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Hydroxychloroquine / azithromycin in COVID-19: The association between time to treatment and case fatality rate

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ABSTRACT

Background: Currently, there is no formally accepted pharmacological treatment for COVID-19.
Materials and methods: We included COVID-19 outpatients of a Peruvian primary care center from Lima, Peru, who were treated between April 30 - September 30, 2020, with hydroxychloroquine and azithromycin. Logistic regression was applied to determine factors associated with case-fatality rate.
Results: A total of 1265 COVID-19 patients with an average age of 44.5 years were studied. Women represented 50.1% of patients, with an overall 5.9 symptom days, SpO2 97%, temperature of 37.3 °C, 41% with at least one comorbidity and 96.1% one symptom or sign. No patient treated within the first 72 h of illness died. The factors associated with higher case fatality rate were age (OR = 1.06; 95% CI 1.01–1.11, p = 0.021), SpO2 (OR = 0.87; 95% CI 0.79–0.96, p = 0.005) and treatment onset (OR = 1.16; 95% CI 1.06–1.27, p = 0.002), being the latter the only associated in the multivariate analysis (OR = 1.18; 95% CI 1.05–1.32, p = 0.005). 0.6% of our patients died.
Conclusions: The case fatality rate in COVID-19 outpatients treated with hydroxychloroquine/azithromycin was associated with the number of days of illness on which treatment was started.

1. Introduction

The rapid spread of the virus referred to as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to a devastating worldwide pandemic. Despite the astonishingly rapid development of effective vaccines, most countries continue to suffer from the tragic consequences of the coronavirus disease 2019 (COVID-19). There is still a need for drugs that effectively control the disease. Unfortunately, COVID-19 has proven elusive and non-responsive to most treatment options as indicated by several clinical trials that failed to demonstrate significant reduction in morbidity and mortality of COVID-19 patients [1,2]. Perhaps most disheartening is the fact that drugs proven to possess strong anti-infectious and anti-inflammatory properties and that have been successfully employed in other viral diseases failed to show statistical improvement in several clinical trials in COVID-19 patients. Two concrete examples are Chloroquine (CQ) and its metabolite Hydroxychloroquine (HCQ). Successfully used to prevent and treat malaria and amebiasis for many years [3], these drugs yielded conflicting results in various clinical trials [4]. furthermore, its usage or treatment interruption could be confounded by the known side effects of CQ and HCQ which include mild gastrointestinal and more serious cardiovascular and neurological effects. This is a particularly important consideration when treating patients at risk of developing severe forms of COVID-19 [4,5].

However, notwithstanding the known limitations and well-justified reservation for the use of these drugs, there is one aspect that requires further investigation: the fact that in viral infections such as influenza,

Abbreviations: Hydroxychloroquine, (HCQ); Azithromycin, (AZIT); Severe acute respiratory syndrome coronavirus 2, (SARS-CoV-2); Coronavirus disease 2019, (COVID-19); Angiotensin converting enzyme 2, (ACE2); Selectivity Index, (SI); Mean Maximum Inhibitory Concentration, (MIC50%); Mean Cytotoxic Concentration, (CC50%); Mean Effective Concentration, (EC50); Maternal-Infant Center, (MIC).

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there is a relationship between early antiviral therapy and survival. It is the rapid elimination of pathogens and the early reduction in the viral load that seems to be decisive to avoid irreversible injury due to progression of the disease [6]. This is particularly relevant for the use of CQ and HCQ in the context of COVID-19 [7], and much can be learned from countries that have considerable clinical experience with the use of these drugs in the treatment of malaria and other infectious diseases. Indeed, the healing properties of the bark of the tree Cinchona officinalis, the source of the natural quinine, was first discovered by the Incas, and clinically applied to cure malaria as early as the 1600's, making the Cinchona tree the national tree of Peru.

Thus, when the first case of COVID-19 was diagnosed in Peru on March 6th, 2020, clinicians experienced with the use of HCQ in the treatment of other diseases employed this drug to combat COVID-19. They based its use on their clinical experience and the knowledge that HCQ significantly decreases viral load in particular when associated with azithromycin (AZIT). To these clinicians, the long-term use of low doses of AZIT was known to reduce exacerbations of poorly controlled asthma, which has been attributed to the suppressive effect of AZIT on the inflammatory TNF pathways [8]. The use of this treatment regimen was further encouraged by early reports that HCQ not only decreased significantly the viral load, but when associated with AZIT, was also able to control the infection in COVID-19 patients [9].

