Journal of Cardiovascular Disease Research

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Original Article

Clinical Effect of the Combination Therapy of Hydroxychloroquine, Azithromycin and Ivermectin in Patients with COVID-19

SatyanandSathi[1], Anil Kumar Garg[1], Amit Mittal[2], Savita Verma*[4], Virendra Singh Saini[1], Manoj Kumar Singh[1], Devinder Vohra [1] , Sushil Kumar [3]

[1]Department of Medicine, S.M.M.H. Government Medical College Saharanpur, Uttar Pradesh, India
[2]Department of Community Medicine, S.M.M.H. Government Medical College Saharanpur, Uttar Pradesh, India
[3]Department of Paediatrics,S.M.M.H. Government Medical College Saharanpur, Uttar Pradesh, India
[4]M.S. Obstetrics and Gynaecology, S.M.M.H. Government Medical College Saharanpur, Uttar Pradesh, India

***Corresponding author**:
Dr. Savita Verma, Assistant Professor, M.S. Obstetrics and Gynaecology, S.M.M.H. Government Medical College, Ambala Road, Saharanpur, Uttar Pradesh, India,
Email : Savita.sathi@gmail.com

Abstract
Background: The ongoing pandemic has highlighted the need for an effective treatment of COVID-19 patients and prevention of SARS-CoV-2 community transmission.Methods: We conducted a prospective observational study on a cohort of 85 COVID-19 patients (80% males, median age 46 years, range 18–80 years). Patients were treated with a triple drug therapy: ivermectin 12 mg once a week, hydroxychloroquine 400 mg twice a day on the first day and 200 mg twice a day for the next 4 days, and azithromycin 500 mg once a day for 5 days. Endpoints were assessed by clinical outcomes, death, negative SARS-CoV-2 RNA-PCR test on the tenth day, and length of the hospital stay.Results: All patients improved except one 70-year-old female, who died on the third day of admission. The clinical outcome was considered good as 95.24% (80/84) of patients presented a negative SARS-CoV-2 RNA-PCR test on the tenth day of admission and 90.48% (76/84) were discharged in stable condition.Conclusions: The response must focus on immediate isolation of COVID-19 patients and their early treatment to prevent irreversible severe respiratory injury. Our study shows the beneficial effect of triple drug therapy in terms of clinical recovery, shorter duration of viral carriage, community spread prevention, and minimal cost of therapy.
KeywordsHydroxychloroquine, Azithromycin, Ivermectin, COVID-19 patients

Introduction

Beginning in the middle of December 2019, several cases of atypical pneumonia were diagnosed in Wuhan, China. Most patients presented with fever, dry cough, and breathlessness[1]. Others presented with fatigue, malaise, sore throat, and a minority reported diarrhea. Some patients developed quick dyspnea leading to acute respiratory distress syndrome. On December 31, 2019, samples of bronchoalveolar lavage fluid were taken from patients diagnosed with pneumonia of unknown etiology[1]. Virus culture, real-time reverse transcription polymerase chain reaction (RT-PCR), and whole-genome sequencing pointed to a new coronavirus, distinct from severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) coronavirus. The first case was confirmed in Anhui province on January 22, 2020[1]and the National Health Commission of China named it Novel Coronavirus Pneumonia on February 7, 2020. The World Health Organization coined the official term coronavirus disease-19 (COVID-19) on February 11, 2020 and declared the COVID-19 pandemic on March 12, 2020[1, 2].

Clinical symptoms are mild in the majority of patients and the case fatality rate is only 2.3% [3]. The latter may rise to 8% in the 70–79 years age group and 14.8% in those over the age of 80 [3]. However, the mortality rate is probably overestimated as numerous asymptomatic carriers in the general population go undetected. Curing symptomatic and asymptomatic patients could shorten virus carriage duration and effectively restrict community transmission of the disease.

