



Comparing outcomes of hospitalized patients with moderate and severe COVID-19 following treatment with hydroxychloroquine plus atazanavir/ritonavir

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Abstract

Background The role of the antiviral therapy in treatment of COVID-19 is still a matter to be investigated. Also efficacy and safety of antiviral regimens were not compared according severity of the disease. In this study the efficacy and safety of hydroxychloroquine plus atazanavir/ritonavir was compared in patients with moderate and severe COVID-19.

Methods We prospectively evaluated the clinical outcomes of 213 patients with COVID-19 during the hospitalization course and up to 56 days after the hospital discharge. The disease was categorized to moderate and severe based on the severity of pneumonia and peripheral oxygen saturation (SpO₂). The patients received the national treatment protocol containing hydroxychloroquine (400 mg BD in first day and then 200 mg BD) plus atazanavir/ritonavir (300/100 mg daily) for 7 days. Main outcomes included discharge rates at day 7, 14 and 28, 28-day mortality, rate of intensive care unit (ICU) admission and intubation, length of hospital and ICU stay and incidence of adverse events.

Results The mean (SD) age of patients was 60(14) years and 53% were male. According to WHO definition, 51.64% and 48.36% of the patients had moderate (SpO₂ ≥ 90%) and severe disease (SpO₂ < 90%) at baseline, respectively. The discharge rate of the moderate group was significantly higher than the severe group at day 7, 14 and 28 (HR = 0.49; 95% CI: 0.35–0.69, p = < 0.001 at day 7, HR = 0.48; 95% CI: 0.35–0.66, p = < 0.001 at day 14 and HR = 0.49; 95% CI: 0.36–0.67, p = < 0.001 at day 28). The 28-day mortality of the severe group was six times higher than the moderate group (HR = 6.00; 95% CI: 2.50–14.44), p = < 0.001).

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The need of admission in ICU for the severe group and the moderate group was 37.86% and 18.18% of the patients. Length of hospital stay was significantly shorter in the moderate group in comparison with the severe group (5 ± 4 vs. 8 ± 6 days, $p < 0.001$). Patients in the moderate group experienced the serious adverse events and complications less than the severe group. The discharged patients were followed up to 56 days after discharge. Some of the patients complained of symptoms such as exertional dyspnea, weakness and new-onset hair loss.

Conclusion Our study did not support the use of hydroxychloroquine plus atazanavir/ritonavir in patients who had $\text{SpO}_2 < 90\%$ at the time of hospital admission. SpO_2 was the only predictor of clinical outcomes (duration of hospital stay, discharge from the hospital and mortality) in patients treated with hydroxychloroquine plus atazanavir/ritonavir.

Keywords COVID-19 · Hydroxychloroquine · Atazanavir/ritonavir · Outcomes

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the emerged life threatening coronavirus, has been known as a serious concern worldwide. It received attention when a series of ‘unknown viral pneumonia’ in China’s Hubei province in late December, 2019 were identified [1]. The century witnesses its third corona virus outbreak i.e. COVID-19 (Coronavirus Disease 2019) after SARS (Severe Acute Respiratory Syndrome) in 2003 and MERS (Middle East Respiratory Syndrome) in 2012 [2]. Although many attempts have been made to slow the spread of infection, the World Health Organization (WHO) declared it as a pandemic on 11th March, 2020 [3]. The virus has now infected more than fifteen million people around the world [4].

Like the other outbreaks of coronavirus, the definite treatment of COVID-19 is yet to be known [5]. Supportive cares and the respiratory supports are the recommended resuscitative interventions now. The role of the antiviral therapy in treatment of COVID-19 is still a matter to be investigated. Also efficacy and safety of antiviral regimens were not compared according severity of the disease. In this study the efficacy and safety of hydroxychloroquine plus atazanavir/ritonavir were compared in patients with moderate and severe COVID-19.

Materials and methods

Study design and participants

Patients with moderate and severe COVID-19 who were admitted to Imam Khomeini Hospital Complex during 20 February to 29 March 2020 were enrolled in this prospective cohort study. The hospital is one of the major hospitals for admission and care of patients with COVID-19 in Tehran, capital of Iran. According to the national committee of COVID-19, the admission indications were the presence of one or more of the subsequent conditions: (1) peripheral oxygen saturation (SpO_2) less than 93% in ambient air (2)

respiratory rate more than 30 breath per minute (3) lung infiltration highly suspicious for COVID-19 in chest imaging. Discharge criteria included all of the following: (1) improvement in clinical signs and symptoms based on decision making of physician (2) afebrile status for 72 h without antipyretics (3) SpO_2 more than 93% in ambient air without supplemental oxygen.

