COVID-19-induced prothrombotic activation and development of ALI, ARDS and multi-organ injury [30].

On the other hand, the present study revealed that colchicine when combined with doxycycline in the second week of COVID-19 therapy led to a dramatic improvement and rapid recovery in the reported case-series. Montealegre-Gomez et al. [21] reported 5 patients on the colchicine therapy for 1–3 weeks prior development of COVID-19 pneumonia, found mild symptoms. Different studies have illustrated that colchicine therapy reduced the activation of IL-1/IL-6 axis and NLRP3 inflammasome that are involved in the development of CS-induced endothelial dysfunction and coagulopathy in patients with COVID-19 [31]. The potential advantage of colchicine therapy in patients with COVID-19 pneumonia was also addressed in a clinical trial that involved 100 COVID-19 patients (Clinical Trial.gov Identifier: NCT04322565), where a significant clinical improvement following colchicine therapy was stated [32].

Scarsi et al. [33] described the potential benefit of colchicine therapy in patients with severe COVID-19 at the 8th day of Covid-19 compared to the standard therapy, through inhibition of major pro-inflammatory cytokines engaged with development of ALI and ARDS. It has been reported that cytokine storm and uncontrolled hyperinflammation are linked with multi-organ damage and ARDS. Therefore, anti-cytokine therapy, such as anakinra (IL-1 receptor antagonist), mavrilimumab (colony stimulating factor antagonist) and tocilizumab (IL-6 receptor antagonist) are theoretically effective in COVID-19, but not prompted full recovery as expected due to late administration of these agents when ALI was developed [34]. Similarly, sarilumab is a human anti-IL-6 receptor monoclonal IgG1 antibody, revealed to be effective against the development of ALI and lung consolidation in COVID-19 pneumonia as reported by Della-Torre et al. [35]. Della-Torre et al. [36] also illustrated that a timely administration of colchicine in COVID-19 patients is important and should be given at 5th day of spiking fever or after 8th day of influenza-like symptoms to attenuate CS-induced ALI and multi-organ damage, since early colchicine therapy in COVID-19 may impairs the immune response to SARS-CoV-2 [36].

Furthermore, Deftereos et al. [37], performed a randomized clinical trial with 105 COVID-19 patients on colchicine therapy compared with standard care control; found that colchicine is an effective agent in reducing ALI and cardiac injury in the early course of COVID-19 due to its potent anti-inflammatory effects. Likewise, Dupuis et al. [38], described that colchicine is effective in the management of ALI and

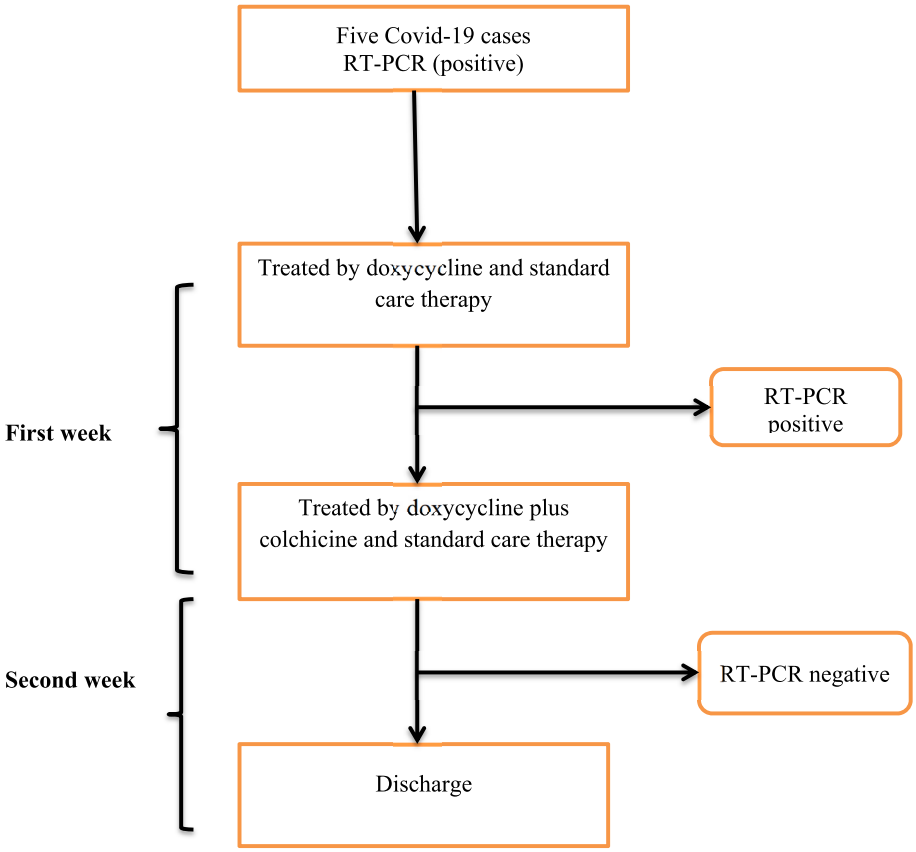

Fig. 1. Clinical course of reported COVID-19 cases.