Chloroquine and its analogue hydroxychloroquine have been used *in vitro* against SARS-CoV[4]. Hydroxychloroquine has a better efficacy and clinical safety profile compared to chloroquine[5-7.] A Chinese study contradicted the efficacy of hydroxychloroquine and reported no significant difference with respect to pharyngeal carriage of viral RNA on day 7 between patients treated with 400 mg hydroxychloroquine per day for 5 days and controls [8]. *In vitro* antiviral activity and effectiveness of azithromycin have been established for

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

Ebola and Zika viruses as a means to prevent severe respiratory tract infections [9–11]. Based on these findings, a combination of hydroxychloroquine and azithromycin was deemed more effective than each drug alone. In spite of documented *in vitro* antiviral activity of hydroxychloroquine, Molinaa and co-workers[12] did not find strong clinical benefits of a combination of azithromycin and hydroxychloroquine in patients with severe COVID-19 infection. *In vitro* antiviral activity of the FDA-approved drug ivermectin was demonstrated against SARS-CoV-2 [13] and was particularly effective against the clinical isolate Australia/VIC01/2020. Supernatant and cell pellets were harvested on days 0 to –3 and analyzed by RT-PCR to detect SARS-CoV-2 RNA. At 24 h, viral RNA present in the supernatant (indicative of released virions) was 93% lower in samples treated with ivermectin compared to those given the vehicle (DMSO). The study hypothesized that ivermectin inhibited importin α/β1-mediated nuclear import of viral proteins [13].

The above evidence provides a strong case for applying a triple combination of hydroxychloroquine, azithromycin, and ivermectin to cure COVID-19 patients. According to a Chinese survey of COVID-19 survivors, viral shedding ranged between 8 and 37 days [14]. Thus, curing COVID-19 patients at an early stage of disease is paramount to limit community transmission of the virus. The present study tested whether a triple combination of the above drugs could effectively reduce viral loads at an early stage of the disease and prevent man-to-man transmission of COVID-19.

Materials and Methods

The study was an uncontrolled, noncomparative, prospective, observational study. Eighty-five COVID-19 patients aged 18 years and above, who gave written informed consent, were enrolled prospectively. The following exclusion criteria were applied: age < 18 years, known allergy to hydroxychloroquine, ivermectin or azithromycin, prolonged QT interval on the electrocardiogram (ECG), pregnancy, and history of glucose-6-phosphate dehydrogenase deficiency. The study was approved by the institutional ethics committee of Shaikh-Ul-Hind MaulanaMahmoodHasan (SMMH) Government Medical College, Saharanpur, UP, India (Ref NoIEC 15-28-05-2020).

Study design

The study was carried out at SMMH Government Medical College. Patients testing positive for COVID-19 and hospitalized either in the isolation ward or intensive care unit (ICU) were enrolled in the study. Nasopharyngeal and oropharyngeal samples for SARS-CoV-2 RNA-PCR were taken on the tenth day of admission. If the result was positive, then testing was repeated every 48 h until a negative outcome was recorded.

Clinical classification

Patients were grouped into three categories at the time of admission: (1) asymptomatic; (2) upper respiratory tract infections, who presented with isolated low-grade fever, rhinitis, pharyngitis, sore throat, and myalgia; and (3) lower respiratory tract infection, who presented with breathlessness, bronchitis, and symptoms of pneumonia. The time between the onset of symptoms and start of treatment was recorded. All enrolled patients were evaluated for risk factors of severe COVID-19 disease, such as low oxygen saturation (SpO2 < 94%), elderly age, chronic smoker, chronic obstructive pulmonary disease, hypertension, diabetes, coronary artery disease, malignancy, and history of immunosuppressive therapy. Duration of hospital stay, need for oxygen therapy, negative SARS-CoV-2 RNA-PCR on the tenth day, and death were recorded. Patients who required oxygen therapy, showed pneumonia on a chest X-ray within 72 h of admission, and had a prolonged hospital stay (>12 days) were considered as having poor clinical outcome. The clinical outcome for the remaining patients was considered good. A chest X-ray was performed on all patients on the day of admission and was repeated whenever required on a clinical basis. The chest X-ray was carried out every second day for ICU patients.