Cloroquine, the derivate of the natural quinine, is a 9-aminoquinoline that has first been described in 1934, and used to combat various viruses since 1960 [10]. In 1978 it was demonstrated that CQ is an acidotropic dibasic agent that increases the pH of lysosomes [11], and alters cellular metabolism [12]. This pH effect in lysosomes and other cytoplasmic organelles contributes to the suppression of viral replication. Together with its anti-inflammatory action as a suppressor of TNF alpha and Interleukin 6, this drug seems to be an ideal candidate to treat patients infected with SARS-CoV-2 [13]. In 2003 it was discovered that the S1 domain of the SARS-CoV protein binds to angiotensin-converting enzyme 2 (ACE2) for cell entry which opened the way for further in-depth mechanistic in vitro studies [14]. One of these studies demonstrated that CQ is an effective inhibitor of replication of the coronavirus SARS-CoV. These cell culture experiments demonstrated that the IC50 for the antiviral activity of CQ was significantly lower than its cytostatic activity, which was reflected in a high selectivity index of 30. Specifically, these studies indicated that the maximal concentration of its antiviral action (8.8 μM) was much lower, than the concentration required for its mean cytotoxic effect (261.3 μM). Clearly, as is the case for all in vitro assays, there are numerous caveats associated with the translation of such preclinical findings into the clinic. Nevertheless, such experiments are encouraging, since concentrations (CC50%) of 261.3 μM are much higher than those achieved in the blood at the therapeutic level of MIC50%, suggesting that CQ could potentially be an effective clinical agent against SARS-CoV [15]. Moreover, as demonstrated in another study cells previously treated with CQ are refractory to SARS-CoV infection, and when cells are already infected, CQ can prevent viral replication [16].

These encouraging results laid a solid scientific foundation for the use of CQ in the treatment of SARS-CoV-2. Indeed, various studies confirmed that the known antiviral properties of CQ also potently blocked SARS-CoV-2 infection at low concentration, with mild cytotoxicity and a high selectivity index (mean effective concentration (EC50%) = 1.13 μM; CC50 > 100 μM, SI > 88.50) [17]. Moreover additional mechanisms have been identified. One study suggests that binding of HCQ/CQ to sialic acids and ECA-2 receptor gangliosides prevents viral S protein from entering the cell [18], and a cell culture assay confirmed that HCQ/CQ blocks the transport of SARS-CoV-2 from early to late endosomes [19]. Another study tested 1520 compounds that are in clinical use and identified fifteen products effective against SARS-CoV-2. Among these products HCQ and AZIT showed the highest antiviral activities (CE50% = 4.17 μM and 2.12 μM, respectively) and highest SIs [20]. In both plasma and lung, CQ/HCQ have mean/median Cmax concentrations above the EC50, and both plus AZIT would reach lung concentrations 10 times higher than the EC50 [21]. Structurally, AZIT resembles the GM1 ganglioside of the ECA2 receptor, so it binds to the tip of the spike protein, while the CQ/HCQ molecules bind to the virus binding sites of sialic acids and ECA-2 gangliosides, generating a synergistic antiviral mechanism [22]. These in vitro studies suggest that the HCQ-AZIT combination has a synergistic effect on SARS-CoV-2 at concentrations that are compatible with those obtained in the human lung [23]. Furthermore, by binding to Sigma 1 and Sigma 2 receptors, HCQ effectively reduces the infectivity of SARS-CoV-2 [24].

The scientific data obtained for the Coronavirus as early as 2006 and confirmed for SARS-CoV-2 at the beginning of the pandemic, together with our extensive clinical experience in the use of CQ in treating malaria and other infectious diseases [3] provided a strong rationale for the therapeutic use of CQ in patients infected during the new coronavirus epidemic COVID-19. It also provided an impetus to test QC immediately in clinical trials [25].