According to the disease severity and WHO definitions [6], the patients were categorized to moderate or severe group. Patients in the moderate group had primary symptoms of pneumonia (dyspnea, cough and fever) and $\text{SpO}_2 \geq 90\%$ in ambient air and patients in the severe group had profound pneumonia and $\text{SpO}_2 < 90\%$.

The sampling for diagnosis of infection was performed according to the WHO interim guidance [7]. The diagnosis was according to positive reverse transcriptase-polymerase chain reaction (RT-PCR) of nasopharyngeal secretions (197 patients) or clinical data plus chest CT scan findings that were highly suggestive for COVID-19 (16 patients).

Ethics Committee of Tehran University of Medical Sciences approved the study (approval ID: IR.TUMS.VCR.REC.1398.1058). Written informed consents were obtained from the patients or one of the first-degree family members if patient was unconscious. The study protocol was also registered as IRCT20100228003449N30.

Procedures

Hydroxychloroquine (Modaquenil®, Mofid Pharmaceutical co., Iran) as 400 mg BD in first day and then 200 mg BD plus atazanavir/ritonavir (ATAZOR-R, Emcure Pharmaceutical co., India) as 300/100 mg daily were started within 24 h of the hospital admission for all patients. The duration of treatment was 7 days. Patients completed the duration of treatment at home if they were discharged before fulfillment of treatment in hospital. Other treatments that were considered for patients such as other antivirals, corticosteroids, intravenous immunoglobulin (IVIG), vitamin C, antibiotics, analgesic agents, anti-nausea/vomiting agents, cardiovascular drugs,

deep vein thrombosis and stress ulcer prophylaxis were recorded.

Data collection

Demographic, clinical presentations of the diseases, baseline diseases, drug history, laboratory and medical data were collected. The patients' symptoms such as fever, cough, dyspnea, myalgia, arthralgia, gastrointestinal (GI) complaints and sings including SpO₂, pulse rate, respiratory rate, blood pressure and temperature were recorded at the time of hospital admission. Laboratory tests such as white blood cells, serum electrolytes, liver and kidney enzymes and inflammatory biomarkers consisting C- reactive protein (CRP) and Erythrocyte Sedimentation Rate (ESR) were extracted from the Hospital Information System (HIS).

Definitions

ARDS was defined based on the Berlin criteria and the risk was stratified according to the SpO₂/FiO₂ ratio [8]. Acute kidney injury (AKI) was defined according to the Kidney Disease Improving Global Outcomes (KDIGO) criteria [9]. Acute hepatic injury (AHI) was considered when hepatic aminotransferases serum levels raised more than three times the upper limit of normal or serum total bilirubin above 2 mg/dl [10]. Acute cardiac injury (ACI) was described as troponin serum level above the 99th-percentile the upper limit normal, regardless of presence or absence of the abnormal findings in echocardiography or electrocardiography [11].

Outcomes

Efficacy outcomes were defined as discharge rate at days 7, 14 and 28, 28-day mortality, rate of intensive care unit (ICU) admission and intubation, length of hospital and ICU stay. Complications during hospitalization such as GI problems, ARDS, AKI, AHI and ACI were considered as safety outcomes.

Follow up

Discharged patients were followed at days 28 and 56 by phone call. Time to the functional recovery, complications, readmission and death were assessed at those times.

Statistical analysis

All statistical analysis was performed using SPSS version 21.0. The continuous variables were presented as mean \pm standard deviation, median and interquartile range (IQR) and categorical variables were described as number and frequency

rate (percentage). If the distribution of data was normal (pass the normality test), independent samples t-test was applied to compare the continuous variables, otherwise Mann-Whitney U test was used. Categorical variables were compared by Chi-square test. The Hazard Ratio (HR) and 95% CI for discharge and mortality rates were calculated by Cox proportional hazards regression analysis. Effects of demographic variables (age and body mass index), comorbid conditions (ischemic heart disease and hypertension), laboratory parameters (CRP) and treatment modalities (corticosteroids, IVIG, vitamin C, ACEI, ARB) on mortality were assessed by adjusted Cox proportional hazards regression models as confounding factors. Finally, Kaplan-Meier cumulative survival plot was created.