COVID-19 patients treatment

Patients were administered a triple drug therapy consisting of ivermectin 12 mg once a week, hydroxychloroquine 400 mg twice a day on the first day and 200 mg twice a day for the next 4 days, and azithromycin 500 mg once a day for the next 5 days. If on the tenth day SARS-CoV-2 RNA-PCR was positive, then a second dose of ivermectin (12 mg) was given and hydroxychloroquine 200 mg BD was continued. Hydroxychloroquine and azithromycin were not given simultaneously as both drugs may cause a prolonged QT interval. For patients with pneumonia, septicemia, and moderate to severe illness, intravenous injection with piperacillin-tazobactam and subcutaneous enoxaparin (40 mg) once a day was added to the above regimen. Injection of hydrocortisone 100 mg thrice a day was added to patients presenting severe respiratory illness. A 12-lead ECG was performed on each patient before treatment and 3 days after its start. Treatment was not

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

started if QTc (Bazett's formula) was > 500 ms.The endpoints were clinical outcome, negative SARS-CoV-2 RNA-PCR, length of stay in the hospital isolation unit, and death.

Data analysis

Data were analyzed by SPSS software (version 18.0 for Windows). Values are presented as mean ± standard deviation/median interquartile range. In case a normal distribution for categorical variables could not be established, frequency (%) is presented instead.

Results

Demographic characteristics and comorbid conditions of patients

Initially, 85 patients with confirmed COVID-19 tests were admitted to the isolation ward and ICU at SMMH Government Hospital, Saharanpur, between April 13, 2020 and June 27, 2020. All patients were treated with hydroxychloroquine, azithromycin, and ivermectin. As one elderly patient died on the third day of admission, a total of 84 patients completed the entire treatment protocol. All patients followed for at least ten days were included in the analysis. The median age of patients was 46 years (range 18–80), 68 patients were male (80.95%) and 16 were female (19.05%), and 13 patients (15.48%) were elderly (> 60 years of age) (Table 1). Associated comorbidities included diabetes mellitus, chronic respiratory disease, coronary artery disease, hypertension, chronic kidney disease, and malignancy (Table 1 and Fig 1). These comorbidities are known to be risk factors for a severe type of COVID-19.

Clinical condition of patients at time of admission

As summarized in Table 2, 58.33% of patients were asymptomatic at the time of admission, 28.57% presented with upper respiratory tract infection symptoms, 15.48% presented with fever, and 13.10% displayed lower respiratory tract infection symptoms. Overall, 41.67% of patients had a history of smoking. Oxygen saturation was > 94% in 85.71% of patients and < 94% in 14.29% of cases. Chest X-rays were consistent with pneumonia in 11.90% of patients. Similarly, 11.90% of patients were hypertensive, of which seven were already taking antihypertensive drugs.

Clinical outcome and treatment

Only 14.28% of patients required oxygen therapy during their hospital stay. As reported in Table 3, RT-PCR revealed that 80 patients (95.24%) were negative for SARS-CoV-2 RNA on the tenth day of admission. The majority of patients (76/84, 90.48%) had good clinical outcomes and were discharged on the tenth day of admission in stable condition. Another four patients (4.76%) were negative for SARS-CoV-2 RNA on the fourteenth day of admission. Eight patients (9.52%) were discharged only after 12 days of admission as they presented poor clinical outcomes: four of them because of positive SARS-CoV-2 RNA-PCR on the tenth day and four because of associated comorbid conditions. The longest permanence in the hospital was 16 days (two patients). Median length of the hospital stay was 13.5 days with mean ± SD of 13.25 ± 2.16. Finally, 80 patients (95.24%) received a single 12-mg dose of ivermectin; whereas four patients (4.76%) received also a second 12-mg dose of ivermectin due to positive COVID-19 results on the tenth day of admission, as well as extra doses of hydroxychloroquine (200 mg twice a day) until SARS-CoV-2 RNA-PCR was negative.