In Peru, the Ministry of Health decided to use the HCQ/CQ combination with AZIT [26]. In the absence of clinical trial results during the early phase of the pandemic, physicians were instructed to apply their extensive clinical experience with the use of this drug combination in the context of the emerging understanding of the pathophysiology of SARS CoV-2 infection in order to determine the impact of early outpatient treatment on hospitalization and mortality [27]. In this report we present the results in 1265 patients treated on an outpatient basis at the Centro Materno-Infantil (CMI) de Tahuantinsuyo Bajo, a I-4-level health center in the city of Lima.

2. Material and methods

The present study analyzed anonymized data from the database of COVID-19 patients attended at the CMI Tahuantinsuyo Bajo, a primary care facility in the city of Lima, between April 30 and September 30, 2020.

Patients arrived at a dedicated triage site for patients with suspected COVID-19 infection. There, vital signs were taken, including SpO_2, and the attending physicians took the patient history and performed a clinical examination to determine whether they met COVID-19 patient clinical criteria according to the guidelines of the Peruvian Ministry of Health. All patients were registered in the respective epidemiological data file and the information included vital signs, comorbidities, symptoms and treatment onset, consisting of 200 mg HCQ every 8 h for 7–10 days in combination with 500 mg AZIT on the first day, followed by 250 mg for 4 days. Data on days from symptom onset to treatment was collected as well. The patients were followed up with daily telephone controls and if any symptoms of deterioration or side effects appeared, they were summoned to the clinical facility. Follow-up was carried out not only with the patients but also with their contacts, with the aim of providing treatment as soon as the first symptoms appeared. Every day the epidemiology team recorded and shared patient's information to the physician coordinating the COVID-19 registry. The information was transcribed into an Excel spreadsheet and the cases were followed up after discharge until they were sure of their condition. If the information could not be obtained by telephone, a home visit was done by the rapid a response team also stablished under Peruvian COVID-19 guidelines.

The treatment started as soon as the attending physician determined that the patient exhibited symptoms that met the COVID-19 patient clinical criteria according to the guidelines of the Peruvian Ministry of Health. Some of these patients arrived at the hospital with a positive test, but most did not. Those that were not tested before arrival were asked to take the test. This test was not readily available at the center, albeit it continues to be offered at no cost at some government testing sites. Given that during the study period it could take almost a week to process and register the result of the NAAT test, and because tests tend to be less accurate within three days of exposure, the treatment regimen was

started irrespective of any result if a patient met all the clinical criteria for COVID-19. Statistical analysis was performed with the Stata 14 statistical package (Stata Corporation, College Station, Texas, USA). Categorical variables were presented as frequencies and percentages and their respective 95% confidence intervals (95%CI), continuous variables as means or medians along standard deviations (SD) or interquartile ranges (IQR). To determine the risk factors associated with death, a logistic regression analysis was performed, odds ratios were presented with their respective 95%CI and a p value of less than 0.05 was considered statistically significant.

This study was approved by the Institutional Human Ethics Committee of the Universidad Peruana Cayetano Heredia (approval code: 203939). This study did not require individual consent from the participants because it analyzed de-identified data from an already existing database. Cayetano Heredia University's researchers analyzed the information that was previously registered and systematized by the team of physicians in charge of primary care at the health center.

3. Results

A total of 1265 clinically diagnosed COVID-19 patients were studied with an average age of 44.5 years, 50.1% being women, with a time of symptom onset to treatment of 5.9 days, SpO2 of 97%, temperature of 37.3 °C, with 41% with at least one comorbidity and 96.1% with at least one symptom or sign (**Table 1**). The most common comorbidities were obesity (17.3%), hypertension (8.3%), chronic respiratory disease (7.2%) and diabetes (6.1%) (**Table 2**). The most common symptoms were cough (85.1%), malaise (81.7%), sore throat (76.7%), sensation of thermal rise (54.2%) and dyspnea (33.8%) (**Table 3**).