Results

Participants

During the study period, 110 (51.64%) and 103(48.36%) patients with moderate and severe disease were included respectively. Mean age \pm SD of patients was 60 \pm 14 years and number of males was slightly more than females (113 vs. 100). The most common baseline diseases among the patients were hypertension (39.90%), diabetes mellitus (32.86%), cardiovascular diseases other than hypertension (28.63%) and hypothyroidism (10.79%). The most frequent symptoms were dyspnea (61.97%), cough (59.62%) and fever (58.21%) as presented. Patients in severe group were older than the moderate group (61 \pm 12 vs. 58 \pm 15 years, $p = 0.085$). Baseline characteristics, baseline diseases (except ischemic heart disease) and drug history were not different between the groups (Table 1).

Vital signs and laboratory data of the patients were compared between the groups (Table 2).

All admitted patients required a type of the respiratory supports including nasal cannula, face mask, non-invasive positive pressure ventilation (NIPPV) and invasive mechanical ventilation (IMV). Ribavirin, interferon, Azithromycin, corticosteroid, IVIG, or vitamin C were considered for patients according the experts' opinions. A corticosteroid was administered in 10.0% and 23.30% of patients in the moderate and severe groups respectively ($p = 0.01$). Also, 12.62% of patients in the severe group and 4.54% of patients in the moderate group received IVIG ($p = 0.047$). Intravenous vitamin C was administrated for 27% and 9.09% of patients in the severe and moderate groups respectively ($p = 0.001$). Patients in the severe group received more antibiotics than in the moderate group (60.19% vs. 32.72%, $p < 0.001$). Other findings are summarized in Table 3.

Table 1 Baseline characteristics of patients

Characteristic	Patients with moderate disease (N = 110)	Patients with severe disease (N = 103)	p value
Age (year), mean ± SD	58 ± 15	61 ± 12	0.085
Male sex, n (%)	63(57.27)	50(48.54)	0.218
Female sex, n (%)	47(42.73)	53(51.46)	
BMI (Kg/m ²), mean ± SD	26 ± 4	27 ± 4	0.121
Baseline diseases, n (%)			
Hypertension	38(34.54)	47(45.63)	0.123
Diabetes mellitus	33(30)	37(35.92)	0.384
Ischemic heart disease	22(20)	39(37.86)	0.006
Hypothyroidism	13(11.81)	10(9.70)	0.664
Malignancy	8(7.27)	4(3.88)	0.377
Neuropsychiatric disorders	5(4.54)	8(7.76)	0.397
Renal disease	3(2.72)	6(5.82)	0.320
Rheumatoid Arthritis	4(3.63)	3(2.91)	1.00
Other rheumatologic disease	3(2.72)	3(2.91)	1.00
Asthma	2(1.81)	1(0.97)	1.00
Transplantation	2(1.81)	1(0.97)	1.00
COPD	1(0.90)	2(1.94)	0.611
Liver disease	0	2(1.94)	0.233
History of drugs, n (%)			
ARB	31(28.18)	32(31.06)	0.655
Metformin	19(17.27)	28(27.18)	0.099
Aspirin	18(16.36)	27(26.21)	0.094
Beta-blockers	14(12.72)	21(20.38)	0.143
Insulin	11(10)	11(10.67)	1.00
Corticosteroids	8(7.27)	5(4.85)	0.572
Hydroxychloroquine	7(6.36)	10(9.70)	0.451
Immunosuppressants	5(4.54)	3(2.91)	0.723
NSAIDs	4(3.63)	1(0.97)	0.370
Methotrexate	4(3.63)	1(0.97)	0.370
ACEI	3(2.72)	6(5.82)	0.320
Azathioprine	1(0.90)	0	1.00
Sulfasalazine	0	2(1.94)	
Symptoms at the time of hospital admission, n (%)			
Dyspnea	66(60)	66(64.07)	0.574
Cough	63(57.27)	64(62.13)	0.488
Fever	62(56.36)	62(60.19)	0.581
Chills	38(34.54)	33(32.03)	0.772
Myalgia	38(34.54)	44(42.71)	0.260
Malaise	24(21.81)	31(30.09)	0.210
Nausea	22(20)	18(17.47)	0.726
Vomiting	19(17.27)	18(17.47)	1.00
Anorexia	15(13.63)	16(15.53)	0.703
Chest discomfort	12(10.90)	8(7.76)	0.487
Diarrhea	10(9.09)	6(5.82)	0.441
Headache	8(7.27)	11(10.67)	0.473
Sore throat	8(7.27)	2(1.94)	0.103
Abdominal pain	7(6.36)	5(4.85)	0.769
Anosmia	1(0.90)	0	1.00
Days of symptoms before the hospital admission, median (IQR)	7(4–10)	7(4–10)	1.00