Eight patients (9.52%) showed adverse effects possibly related to the triple drug therapy; these included diarrhea, abdominal pain, nausea, vomiting, headache, rash, and transient blurring of vision (Table 4). No patient displayed a prolonged QT interval on the ECG during therapy.

Discussion

At present, there is no specific antiviral treatment for COVID-19[15]. SARS-CoV-2 infection encompasses two phases: an early phase lasting up to 7–10 days and characterized by high viral loads in the upper and lower respiratory tract [16]; and a second phase, which can trigger viral pneumonia as well as the host's inflammatory and procoagulant responses. The latter can include shock, cardiac failure, systemic inflammatory response syndrome, and acute respiratory distress syndrome[16]. Therefore, the main focus must be on initial management of COVID-19 patients through early identification of the suspect, their immediate isolation, and early onset of treatment [15]. Such strategy avoids respiratory complications to the patient and prevents the disease from spreading. There is some controversy among the medical establishment regarding the effect of hydroxychloroquine and azithromycin combination therapy against COVID-19. To overcome these issues, the patients in this study received a third drug: ivermectin.

Although our study is not a randomized control trial, it nevertheless enriches clinical evidence describing the treatment of COVID-19 patients. Specifically, we present the outcomes of 85 patients treated with a combination of hydroxychloroquine, azithromycin, and ivermectin. Because one 70-year-old female patient died

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

on the third day of admission due to septic shock and respiratory failure, only 84 patients completed the study protocol. The proposed triple drug therapy revealed an improvement in all 84 patients, as all were discharged in stable condition. Andreani and co-workers[17] showed synergistic effect of hydroxychloroquine and azithromycin *in vitro* on SARS-CoV-2 at concentrations compatible with those recorded in human lungs. Our study revealed a relatively rapid decrease in viral RNA loads, with 95.24% of study patients reporting a negative SARS-CoV-2 RNA-PCR on the tenth day of admission following nasopharyngeal and oropharyngeal swabs. These results compare favorably to existing reports, which indicate that absence of specific treatment causes viral RNA loads to remain high for approximately three weeks and more than a month in some cases[18,19]. Another study in 16 Chinese COVID-19 patients showed that even after the disappearance of symptoms, 50% of patients remained positive for SARS-CoV-2 RNA for a median of 2.5 days and a maximum of 8 days [20]. In our study, only 4.76 % of patients resulted in a positive SARS-CoV-2 RNA-PCR on the tenth day of admission. This value is substantially lower compared to that of a Chinese study identifying the shortest period of viral shedding as 8 days and the longest as 37 days [14]. Thus, our study appears to confirm the effectiveness of triple drug therapy.

At the time of admission, 58.33% of study participants were asymptomatic and 41.67% were symptomatic. Of the latter, only 9.52% were admitted to the ICU. The proposed triple drug therapy showed a marked improvement in the condition of ICU patients. As reported by Gautret and co-workers [21], a combination of hydroxychloroquine and azithromycin produced a favorable outcome in 65 out of 80 COVID-19 patients (81.3%). Here, 90.48% of patients presented good clinical outcomes and were discharged in stable conditions on the tenth day of admission, with the remaining patients discharged soon after and no longer than 16 days after hospitalization. Million and co-workers [22] performed a retrospective analysis of 1061 COVID-19 patients treated with a combination of hydroxychloroquine and azithromycin, and showed that a virological cure was achieved in 91.7% of patients within ten days. Here, this marker was achieved by 95.24% of patients. Based on improved clinical outcome and lower viral presence compared to previous combinations of hydroxychloroquine and azithromycin only, addition of ivermectin provided extra benefit and synergistic effect in terms of clinical recovery.

