Therapeutic efficacy of macrolides in management of patients with mild COVID-19

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Abstract

Evidence on the efficacy of adding macrolides (azithromycin or clarithromycin) to the treatment regimen for COVID-19 is limited. We testify whether adding azithromycin or clarithromycin to a standard of care regimen was superior to standard of supportive care alone in patients with mild COVID-19.The study included three groups of patients with COVID-19. The azithromycin group included, 107 patients who received azithromycin 500 mg/24 h for 7 days, the clarithromycin group included 99 patients who received clarithromycin 500 /12 h for 7 days, and the control group included 99 patients who received standard care only. All three groups received only symptomatic treatment for control of fever and cough .Clinical and laboratory evaluations of the study participants including assessment of the symptoms duration, real-time reverse transcription-polymerase chain reaction (rRT-PCR), C-reactive protein (CRP), serum ferritin, D-dimer, complete blood count (CBC), non-contrast chest computed tomography (CT), were performed.The overall results revealed significant early improvement of symptoms (fever, dyspnea and cough) in patients treated with either azithromycin or clarithromycin compared to control group, also there was significant early conversion of SARS-CoV-2 PCR to negative in patients treated with either azithromycin or clarithromycin compared to control group (p□0.05 for all).There was no significant difference in time to improvement of fever, cough, dyspnea, anosmia, GIT symptoms and time to PCR negative conversion between patients treated with azithromycin compared to patients treated with clarithromycin (p□0.05 for all). Follow up chest CT done after 2 weeks of start of treatment showed significant improvement in patients treated with either azithromycin or clarithromycin compared to control group (p□0.05 for all).Adding Clarithromycin or Azithromycin to the therapeutic protocols for COVID-19 could be beneficial for early control of fever and early PCR negative conversion in Mild COVID-19

Introduction

SARS-CoV-2 infection is major global health emergency with many countries still experiencing an increase in cases and related fatalities [1, 2].

As of December 30, 2020, Egypt has reported 135,333 cases of COVID-19 and 7,574 deaths. Most cases were asymptomatic or had mild to moderate symptoms as dyspnea, fever, dry cough, sore throat, or malaise [3–6]. Mild cases pass unnoticed but they can transmit the infection and increase the disease burden.Therapeutic strategies vary between using newly developed medications and repurposing existing medications for COVID-19. Drugs as Remdesivir, Lopinavir/Ritonavir; (anti HIV drugs), Chloroquine and Hydroxychloroquine (antimalarial drugs) were recommended in COVID-19 treatment due to their antiviral activity [4–6]. Other drugs still under trial as tocilizumab and sarilumab can attenuate COVID-19 and associated cytokine storm by interfering with interleukin-6 [7, 8], also the mesenchymal stem cell therapy described earlier [9].

Azithromycin is a widely available drug that might decrease viral load when added to hydroxychloroquine in patients with non-severe COVID-19 [10, 11]. Furthermore, preclinical studies have reported

immunomodulatory effects of azithromycin and other macrolides, which could control the intense inflammatory responses that might cause progression to organ failure [4–7].

Macrolides are bacteriostatic antibiotics, which are widely used in clinical practice against respiratory tract pathogens as Gram-positive and atypical bacterial have been shown to have immunomodulatory and anti-inflammatory effects [12–14].

To date, evidence on the efficacy and safety of adding azithromycin or clarithromycin to the treatment regimen for mild/ moderate COVID-19 is limited by low-quality studies [10, 11, 14]. The main objective of the current research is to address the effectiveness of these drugs in improving the clinical status and fasten PCR negative conversion in patients with mild COVID-19.

Patients And Methods

Study design and participants

The current study is a clinical trial conducted at Qena Governorate, Egypt, during the period from May 2020, to September 2020. An institutional ethical committee approval, Faculty of Medicine, South Valley University, Qena, Egypt, was taken, Ethical approval code: SVU202(SVU-CHT019-420860). A written informed consent was taken from all the patients participate in this study and the procedures were in accordance to Helsinki guidelines for human research.