COPD: chronic obstructive pulmonary disease, ARB: Angiotensin II Receptor Blocker, ACEI: angiotensin converting enzyme inhibitor, NSAID: non-steroidal anti-inflammatory drug

Outcomes

In the moderate group, 77.27% of patients were discharged at day 7 while this value was 49.51% in the severe group (HR =

0.49; 95% CI: 0.35–0.69, $p < 0.001$). Furthermore, discharge rates were significantly higher in the moderate group compared with the severe group at days 14 and 28 (HR = 0.48; 95% CI: 0.35–0.66, $p < 0.001$ at day 14 and HR = 0.49; 95% CI: 0.36–0.67, $p < 0.001$ at day 28). The 28-day mortality rate was much higher in the severe group in comparison with the moderate group (HR = 6.00; 95% CI: 2.50–14.44), $p < 0.001$) (Fig. 1). Out of patients in the moderate and severe groups, 18.18% and 37.86% were admitted to ICU respectively ($p = 0.002$). Of these patients, 40% and 74.35% were intubated in the moderate and severe groups respectively. Average ICU length of stay was not significantly different between the groups. However, mean ± SD of hospital stay was statistically longer in the severe group than moderate group (8 ± 6 vs. 5 ± 4 days, $p < 0.001$). Nausea, vomiting, dyspepsia, abdominal pain, diarrhea were GI complaints in 16.90% of patients. During the hospitalization course, 47 (45.63%) and 8 (7.27%) of patients in the severe and moderate groups experienced ARDS respectively. Prevalence of AKI was significantly higher in the severe group than moderate group (24.27% vs. 7.27% respectively, $p = 0.001$). Incidence of AHI (15.53% vs. 5.45%, $p = 0.023$) was significantly higher in the severe than moderate groups. The incidence of ACI was not statistically different between the groups (22.33% vs. 16.35%, $p = 0.300$) (Table 4).

Adjusted cox proportional regression models

To evaluate the effect of confounding factors on mortality rate, Cox regression models were adjusted for these variables. However, significant deference in mortality rate was detected between the groups (HR = 3.89; 95% CI: 1.36–11.11, $p = 0.011$).

28-day and 56-day follow up

Of 176 discharged patients, 3 patients died at day 28. Other 173 patients were alive on day 56. Readmission rates were 3.97% and 1.70% at days 28 and 56 respectively. The median (IQR) time to functional recovery was 7 (0–14) days. At day 56, 3 and 6 patients reported significant hair loss and exertional dyspnea respectively. Moreover, four patients complained about generalized weakness and dizziness. Up to June 10, 2020, 173/213(81.22%) patients were alive.

Discussion

To the best of our knowledge, for the first time, outcomes of hospitalized patients with moderate and severe COVID-19 treated with hydroxychloroquine plus atazanavir/ritonavir, were compared.

