ORIGINAL RESEARCH PAPER

Medicine

KEY WORDS:

IVERMECTIN AS ADJUVANT TO HYDROXYCHOLOROQUINE IN PATIENTS RESISTANT TO STANDARD TREATMENT FOR SARS-CoV-2: RESULTS OF AN OPEN-LABEL RANDOMIZED CLINICAL STUDY

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BACKGROUND:

A cluster of pneumonia cases of unknown etiology was reported from the city of Wuhan, in the Hubei province of China, in December 2019. A novel coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative agent of the disease which was subsequently termed as the coronavirus disease (COVID-19) by the World Health Organization (WHO). SARS-CoV-2 mainly affects the lower respiratory tract and manifests as pneumonia in humans.[1]

The incidence of COVID-19 continues to rise, with 9,653,048 laboratory-confirmed cases and 491,128 deaths worldwide and India has reported 508,953 cases and 15,685 deaths till 27th, June 2020.[2]

The clinical manifestations of COVID-19 range from fever, cough, fatigue, sore throat, shortness of breath and less common symptoms such as headache, nausea and diarrhea.[3,4] The most common abnormalities in vital signs are increased temperature and tachypnea. The most common radiological findings are bilateral pulmonary infiltrates, ground glass opacities and consolidation. The most common findings associated with severe disease are older age, d- dimer levels greater, higher SOFA score, elevated IL-6, increased Lactate Dehydrogenase, hyperferritinemia and lymphopenia on admission.[5,6]The most common complications are sepsis, respiratory failure, acute respiratory distress syndrome (ARDS), cardiac injury and acute kidney injury.

As per WHO the mild COVID-19 illness may include: uncomplicated upper respiratory tract viral infection symptoms such as fever, fatigue, cough (with or without sputum production), anorexia, malaise, muscle pain, sore throat, dyspnea, nasal congestion, or headache. Rarely, patients may also present with diarrhoea, nausea, and vomiting.[7]

According to a recent Chinese study, about 80% of patients present with mild disease and the overall case-fatality rate is about 2.3% but reaches 8.0% in patients aged 70 to 79 years and 14.8% in those aged ≥80 years.[7] However, there is probably an important number of asymptomatic carriers in the population, and thus the mortality rate is probably overestimated. India is facing the COVID-19 wave with more than 145,380 cases, as of May 2020. Thus, there is an urgent need for an effective treatment to treat asymptomatic patients and to decrease the duration of virus carriage in order to limit the transmission in the community. Among candidate drugs to treat COVID-19, repositioning of old drugs for use as antiviral treatment is an interesting strategy because knowledge on safety profile, side effects, posology and drug interactions are well known.[8,9]

In India, as per guidelines of Ministry of Family Health and Welfare, for COVID-19 patients, Hydroxycholoroquine 400 mg twice a day for day one followed by 200 mg twice a days for next 4 days plus Paracetamol 500 mg as required plus tab. Vitamin C one tablet twice a day is advised.[10]It is observed that several patients are found to be positive even after completing the course of the standard treatment. Thus there is urgent need to discover drugs that can control the viral load and in turn the spread of viral infection. Since development of new drug or vaccine is a time consuming process, thus at present equal importance is being given to existing drugs with anti-viral activity against SARS-CoV-2.

Ivermectin, an anti-parasitic has shown to have broad-spectrum anti-viral activity in vitro, is an inhibitor of the causative virus (SARS-CoV-2), with a single addition to Vero-hSLAM cells 2 h post infection with SARS-CoV-2 able to effect ~5000-fold reduction in viral RNA at 48 h. Ivermectin therefore warrants further investigation for possible benefits in humans.[11]Another report on ivermectin suppression of SARS-CoV-2 viral replication in cell cultures has been published, and the use of this medication seems to be potentially useful for the therapy.[12]

Ivermectin (IVM) safety profile and IVM wide spectrum enables to move forward with the investigation in patients infected by SARS- CoV-2 for its possible use in the management of patients with COVID-19, given the current pandemic situation. The present study was designed to assess the efficacy of ivermectin as adjuvant drug in patients resistant to standard treatment for SARS-Co-2and to compare the efficacy of ivermectin plus hydroxychloroquine with hydroxychloroquine alone in patients resistant to standard treatment for SARS-CoV-2

METHODS:

Setting:

We conducted this study in the in the department ofmedicine, MDM Hospital Associated with Dr. S.N. Medical College,

Jodhpur. This study is a randomized, open label study to evaluate the efficacy of ivermectin as adjuvant to hydroxychloroquine in hospitalized adults diagnosed with COVID-19.

Study Population:

Hospitalized COVID-19 patients fulfilling the inclusion criteria:i) patients of both genders, aged between 18 and above tested positive after completion of standard care treatment for SARS-CoV-2 confirmed by reverse-transcriptase–polymerase- chain-reaction (RT-PCR) assay; ii) Mild/ Asymptomatic patients. iii) No comorbidities affecting the patient s prognosis, rendering them high-risk patients; iv) Documented acceptance to participate by means of the execution of the Informed Consent.