Four patients (4.76%) had positive RT-PCR RNA reports for SARS-CoV-2 on the tenth day of admission and suffered from associated comorbid conditions. Specifically, two presented malignancy and were on immunosuppression therapy; whereas the other two had diabetes mellitus, hypertension, and a history of chronic smoking. These four patients received an extra single dose of ivermectin and extra doses of hydroxychloroquine until the fourteenth day, when they became negative for SARS-CoV-2 RNA.

In our experience, the therapy was well tolerated, causing minor side effects in only 9.52% of patients. All side effects were mild and there was no discontinuation of treatment among study participants. There are anecdotal reports of heart complications with the use of hydroxychloroquine in patients with underlying comorbid conditions[23]; hence, we suggest performing an ECG before initiating the treatment and applying continuous ECG monitoring for possible cardiac side effects. As hydroxychloroquine and azithromycin may both prolong the QT interval, we administered the drugs over separate periods: hydroxychloroquine for the first 5 days and azithromycin for the next 5 days. We did not detect a prolonged QT interval in any of our study patients.

Given that 41.67% of study patients were smokers, it appears that smoking may predispose individuals to COVID-19 infection. Lung angiotensin-converting enzyme 2 (ACE 2) levels do not vary by age or sex, but smokers exhibit upregulated ACE2[24]. Smith and co-workers [24] reported that smokers' lungs harbored higher levels of the coronavirus receptor ACE2. Moreover, ACE2 was expressed in a subpopulation of respiratory secretory cells that expanded in response to smoke exposure. Finally, they established that *ACE2* expression was stimulated by interferon, resulting in overexpression following viral infections. Taken together, these results may partially explain why smokers are particularly at risk for severe SARS-CoV-2 infections [24].

Widely varying COVID-19-related fatality rates were reported across the world. On March 3, 2020, the World Health Organization reported a mortality rate of 3.4%[25]. Hu and co-workers[26] carried out a systematic review and meta-analysis for the prevalence and severity of COVID-19; they found a severity risk of 12.6–23.5% and a mortality risk of 2.0–4.4%. In the present study, 13 out of 85 patients (15.29%) required oxygen therapy. The case fatality rate was 1.17% and approximately 15% of patients manifested a severe form of COVID-19 disease.

Our study has also some limitations. Computed tomography scans and serum drug levels were not available for all patients. The study was of an observational prospective nature and only 85 patients were enrolled. The majority of patients had relatively mild clinical presentations. We did not take into consideration the possible

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

confounding effect of disease severity in relation to comorbid conditions. Finally, the triple drug therapy should be verified in randomized controlled trials.

Conclusion

Managing COVID-19 patients should focus on identification of the suspect in the early phase of viral syndrome, their immediate isolation, and early onset of treatment. This approach can effectively prevent the spreading of disease and the occurrence of irreversible severe respiratory injury. Our study demonstrates the beneficial effect of triple drug therapy in terms of clinical recovery, shorter duration of viral carriage, community spread prevention, and minimal cost of therapy. The drug ivermectin in combination with hydroxychloroquine and azithromycin must be evaluated further in controlled trials.

Conflict of Interest

The authors have no conflicts of interest to declare.

Funding Statement

The authors declare that they have no financial interests related to the material in the manuscript.

Acknowledgments

The authors express their gratitude to the patient and his guardians for providing medical records to prepare the manuscript. We are also highly thankful to Professor Dr Arvind Trivedi and Dr C M Kamal for encouragement.

Ethics Statement

The authors followed the guidelines for human studies and the research was conducted in accordance with the Declaration of Helsinki (1964). Information revealing the subject's identity was avoided. Patients gave written informed consent for the study and publication of study data.