Table 2 Vital signs and laboratory data

Vital sign	Mean ± SD in patients with moderate disease	Mean ± SD in patients with severe disease	p value
Temperature (°C)	37.87 ± 0.84	37.87 ± 0.84	0.989
Heart rate (beats /minute)	91 ± 14	91 ± 15	0.973
Respiratory rate (breaths /minute)	21 ± 4	21 ± 4	0.478
Systolic blood pressure (mm Hg)	124 ± 19	121 ± 16	0.265
Laboratory data			
White Blood Cell (cells / μ l)	7090 ± 3887	7063 ± 3904	0.960
Acute Lymphocyte count (cells / μ l)	1086 ± 480	1133 ± 1049	0.677
Hemoglobin(g/dl)	13.07 ± 2.33	13.46 ± 2.01	0.204
Platelet count (cells $\times 10^3$ / μ l)	205 ± 74	199 ± 73	0.598
Blood Urea Nitrogen (mg/dl)	17 ± 13	19 ± 12	0.293
Creatinine(mg/dl)	1.30 ± 0.78	1.14 ± 0.42	0.082
Aspartate aminotransferase(u/l)	44 ± 29	45 ± 32	0.900
Alanine aminotransferase(u/l)	38 ± 27	36 ± 20	0.524
Alkaline phosphatase(u/l)	197 ± 122	175 ± 77	0.207
Total bilirubin(mg/dl)	0.74 ± 0.50	0.89 ± 0.77	0.180
C-reactive protein(mg/dl)	94 ± 78	117 ± 75	0.053
Erythrocyte sedimentation rate(mm/h)	65 ± 32	66 ± 27	0.823

Table 3 Respiratory support and medications

Parameter	Patients with moderate disease (N = 110)	Patients with severe disease (N = 103)	p value
Respiratory support, n (%)			
Nasal cannula	20(18.18)	11(10.67)	0.001
Face mask	82(74.54)	63(61.16)	
IMV	8(7.27)	29(28.15)	
Applied medications during hospitalization, n (%)			
Sofosbuvir	3(2.72)	7(6.79)	0.203
Interferon beta	1(0.90)	5(4.85)	0.101
Ribavirin	0	8(7.76)	0.003
Corticosteroids	11(10)	24(23.30)	0.01
IVIG	5(4.54)	13(12.62)	0.047
Vitamin C	10(9.09)	28(27.18)	0.001
Antibiotics, n (%)	36(32.72)	62(60.19)	< 0.001
NSAID (naproxen), n (%)	52(47.27)	56(54.36)	0.338
Diphenhydramine, n (%)	49(44.54)	59(57.28)	0.075
PPIs	89(80.90)	95(92.23)	0.017
H ₂ RAs	12(10.90)	5(4.85)	0.131
UFH	84(76.36)	88(85.43)	0.118
LMWH	6(5.45)	6(5.82)	1.00
ARBs	19(17.27)	19(18.44)	0.859
Statins	17(15.45)	27(26.21)	0.063
ACEIs	3(2.72)	4(3.88)	0.714

IMV: invasive mechanical ventilation, NSAID: non-steroidal anti-inflammatory drug, H₂ RA: H₂ receptor antagonists, PPI: proton pump inhibitor, UFH: Unfractionated heparin, LMWH: Low molecular weight heparin, ARB: Angiotensin II Receptor Blocker, ACEI: angiotensin converting enzyme inhibitor