Patients were excluded if they had: i) allergy or hypersensitivity to ivermectin and/or its inactive ingredients; ii) respiratory distress or requiring intensive care; iii) used immunosuppressants (including systemic corticosteroids) in the last 30 days; iv) known HIV infection with CD4 count <300 cell/ L; v) pregnancy or lactating patients; v) medical conditions such as mal-absorption syndromes affecting proper ivermectin absorption; vi) autoimmune disease and/or decompensated chronic diseases; vii) Uncontrolled, intercurrent diseases including renal impairment, hepatic impairment, symptomatic congestive heart failure, unstable chest angina or heart arrhythmia; viii) treated in any other study in the previous 30 days; ix) concomitant administration of enzyme inducers (such as carbamazepine) that could affect the effectiveness of the drug and those receiving CYP3A4 substrates (such as statins) due to the risk of increased toxicity.

Informed consent

Before being included in the study, patients meeting inclusion criteria had to give their written informed consent to participate to the study. Written informed signed consent was obtained from all participants. Patient Information Sheet that indicated the risks and the benefits associated with the participation to the study was shared with all patients. This study was conducted in accordance with the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines of good clinical practice, the Helsinki Declaration, and applicable standard operating procedures.

Procedure:

Patients were seen at baseline for enrolment, initial throat swab test for COVID -19 was done at day-0, and again after 48 hours and 96 hours. Each day, patients received a standardized clinical examination. All clinical data were collected using WHO standardized form.

Recruited patientswere randomly divided into two groups- Control group receiving standard care and the test group receiving ivermectin 12 mg along with standard care. The treatment schedule of both groups is shown in Table 1.

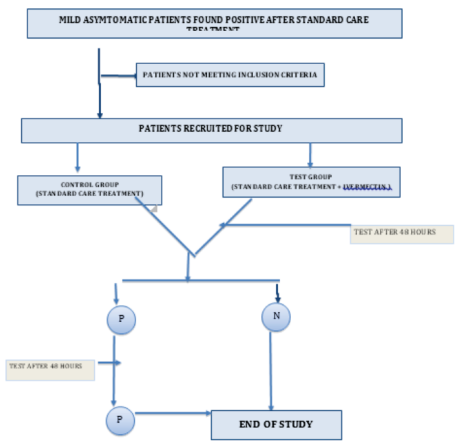
TABLE 1: Treatment Schedule for Control and Test Group

Day	Control Group	Test Group
Day -1	Hydroxychloroquine400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day	Ivermectin 12 mg single dose Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day
Day -2-5	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day
Day-3	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as require Vitamin C 1tab twice a day
Day-4	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day
Day-5	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day	Hydroxychloroquine 400 mg twice a day Paracetamol 500mg as required Vitamin C 1tab twice a day
On daily basis	Plenty of water with caloric diet intake Temperature and spo2 monitoring Good oral hygiene	Plenty of water with caloric diet intake Temperature and spo2 monitoring Good oral hygiene

Randomization:

Patients were randomized at a one to one ratio to hydroxychloroquine alone or ivermectin plus hydroxycholoroquine. The randomization list was generated by a computerized system by a unit independent of the study team The randomization codes was kept in sealed sequentially numbered opaque envelopes and wasnot be opened until the patient showsthroat swab positive test after completion of study for SARS-CoV-2 confirmed by reverse-transcriptase–polymerase- chain-reaction (RT-PCR) assay.

Figure 1: Study Cohort And Testing Schedule



Data collection

Data was collected in " Novel Coronavirus (COVID-19)- Rapid version" Case Record Form prescribed by WHO. All the clinical investigations were carried out pre and post enrollment in the study as per WHO CRF.

End Points

The primary endpoint for the study was negative throat swab report for SARS-CoV-2 conducted by RT-PCR after 48 hours of day one of research therapy. However if patient was tested positive on the then the test was repeated again after 48 hours. After this test the study on patient ended regardless of positive or negative result.

RESULTS:

Demographics and clinical presentation

We enrolled 32 patients meeting the inclusion criteria in this study that had at least six days of follow-up at the time of the present analysis. A total of 19 patients were enrolled in test group, they received hydroxychloroquine along with ivermectin and 16 patients were in control group, they received hydroxychloroquine alone. Basic

demographics and clinical status are presented in Table 2. Mean age of patients in test group was 39.5 (t value -0.588, p value-0.560) and that in the control group was 37.0 years (p value- 0.783).

TABLE 2: Demographic features of patients under study

	Test Group	Control Group
Age in years		
≤ 20	02(10.5%)	01(7.7%)
21-40	08(42.2%)	09(69.2%)
41-60	07(36.8%)	03(23.1%)
>60	02(10.5%)	00
Total	19(100%)	13(100%)
GENDER		
Male	14(73.7%)	09(69.3%)
Female	05(26.3%)	04(30.7%)

Comparison of Hydroxychloroquine plus Ivermectin and Hydroxychloroquine alone on throat swab test.