Tables

Table. 1 Sociodemographic characteristics and comorbid conditions

	n	%	Mean ± S.D.	Median	IQR
Age (years)					
10-20	6	7.14	18.5 ± 0.837	18	1.25
20-30	24	28.57	23.46 ± 2.30	26	3
30-40	21	25.00	36 ± 3.06	36	6
40-50	12	14.29	46.66 ± 2.57	46	4.5
50-60	8	9.52	56.25 ± 3.37	55	5.75
60-70	10	11.90	64.1 ± 3.21	62.5	5.5
70-80	3	3.57	78.33 ± 2.89	80	5.5
Male	68	80.95			
Female	16	19.05			
Elderly (>60 years)	13	15.48			
Comorbid Conditions					
Diabetes Mellitus	13	15.48			
Hypertension	10	11.90			
Coronary artery disease	11	13.10			
Chronic respiratory diseases	13	15.48			
Chronic kidney disease	4	4.76			
Malignancy	2	2.38			
Immunosuppressive therapy	2	2.38			
Thyroid disorders	5	5.95			
Fungal infections	2	2.38			

Table. 2 Clinical condition of patients at the time of admission

Clinical presentation	N	%
Asymptomatic	49	58.33
Upper respiratory tract infection symptoms	24	28.57
Lower respiratory tract infection symptoms	11	13.10

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

History of smoking	35	41.67
Time between onset of symptoms and treatment		
Treatment initiated on day 1	49	58.33
Treatment initiated on day 2	16	19.05
Treatment initiated on day 3	19	22.62
Chest X-ray within 72 hours of admission		
Consistent with pneumonia	10	11.90
Not consistent with pneumonia	74	88.10
SpO2 during hospital stay		
>94%	72	85.71
<94%	12	14.29
Patients on antihypertensive medication	7	8.33

Table. 3 Clinical outcome and treatment

	N	%
Patients who required oxygen therapy		
ICU	8	9.52
Isolation Ward	4	4.76
Negative RT-PCR by nasopharyngeal and oropharyngeal swabs		
On tenth day	80	95.24
On fourteenth day	4	4.76
Patients who received Ivermectin 12 mg once a week		
Single dose	80	95.24
Two doses	4	4.76
Length of hospital stay		
>12 days	8	9.52
10 days	76	90.48
Good clinical outcome	76	90.48
Poor clinical outcome	8	9.52

Table. 4 Possible adverse effects drugs

Patients without any adverse effect	76	90.48
Patients with possible adverse effects related to therapy	8	9.52
Diarrhea	2	
Pain in abdomen	1	
Nausea and vomiting	2	
Headache	1	
Rash	1	
Transient blurring of vision	1	
Prolongation of QT-interval	None	

References

1. Wang R, Pan M, Zhang X, Fan X, Han M, Zhao F, et al. Epidemiological and clinical features of 125 hospitalized patients with COVID-19 in Fuyang, Anhui, China. Int J Infect Dis 2020;95:421–428.

2. WHO Director-General's opening remarks at the media briefing on COVID-19 –11 March 2020.

3. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72314 cases from the Chinese center for disease control and prevention. J Am Med Assoc 2020.

4. Biot C, Daher W, Chavain N, Fandeur T, Khalife J, Dive D, et al. Design and synthesis of hydroxyferroquine derivatives with antimalarial and antiviral activities. J Med Chem 2006;49:2845-2849.

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

5. Expert consensus on chloroquine phosphate for the treatment of novel coronavirus pneumonia. ZhonghuaJiehe He HuxiZazhi 2020;43:185–8.

6. Gautret P, Lagier JC, Parola P, Hoang VT, Meddeb L, Mailhe M, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 2020:105949.

7. Marmor MF, Kellner U, Lai TY, Melles RB, Mieler WF; American Academy of Ophthalmology. Recommendations on Screening for Chloroquine and Hydroxychloroquine Retinopathy (2016Revision). Ophthalmology.2016Jun; 123(6):1386- 94.

8. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, et al. A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J of Zjeijang University; 2020

9. Retallack H, Di Lullo E, Arias C, Knopp KA, Laurie MT, Sandoval-Espinosa C, et al. Zika virus cell tropism in the developing human brain and inhibition by azithromycin. ProcNatlAcadSci U S A. 2016 Dec 13;113(50):14408-14413.