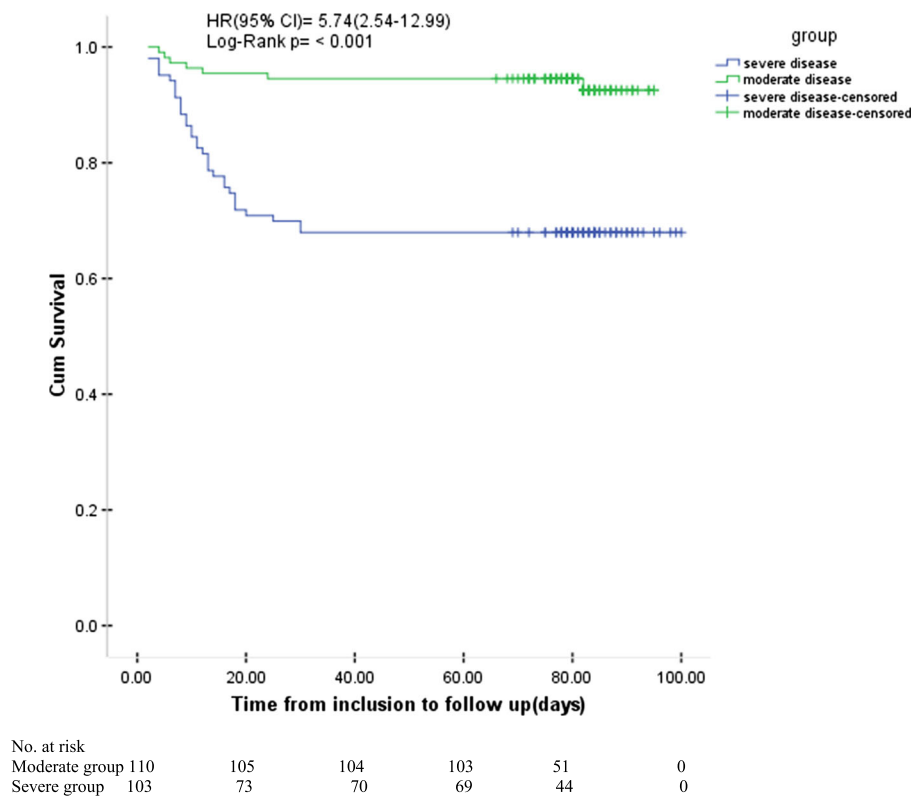
Table 4 Efficacy and safety outcomes

Parameter	Patients with moderate disease (N = 110)	Patients with severe disease (N = 103)	p value
Efficacy outcomes			
Discharge rate at day 7, n (%)	85(77.27)	51(49.51)	< 0.001
Discharge rate at day 14, n (%)	100(90.90)	65(63.10)	< 0.001
Discharge rate at day 28, n (%)	104(94.54)	72(69.90)	< 0.001
28-day mortality, n (%)	6(5.45)	29(28.15)	< 0.001
56-day mortality, n (%)	7(6.36)	33(32.03)	< 0.001
Duration of hospital stay, Mean ± SD (days)	5 ± 4	8 ± 6	< 0.001
Duration of ICU stay, Mean ± SD (days)	6 ± 5	8 ± 6	0.191
Required ICU care, n(%)	20(18.18)	39 (37.86)	0.002
Required IMV, n (%)	8(7.27)	29(28.15)	< 0.001
Safety outcomes			
GI adverse effects	14(12.72)	22(21.35)	0.103
ARDS	20(11.36)	35(33.98)	0.012
AKI	8(7.27)	25(24.27)	0.001
AHI	6(5.45)	16 (15.53)	0.023
ACI	18(16.36)	23 (22.33)	0.300

IMV: invasive mechanical ventilation, AKI: acute kidney injury, AHI: acute hepatic injury, ARDS: acute respiratory distress syndrome, ACI: acute cardiac injury, GI: gastrointestinal

In this study, treatment with medications that were recommended in the national treatment protocol significantly improved discharge rates at days 7, 14 and 28 and reduced 28-

day mortality, need for admission in ICU and length of hospital stay in patients with moderate COVID-19 in comparison with patients with severe disease. Furthermore, patients with

Fig. 1 Kaplan-Meier cumulative survival plot

moderate disease experienced less serious adverse events and complications than patients with severe disease.

The demographic features, baseline diseases, drug history, symptoms at the time of hospital admission, time from symptoms' onset to hospital admission, initial vital signs and laboratory data were not statistically different between groups. Compared with patients with SpO₂ < 90%, those with SpO₂ ≥ 90% experienced more benefits following treatment with hydroxychloroquine plus atazanavir/ritonavir regimen. This finding will be important in management of patients with COVID-19 particularly in countries where this regimen is still in their protocols. This change in practice can have following benefits. First, the probability of drug shortage will decrease in future and drugs will be saved for main indications. Second, patients with severe disease are more prone to developing serious adverse effects such as cardiac side effects. So the selection of safe drug regimens is crucial. Third benefit of this change is prevention of drug interactions.

Although supportive cares are the cornerstones of management of patients with moderate COVID-19 in outpatient setting, according to the disease severity, further interventions may be considered for the hospitalized patients. According to the National Institutes Health (NIH) guideline [12], patients with lower respiratory tract involvement and SpO₂ ≥ 94% in ambient air are in category of moderate disease while based on the WHO definition [6], patients with moderate disease have SpO₂ ≥ 90%. In most studies and large clinical trials, patients with SpO₂ 90–94% were defined as severe while these patients were in moderate group in our study [13–15]. All patients needed supplemental oxygen in our study and their management was not possible at home. On the other hand, patients without requirement of supplemental oxygen were not admitted owing to limitations in number of hospital beds. Patients with low oxygen saturation are at risk for developing of cytokine release syndrome. Therefore, initiation of antivirals as soon as possible can decrease the virus replication and subsequent complications. According to the RECOVERY trial [16], NIH [12] and IDSA [17], a corticosteroid is recommended for patients requiring oxygen supplementation.