ARDS through reduction of pro-inflammatory CS and inhibition of cellular inflammasomes. Indeed, COVID-19 in FMF patients under colchicine therapy developed mild symptoms without radiological findings [39]. In addition, various studies illustrated that colchicine therapy is an effective agent in prevention of ALI and ARDS.

Yue et al. [40] found that pretreatment with colchicine improves lung oxygenation with a significant reduction of pulmonary edema and neutrophil recruitment, and may prevent the development of ARDS. As well, a meta-analysis by Vrachatis et al. [41] illustrated the beneficial effect of colchicine therapy in the management of COVID-19.

On the other hand, colchicine has important antiviral effects; briefly, it inhibits the replications of flaviviruses, respiratory syncytial virus, hepatitis virus, coronaviruses and human immune deficiency virus through suppression of microtubules polymerization [42]. SARS-CoV-2, like other coronaviruses, its replication is mainly depend on the cytosolic microtubules with formation of membrane vesicles in the infected cells due to interaction of spike protein with tubulin proteins, that facilitates the SARS-CoV-2 entry to the nucleus with further assembly of virions. Therefore, inhibition of microtubules by colchicine may prevent the virus transport and replication with subsequent reduction of tissue damage due to reduction of pro-inflammatory cytokines release [43].

Therefore, the potent anti-inflammatory effect of colchicine along with its antiviral effects made it a possible candidate against SARS-CoV-2 infections [44].

To author's knowledge, there is no any study recommending the combination of doxycycline and colchicine in the management of COVID-19. Nevertheless, one study reported that the combination of doxycycline and colchicine therapy for autoimmune bullous diseases should be continuing during COVID-19 development [45]. However, there are various studies reporting the combination of doxycycline with other agents in the COVID-19 management. Alam et al. [46] showed a significant effect of doxycycline and ivermectin combination in the management of mild-to-moderate COVID-19. Similarly, the combination of doxycycline with famotidine is regarded as a robust chemoprophylaxis against COVID-19 [47].

Thus, depending on both previous and recent studies, we suggest that combination of doxycycline and colchicine lead to the following effects in patients with COVID-19: 1) Synergistic anti-SARS-CoV-2 effects through inhibition of cellular microtubules by colchicine and RNA-dependent polymerase, non-structural proteins by doxycycline [48,49]; 2) Synergistic anti-inflammatory effect through inhibition of NF-κB/NLRP3 inflammasome and of pro-inflammatory cytokines release by both colchicine and doxycycline [50,51].

In the present study, all reported cases were positive for RT-PCR following doxycycline therapy and became negative following combination of doxycycline and colchicine, suggesting that combination therapy was more effective than doxycycline alone in the management of COVID-19 pneumonia.

In the present study, some of the reported cases had certain side effects, like hypoglycemia (case 3 and 3), despite was mild and self-limited. Hypoglycemia during doxycycline therapy is due to DPP4 blocking effect with prolongation of insulin half-life [52]. In addition, there were no significant interactions between colchicine and doxycycline, meaning that this combination can be used safely in the management of COVID-19.

This study has some limitations, including the small sample size, and the fact that both IL-6 and TNF-α levels were not evaluated in relation to doxycycline alone or in combination with colchicine. Though, a final causal relationship between using of colchicine: doxycycline combination to improve COVID-19 could not be confirmed unless documented by large-scale clinical study. Therefore, we suggest that emerging of different clinical trials should be done to assess the clinical efficacy of colchicine: doxycycline combination in the management of COVID-19.

9. Conclusion

In the present study we described a series of case-repots illustrated that the use of colchicine and doxycycline in combination was linked with marked improvements in clinical, laboratory and radiological outcomes in patients with COVID-19 pneumonia. However, we cannot sketch any definitive conclusion from our observation due to the small sample size. Anyway, we hypothesize that combination of colchicine: doxycycline therapy may attenuate and even be an enormous contributor to treat COVID-19. In our opinion the effect of this combination treatment should be evaluated in a clinical trial and large-scale prospective clinical studies.

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Declaration of competing interest

Nil.

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