Our study included 305 confirmed cases of mild COVID-19 according to WHO classification of clinical cases and all of them received the standard care regimen. They have active COVID-19, Oxygen saturation > 90% on room air and imaging shows less than 25% infiltrates on CT chest. They were randomly assigned to one of three study groups. The azithromycin group included, 107 patients who received azithromycin 500 mg/24 h for 7 days, the clarithromycin group included 99 patients who received clarithromycin 500 /12 h for 7 days, and the control group included 99 patients who received standard care only. All three groups received standard care regimen to control fever (paracetamol) and cough, patients were followed in intermediate care facility for quarantine of mild COVID-19 cases. The study flow chart done in intermediate care facility for quarantine of mild COVID-19 cases was presented in (Fig. 1).

All study participants underwent full clinical evaluation including duration of fever, cough, dyspnea, anosmia or GIT symptoms, daily evaluation of symptoms was done, fever chart was plotted 3 times daily measurement, patient was considered a febrile if body temprature < 37.2 C for two consecutive days.

Hematological, biochemical and molecular measurements, and imaging

1. 6 mls of venous blood was withdrawn from every included patients prior to therapy and was divided into 3 parts; (2mls were evacuated into EDTA tubes for CBCs ; 2mls were evacuated into serum gel separator tubes and allowed to be clotted at 37 0C for 30 min and then centrifuged 3500 rpm for 10 minutes and the separated sera were used for C-reactive protein (CRP) and ferritin assays, while the

remaining 2 mls were adjusted to be evacuated into citrate tubes and after centrifugation the separated citrated plasma was used for D- dimmer assays) as follow:

1. CBC with differential: By using cell dyne-Ruby (Abbott Diagnostics-Santa Clara- California-USA), automated cell counter.
2. Determination of D-Dimer: By automated blood coagulation analyzer CS-1600 – Japan. The assay kit was supplied by Spectrum, Egyptian company of biotechnology, Cairo, Egypt, catalogue N.585002. The assay is based upon reinforced immunoturbidimetry monoclonal anti D-dimer antibodies in the reagent react with the D-dimer antigen in the sample, forming antigen/antibody complexes that increase the work solution turbidity. Reference range: up to 0.5 ng/dL.
3. CRP (C-reactive protein): assays were performed using the semi-quantitative latex agglutination test (AVITEX CRP kits; Catalog No. OD023; supplied by Omega Diagnostics, UK). Reference range: 6mg/dL.
4. Serum ferritin assays were performed using commercially available ELISA assay kits (supplied by BIOCHECK, INC,323 VNTAGE Park Dr. Foster City, CA94404-catalog number: BC-1025), by an ELISA multiskan EX microplate photomter, thermo scientific (STAT FAX-2100, USA) according to the manufacturer protocol. Reference range: 10–232 ng/dL.

1. PCR testing was performed on aliquots of Universal Transport Medium (UTM) used for nasopharyngeal swabs' collection (Huachenyang Technology, China). Aliquots were: extracted on the QIA symphony platform (QIAGEN, USA) and tested with real-time reverse-transcription PCR (RT-qPCR) using the QIAamp ® DSP Virus Spin Kit (QIAGEN Hilden, Germany) on a Rotor-Gene Q (QIAGEN Hilden, Germany) [15, 16]. PCR was repeated at 5th day and every 48 h there till 2 consecutive negative PCR obtained.
2. Non-contrast chest computed tomography (CT) which was repeated 2 weeks after start of treatment. Improvement in CT chest was defined as complete resolution of infiltrates in follow up CT chest done after 15 days from start of treatment

Statistical analysis

Statistical package of social sciences (SPSS), version (21) (SPSS: An IBM Company, version 21.0, IBM Corporation, Armonk, NY, USA) was used for data analysis. Quantitative data presented as mean ± standard deviation (SD) and qualitative data presented in the form of frequency and percentage. Independent Sample T-test, ANOVA or Chi-square test was used to determine the statistically significant variances among the study groups. Pearson's correlation was used to compare the studied parameters. $P \leq 0.05$ reflected significance.