Hypertension, diabetes mellitus and cardiovascular diseases other than hypertension were in order the most common baseline diseases in the patients. This is same as Chinese investigations. However, hypertension, cardiovascular diseases, renal diseases and diabetes mellitus in order were common comorbidities in an American population [18, 19]. An interesting finding in this research is the partially high prevalence of hypothyroidism in approximately 10% of cases.

Dyspnea, cough and fever were the most common symptoms among the patients. The findings are similar to the results of other studies regarding fever and cough [19, 20] but dyspnea was marked as the most common symptom in our patients and Cummings et al. study [21]. The difference may be related to the admission protocol of our hospital. Patients who

required respiratory supports had higher priority of the hospital admission. Furthermore, dyspnea is a subjective symptom and it cannot be precisely described like cough or fever. Fever was less common in our patients which may be due to use of analgesic and antipyretic at home.

The overall mortality on day 28 of inclusion in our patients was 16.43%. This value was 86.49% in intubated patients. The mortality rate of COVID-19 is dependent on the severity of the disease and the site of patients' care. Moreover, in most studies the mortality rate was underestimated owing to short follow up period. In Zhang et al. study the overall mortality was 5.4% while 75.6% of their patient were still in hospital [18]. In another study in which outcomes of 1590 patients were reported, the mortality rate was about 3% while only 3.1% of the patients needed IMV. On the other hand, the risk stratification of the patients was different [22]. In Zhou et al. study, the overall mortality and intubation rates were 28% and 17%, respectively. Despite the similarity in the proportions of the intubated patients, the mortality rate was higher than in our study. Although the exact definition of disease severity was not clearly reported, 63% of their patients had severe to critical disease. The overall mortality rate of the study was similar to mortality rate of patients with severe disease in our study [23].

The most frequent side effect of the treatment protocol was gastrointestinal (nausea, vomiting, abdominal discomfort and diarrhea) like other studies [13, 24]. All were detected following initiation of the treatment. Thirty-five patients mainly in the severe group received corticosteroid which was started in the late phase of disease (>10 days after the time of hospital admission). Patients received 125 mg methyl prednisolone daily for 3 days. Most of these patients were in ICU and also experienced ARDS at that time. The effect of corticosteroid administration on mortality must be interpreted with caution as fewer patients in the moderate group received corticosteroid. Furthermore, a substantial number of patients in the severe group developed ARDS and multi-organ failure. However, the use of corticosteroid may non-significantly increase the risk of mortality [25]. Moreover, without reduction in mortality rate, these medications can increase duration of hospital stay [26]. The effects on mortality and outcomes can be generalized to IVIG and vitamin C in our study. IVIG not only did not show the survival benefits in a multicenter retrospective study but also increased total duration of the symptoms and hospital stay [27].

ARDS was the most serious complication that occurred in high number of patients compared with other complications. The incidence rate of ARDS was reported between 17 and 30% [23, 28].

Extra-pulmonary complications can also occur in patients with COVID-19. Renal involvement can happen through

different mechanisms [29]. Hepatic injury is expected following direct invasion of the virus to hepatic cells, like that was described in SARS [30]. Higher rates of organs involvement i.e. 33% for liver and 22.5% for kidney were previously reported in COVID-19 patients [31]. Hospitalized patients, especially critically ill ones, were more prone to incidence of AKI [32]. AKI developed in 89.19% of the intubated patients similar to other studies. In the study of Argenziano et al. AKI was reported in 89.7% of intubated patients [33]. Moreover, in a study from New York 78% of patients admitted in ICU developed AKI. Of note, more than 93% of their patients were intubated [34].