When comparing the effect of hydroxychloroquine treatment as a single drug and the effect of hydroxychloroquine and ivermectin combination, the proportion of patients that had negative RT-PCR results in oropharyngeal samples was not significantly different between the two groups at days 3 and 5 post-inclusion (Table 3). At day 5 post-inclusion, 42.2 % of patients treated with hydroxychloroquine and ivermectin combination were tested negative comparing with 46.2 % in patients treated with hydroxychloroquine only (p value 0.820). These results are summarized in Table 2.

Hospitalization status of the subjects at end of study:

There was no significant difference in terms of hospital stay between the group of patients receiving ivermectin as an adjuvant to hydroxychloroquine and the group of patients receiving hydroxychloroquine alone.

Table: 3: Throat swab test status of COVID 19 post recruitment in the trial:

TEST STATUS	TEST GROUP	CONTROL GROUP
Test results on 3rd day post recruitment:		
Positive	11(57.8%)	07(53.8%)
Negative	08(42.2%)	06(46.2%)
Total	19(100%)	13(100%)
Test results on 5th day post recruitment:		
Positive	01(20%)	00
Negative	04(80%)	06(100%)
Hospitalization status of the subjects at end of study:		
Discharged	08(42.2%)	06(46.2%)
Not Discharged	11(57.8%)	07(53.8%)

DISCUSSION:

In this study we did not observe any benefit of adding ivermectin to the hydroxychloroquine in the management of patients of SARS-CoV-2 resistant to standard care treatment. Our finding are based on small cohort of asymptomatic or patients with mild symptoms of COVID-19. Such patients were recruited when they did not responded to the standard treatment. Ivermectin was tested as an adjuvant drug to the standard treatment with hydroxychloroquine. On comparisons of patients receiving hydroxychloroquine plus ivermectin with the patients receiving hydroxychloroquine alone, no significant difference was observed in the cure rates. There were no significant adverse effects were observed in patients receiving ivermectin.

The use of ivermectin 12 mg single dose as an adjuvant to standard treatment was based on the widespread uncontrolled studies that suggested that the ivermectin has antiviral activity against a broad range of viruses.[13-16].Based on these studies it was concluded that ivermectin's nuclear transport inhibitory activity may be effective against SARS-CoV-2.[11]

Wagstaffet. al. observed that the ivermectin possesses the ability to disassociate the preformed IMP / 1 heterodimer which is responsible for the nuclear transport of viral protein cargos.[16]The inhibition of IMP / 1 leads to disruption of the immune evasion mechanism of the virus.[17] Although the exact mechanism for antiviral activity of ivermectin is still a subject of investigation butit is reported that a single treatment of ivermectin in a COVID-19 patient may cause the effect of a ~5000-fold reduction in viral RNA at 48 hours.[11,18]

These studies claiming large-scale decrease in viral load after single dose of ivermectin, prompted the investigators to initiate human trial of ivermectin for COVID-19. As of now, fifteen clinical trials testing the efficacy of ivermectin in COVID-19 are registered on clinicaltrails.com[19] and five trials are registered on clinical trial registry of India.[20]

Angela Patriet. et.al hypothesized that hydroxychloroquine and ivermectin may act in synergistic manner against COVID-19. Hydroxychloroquine may inhibit the entry of the virus into the host cell, while ivermectin could reduce viral replication.[12] Although all studies revealing antiviral activity of ivermectin are in vitro studies. However our study is first of its kind to report the inefficacy of ivermectin in COVID-19 patients.

The possible cause for ineffective in vivo antiviral activity of ivermectin could be due to lack of effective plasma concentration of the drug required to exhibit antiviral activity.[21]

Since the pharmacodynamics response of any drug is typically dependent on the duration for which the drug concentration is above the minimum therapeutic concentrations. Smith et al in their study showed that in order to achieve clinical translation, the in vivo plasma concentration of ivermectin should be more than at least nine folds of the effective invitro plasma concentration.[22]

The limitations of our study that we included small sample size due to change in guidelines during the study in which the asymptomatic and patients with mild symptoms were recommended to be home isolated and not hospitalized. Since we preferred to keep the recruited patients for study in hospital that was not possible as per the newer guidelines for the management of COVID-19 asymptomatic and mild symptoms patients were to be discharged and thus the sample size was restricted.

In summary, this open label randomized study of patients with COVID-19 found that the use of a regimen containing hydroxychloroquine and ivermectin was associated with no evidence of benefit in comparison to hydroxychloroquine alone. However, it was observed that ivermectin was well tolerated with no serious drug related adverse event thus a large sample sized randomized clinical trial may be initiated to further investigate its efficacy as anti-viral agent inCOVID-19. Another important aspect is to investigate is the effective in vivo therapeutic concentration for ivermectin as antiviral agent within the tolerated dose range.

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