Results

Baseline demographic, clinical, laboratory and imaging data of the study groups

The current study included 305 confirmed cases of mild COVID-19 who were allocated into three groups. The azithromycin group included, 107 patients, mean age 45.8 ± 18 years, 73 male and 34 female, the clarithromycin group included 99 patients mean age 46.1 ± 19 years, 68 males and 31 female, the control group included 99 patients, with mean age 41.1 ± 18 years, 73 male and 28 female with non-significant differences between the study groups regarding to the age and sex, indicating matching, p0.05 for all (**Table.1**).

As regards the baseline clinical and laboratory data, there were no significant differences in the frequency percentage of fever, cough, dyspnea and anosmia, or the mean ± SD of the baseline CRP, serum ferritin, nutrophil/lymphocyte ratio and D-dimer, or the extent of CT chest infiltrates between azithromycin, clarithromycin or control groups, p0.05 for all (Table 1)

Table 1
Baseline characteristics of the study groups

Variables	Azithromycin Group N = 107	Clarithromycin Group N = 99	Control Group N = 99	P value
Age (mean ± SD)	45.8 ± 18	46.1 ± 19	41.1 ± 18	0.098
Sex : N (%)	73(68.2%)	68(68.6%)	73(73.7%)	0.638
• Males	34 (31.8%)	31 (31.4%)	26 (26.3%)	
• Females				
Fever	107 (100%)	99 (100%)	99 (100%)	0.135
N (%)				
Cough	107 (100%)	96 (97%)	96 (97%)	0.154
N (%)				
Dyspnea	78 (73%)	86 (87%)	85 (86%)	0.056
N (%)				
Anosmia	21 (19.6%)	23 (23.2%)	23 (23.2%)	0.768
N (%)				
GIT symptoms	32 (30%)	40 (40%)	33 (33%)	0274
N (%)				
CRP (mean ± SD, mg/dl)	16.3 ± 15.8	20.6 ± 19.4	18.5 ± 18.1	0.216
D-dimer (mean ± SD, ng/mL)	191 ± 291	182 ± 260	237 ± 285	0.334
Neutrophil/lymphocyte ratio (mean ± SD)	3.2 ± 0.9	3.1 ± 1.2	3 ± 1.2	0.598
Ferritin (mean ± SD, ng/mL)	715 ± 417	790 ± 507	690 ± 477	0.292
CT Chest	0	3 (3%)	2 (2%)	0.171
<5% infiltrates	107 (100%)	96 (97%)	97 (98%)	
6–25% infiltrates				

Therapeutic efficacies of macrolids in patients with mild COVID-19 compared to standard supportive therapy

There was significant shorter duration of fever (days), in patients treated with either azithromycin (5.2 ± 2.3) or clarithromycin (4.9 ± 1.5) compared to control group (12.9 ± 2.2), P < 0.000. Duration of cough also showed significant fewer days for improvment in patients treated with either azithromycin (5.4 ± 2.7) or clarithromycin (5.1 ± 2) compared to control group (12.9 ± 2.2) P < 0.0001, in addition, dyspnea duration (days) showed significant early improvement in patients treated with either Azithromycin (4.6 ± 3.3) or Clarithromycin (4.7 ± 2.9) compared to control group (9.3 ± 2.7) ,P < 0.0001 (Table 2). There was significant early conversion (days) of SARS-CoV-2 PCR to negative in patients treated with either azithromycin (8.7 ± 2.8) or clarithromycin (8.3 ± 2.6) compared to control group (13.2 ± 4.2), P < 0.0001 (Table 2).