Lagier et al. assessed the clinical outcomes of patients treated with the combination of hydroxychloroquine/azithromycin and other regimens. The outcomes of their study included mortality rate, ICU admission rate, duration of hospitalization ≥ 10 days and viral shedding. Two clinical outcomes of the study (mortality rate and ICU requirement) can be compared with our study. The case fatality rate was 0.9% that reached 1.1% in the follow up. The overall mortality rate of our patients treated with hydroxychloroquine in combination with atazanavir/ritonavir was 17.37%. This value was 5.45% in patients with moderate disease. The main differences between two studies are the disease severity and clinical presentation of the patients. Eighty-two percent of the patients were treated in a day-care hospital and only 18% of the patients were hospitalized in the wards. However, all of our patients were admitted to hospital and received the supportive care and antiviral agents. On the other hand, only 1.8% of their patients needed admission in ICU. In our study, approximately 28% of the patients required the intensive cares [35]. Geleris et al. evaluated association between hydroxychloroquine use and intubation requirement or death in an observational study [36]. Intubation or death occurred in 32.3% of hydroxychloroquine users. The hazard ratio was 2.37 in crude analysis versus 1.04 in multivariable analysis. Finally, authors concluded that hydroxychloroquine did not increase or decrease the mortality rate. Evaluating the effect of hydroxychloroquine use on mortality was not possible in our study because of all patients receiving it.

Arshad et al. evaluated outcome of 2541 patients treated with hydroxychloroquine alone and in combination with azithromycin during a multicenter retrospective study. Hydroxychloroquine was administered as 400 mg BD on first day then 200 mg BD for 4 days. The dose of azithromycin was 500 mg daily on first day and then 250 mg daily on days 2–5. In-hospital mortality was considered as the primary outcome. The overall mortality rate was 18.1%. This value was 13.5%, 20.1%, 22.4 and 26.4% in the patients who received hydroxychloroquine alone, hydroxychloroquine + azithromycin, azithromycin alone and none of these medications respectively [37]. Same as our study, of these patients 50% had $SpO_2 < 90\%$, 24.2% needed ICU admission and 17.6% were

intubated. Furthermore, the overall in-hospital mortality rates were similar (18.1% versus 17.3%). Nevertheless, the patients were different in terms of underlying diseases and receipt of second antiviral and immunomodulatory medications. Although this study was multicenter, the design was retrospective. Our study was single center but all patients were prospectively monitored during the hospital course and followed up after discharge.

One of the first studies reporting benefits of hydroxychloroquine in the treatment of patients with COVID-19 was the study of Gautret et al. Viral clearance at day 6 of inclusion was considered as primary outcome. Hydroxychloroquine alone and in combination with azithromycin reduced viral load and accelerated viral clearance. Due to small sample size, non-randomization and drop-out of six patients from the analysis, their results should be interpreted with caution [38].

The second common drug in the patients' drug regimens was an antiretroviral. Cao et al. evaluated the efficacy of lopinavir/ritonavir in patients with severe COVID-19. The primary outcome of the study was the time to clinical improvement that was defined according to seven-category scale. Lopinavir/ritonavir did not make a difference in time to clinical improvement compared with the standard of care. Late initiation of the drug in majority of patients (the median time from onset of symptoms to randomization was 13 days) is a major concern in this study. In our study, the antiretroviral agent was initiated as soon as possible following admission. Although mortality rate of that study was similar to our study but there are important differences between them: 1) longer duration of follow up in our study 2) more patients in our study required IMV during the course of hospitalization 3) all recruited patients in our study needed oxygen support. Similar to our study, the most frequent side effect of lopinavir/ritonavir was GI adverse effects [13].

Following limitations should be considered before interpretation of our findings. First, the design of the study was observational. Second, some laboratory tests required for monitoring the treatment regimen were not repeated during the hospitalization course. Third, the cardiovascular side effects of hydroxychloroquine was not reported while they are important and prevalent according to recent studies. Fourth major limitation of the study was the lack of repeated molecular tests and chest imaging during the hospitalization course or at the time of hospital discharge.

Conclusions

Our study did not support the use of hydroxychloroquine plus atazanavir/ritonavir in patients who had $SpO_2 < 90\%$ at the time of hospital admission. SpO_2 was the only predictor of clinical outcomes (duration of hospital stay, discharge from

the hospital and mortality) in patients treated with hydroxychloroquine plus atazanavir/ritonavir.

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Anahid Nourian: Data collection.

Morteza Nabiee: Data collection.

Setayesh Sadeghi: Data collection.

Hossein Khalili: Conceptualization, supervision, review and edit manuscript.

Ladan Abbasian: Clinical assessment of patients.

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Arash Seifi: Clinical assessment of patients.

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Compliance with ethical standards

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