10. Madrid PB, Panchal RG, Warren TK, Shurtleff AC, Endsley AN, Green CE, Kolokoltsov A, et al. Evaluation of Ebola Virus Inhibitors for Drug Repurposing. ACS Infect Dis. 2015 Jul 10;1(7):317-26.

11. Bacharier LB, Guilbert TW, Mauger DT, Boehmer S, Beigelman A, Fitzpatrick AM, et al. Early administration of azithromycin and prevention of severe lower respiratory tract illnesses in preschool children with a history of such illnesses: A randomized clinical trial. JAMA. 2015 Nov 17;314(19):2034-2044.

12. J.M. Molinaa, C. Delaugerreb, J. Le Goffb, B. Mela-LimaaD. PonscarmeaL. GoldwirteN. et al. Letter to the editor ; No evidence of rapid antiviral clearance or clinical ben-efit with the combination of hydroxychloroquine and azithromycin in patients with severe COVID-19 infection. Médecineet maladies infectieuses 50 (2020) 382–387.

13. Caly L, Druce JD, Catton MG, Jans DA, Wagstaff KM. The FDAapproved Drug Ivermectin inhibits the replication of SARS-CoV-2 in vitro. Antivir Res 3 April 2020:104787.

14. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology inWuhan China: the mystery and the miracle. J Med Virol 2020.

15. HarapanHarapan, NaoyaItoh, Amanda Yufika, WiraWinardi, SynatKeam, Haypheng T et al. Coronavirus disease 2019 (COVID-19): A literature review; Journal of Infection and Public Health 13 (2020) 667–673.

16. J.M. Perica`s , M. Hernandez-Meneses, T.P. Sheahan , E. Quintana ,
J. Ambrosioni, E. Sandoval et al. COVID-19: from epidemiology to treatment. European Heart Journal (2020) 41, 2092–2108

17. Julien Andreani, Marion Le Bideau, Isabelle Duflot, Priscilla Jardot, Clara Rolland, Manon Boxberger et al. In vitro testing of combined hydroxychloroquine and azithromycin on SARSCoV-2 shows synergistic effect. Microbial Pathogenesis 145 (2020) 104228

18. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020; 395: 1054–62

ISSN:0975-3583,0976-2833 VOL12,ISSUE05,2021

19. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. Lancet Infect Dis 2020; 20: 565–74.

20. Chang Mo G, Yuan X, Tao Y, Peng X, Wang F, et al. Time kinetics of viral clearance and resolution of symptoms in novel coronavirus infection. Am J RespirCrit Care Med 2020;Volume 201 Number 9

21. Philippe Gautret, Jean-Christophe Lagier, Philippe Parola, Van Thuan Hoang, Line Meddeb, Jacques Sevestre et al: clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: a pilot observational study. Travel Medicine and Infectious Disease 34 (2020) 101663

22. Matthieu Million, Jean-Christophe Lagier, Philippe Gautret, Philippe Colson, Pierre-Edouard Fournier, Sophie Amrane et al. Early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille; France.Travel Medicine and Infectious Disease 35 (2020) 101738

23. Chatre C, Roubille F, Vernhet H, Jorgensen C, Pers YM. Cardiac complications attributed to chloroquine and hydroxychloroquine: a systematic review of the literature. Drug Saf 2018;41:919–31.

24. Joan C. Smith, Erin L, Sausville, VishruthGirish, Monet Lou Yuan, AnandVasudevan, Kristen M. John et al. Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. Developmental Cell June 8, 2020; 53, 514–529

25. WHO Director-General's opening remarks at the media briefing on COVID-19 - 3 March 2020 - World Health Organization, March 3, 2020

26. Y. Hu, J. Sun, Z. Dai, et al., Prevalence and severity of corona virus disease 2019 (COVID-19): a systematic review and meta-analysis, J. Clin. Virol. 2020 Apr 14; 127