Table 2
Duration of symptom improvement and PCR conversion to negative in the study groups among patients with mild COVID-19

Variables	Azithromycin Group N = 107	Clarithromycin Group N = 99	Control Group N = 99	P1 value (1,2)	P2 value (1,3)	P3 value (2,3)	P value ANOVA
Time to PCR –ve (Mean ± SD, days)	(8.7 ± 2.8)	(8.3 ± 2.6)	(13.2 ± 4.2)	0.351	< 0.0001	< 0.0001	< 0.0001
Fever days (Mean ± SD)	(5.2 ± 2.3)	(4.9 ± 1.5)	(12.9 ± 2.2)	0.353	< 0.0001	< 0.0001	< 0.000
Cough days (Mean ± SD)	(5.4 ± 2.7)	(5.1 ± 2)	(12.9 ± 2.2)	0.481	< 0.0001	< 0.0001	< 0.0001
Dyspnea days (Mean ± SD)	(4.6 ± 3.3)	(4.7 ± 2.9)	(9.3 ± 2.7)	0.726	< 0.0001	< 0.0001	< 0.0001
Anosmia days (Mean ± SD)	(0.48 ± 0.9)	(1.2 ± 3)	(0.9 ± 2.3)	0.024	0.208	0.323	0.076
GIT symptoms days (Mean ± SD)	(0.9 ± 1.7)	(1.5 ± 2.4)	(1.2 ± 2)	0.046	0.406	0.250	0.134
CT chest follow-up							
• Improved	70 (65%)	76 (77%)	53 (54%)				0.0001
P1 = Azithromycin treated group vs. clarithromycin treated group.							
P2 = Azithromycin treated group vs. control group.							
P3 = clarithromycin treated group vs. control group.							

There was no significant difference in time to improvement of fever (P = 0.351), cough (P = 0.481), dyspnea (P = 0.726), and time to PCR negative conversion (P = 0.351) between patients treated with azithromycin compared to patients treated with clarithromycin (Table 2).

Follow up chest CT chest done 2 weeks after the start of treatment showed significant improvement in patients treated with either azithromycin 70 (65%) or clarithromycin 76 (77%) compared to control group 53 (54%) $P < 0.0001$, (Table 2).

Discussion

COVID-19 management showed great challenges to physician and intensivists, partially due to the novelty of the disease and the dramatic effects the disease causing. Though vaccination was recently introduced, it is still of limited application. Treating the causative virus using antiviral therapeutics or neutralizing monoclonal antibodies should be started early and may not benefit in treating the associated inflammatory condition. Macrolids' antiviral activity was first reported in treating patients with diffuse pan bronchitis who received low dose of erythromycin and led to decrease inflammation in upper and lower respiratory tract [17]. The efficacy of clarithromycin against viral infection as rhinovirus (RV), respiratory syncytial virus (RSV), and influenza virus was addressed before [18–21].

The anti inflammatory mechanism of clarithromycin through inhibiting the activation of NF-kB in cell nuclei and transcription reduction make it a good candidate drug in COVID-19 setting as most of the disease pathology is due to inflammation [22]. In addition clarithromycin decreases the expression of SAα 2,6Gal, a receptor for human influenza located on human tracheae surface mucosa, and prevents viral invasion. All together ends with inhibiting protein synthesis, translocation of aminoacyl transfer-RNA, and preventing peptide chain elongation. The same scenario can happen in COVID-19 [23]. Studies on the mechanism of action of clarithromycin on SARS-Cov-2 virus are warranted.

In this study; 2 groups of patients randomly received in addition to the standard of care therapeutic either azithromycin or clarithromycin and compared to the third group who receive standard of care only. The average mean of age was comparable among the 3 study groups (Azithromycin : 45.8 years, Clarithromycin, 46.1years and Control: 41.1 years, $P > 0.05$). Clinical and laboratory investigations do not vary among the patients before starting the therapeutic regimen. A significant early improvement of fever, dyspnea and cough in Azithromycin and Clarithromycin groups is measured after adding azithromycin or clarithromycin to the standard of care, also there was significant early conversion of COVID-19 PCR to negative in patients treated with either azithromycin or clarithromycin compared to control group. In a French clinical trial of 20 patients combining azithromycin and hydroxychloroquine led to rapid viral resolution at day 6 than those on :100% of patients treated with the combination vs. 57.1% of patients treated with hydroxychloroquine only and 12.5% of the control group ($P < 0.05$).[11]

In the other hand, Furtado and his colleges in Brazil when studied the efficacy of azithromycin in severe COVID-19 patients, they did not find a significant value of azithromycin [24]. To be noted, their patients' status differs from ours, who are mild cases in whom azithromycin or clarithromycin may still have the chance to clear the virus before inflammation and complications start. Adding to this they didn't evaluate time to PCR negative conversion.

In support of the importance of azithromycin in treating COVID-19 a survey done in 30 countries included 6200 physicians and they reported that, azithromycin was the second most commonly prescribed for COVID-19 treatment [25]. Based on our results we concluded that, azithromycin and clarithromycin when used in mild cases are safe and effective adjunctive therapy in improving the early symptoms, viral shedding that can decrease viral transmission.

Conclusion

The current study provide an evidence regarding to the possible use of macrolids (clarithromycin or azithromycin) in the therapeutic protocols for mild COVID-19 that could be efficient for early control of fever and early PCR negative conversion .

Declarations

Conflict of interest

No potential conflicts of interest between authors to be declare.

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Figures

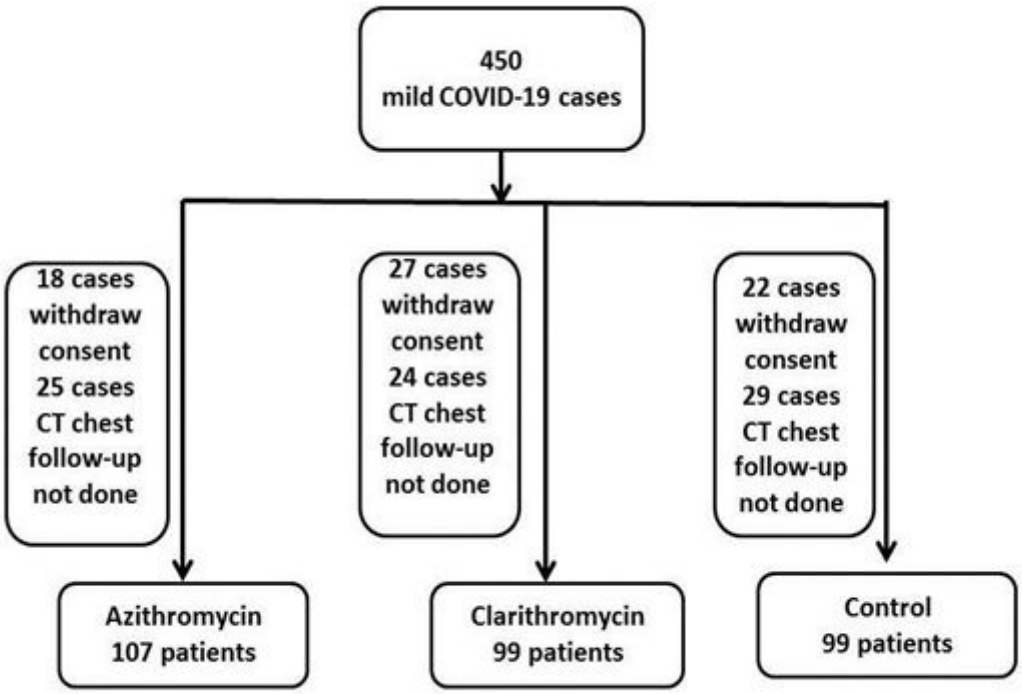

Figure 1

Algorithm of the study design and participants