At follow-up, there were 7 deaths in total, all men with a mean age of 57.7 years, SatO2 96%. Four of the deceased patients carried one known comorbidity (hypertension, obesity, diabetes and chronic respiratory disease), three had no comorbidity. The one aged 29 years old had obesity and a 98% SpO2 at first encounter, while the patient age 39 years old had no known comorbidities and a 98% SpO2.The most common symptoms were cough (100%), malaise (100%), sore throat (85.7%), dyspnea (71.4%), nasal congestion (42.9%) and febrile sensation (42.9%) (Table 3). Logistic regression showed that those factors associated with higher mortality were age (OR 1.06; 95% CI 1.01–1.11, p = 0.021), SpO2 (OR 0.87; 95% CI 0.79–0.96, p = 0.005) and number of days until the start of treatment (OR 1. 16; 95% CI 1.06–1.27, p = 0.002). However, in a multivariate analysis the time of illness elapsed before receiving treatment was the only factor associated with higher mortality (OR 1.18; 95%CI 1.05–1.32, p = 0.005) (**Table 4**).

The case-fatality rate of this cohort of patients treated with HCQ-AZIT was 0.6. No female patient died and the mortality among males was 1.12%. (**Table 5**).

Remarkably, none of those treated in the first 72 h of illness onset died. Deaths occurred on days four, when two died, and on days six, seven, eight, twelve and thirty-one after onset. All but nine patients

Table 1
Characteristics of COVID-19 patients treated with hydroxychloroquine and azithromycin at CMI Tahuantinsuyo Bajo.

Variables	All patients (n = 1265)			Dead (n = 7)	
	n	Mean	SD	Mean	SD
Age (years)	1265	44.5	14.8	57.7	20.6
SpO2 (%)*.	1057	97	2	96	4
Temperature (°C)	1108	37.3	0.8	37.4	0.7
Time from symptom onset to treatment (days)	1202	5.9	4	10.3	9.5
Symptoms (number)*	1265	5	3	6	4
Comorbidities (number)*	1265	0	1	1	1
Height (meters)	420	1.62	0.09	1.64	0.1
Weight (kg)	418	72.9	13	73	25.7
BMI (kg/m2)	418	27.8	4.2	26.8	7.5
Characteristics	n	%	CI 95%	n	%
0–20 years	39/1265	3.1	2.3–4.2%	0	0
20–40 years	439/1265	34.7	32.1–37.4%	2	28.6
40–60 years	590/1265	46.6	43.9–49.4%	2	28.6
60–80 years	177/1265	14	12.2–16.0%	1	14.3
80–100 years	20/1265	1.6	1.0–2.4%	2	28.6
Sex (female)	634/1265	50.1	47.4–52.9%	0	0
At least one comorbidity	470/1265	37.2	34.5–39.9%	4	57.1
SpO2 <92%	32/1265	2.5	1.8–3.6%	1	14.3
Tested for SARS-CoV-2 (any test)	134/1265	10.6	9.2–12.7%	5	3.6
Treatment discontinuation	10/1265	0.8	0.4–1.5%	1	10%

Table 2
Comorbidities in COVID-19 patients who were treated with hydroxychloroquine and azithromycin.

Comorbidities	All patients (n = 1265)				Dead (n = 7)	
	n	%	IC 95%		n	%
Obesity	219	17.3	15.3	19.5	1	14.3
Hypertension	105	8.3	6.9	10	1	14.3
Respiratory disease	91	7.2	5.9	8.8	1	14.3
Diabetes	77	6.1	4.9	7.5	1	14.3
Endocrinological disease	18	1.4	0.9	2.2	0	0
Cardiovascular disease	13	1	0.6	1.8	0	0
Gastrointestinal disease	12	0.9	0.5	1.7	0	0
Neurological disease	10	0.8	0.4	1.5	0	0
Rheumatologic disease	7	0.6	0.3	1.2	0	0
Immunosuppression	7	0.6	0.3	1.2	0	0
Pregnant women	6	0.5	0.2	1.1	0	0
Psychiatric disease	5	0.4	0.2	0.9	0	0
Surgical pathology	4	0.3	0.1	0.8	0	0
Hematologic disease	3	0.2	0.1	0.7	0	0
Renal disease	2	0.2	0	0.6	0	0
Neoplastic disease	2	0.2	0	0.6	0	0

Table 3
Symptoms presented by COVID-19 patients who were treated with hydroxychloroquine and azithromycin.

Symptoms	All patients (HCQ + AZIT) (n = 1265)				Dead (n = 7)	
	n	%	CI 95%		n	%
Cough	1076	85.1	83	86.9	7	100
General discomfort	1034	81.7	79.5	83.8	7	100
Sore throat	970	76.7	74.3	78.9	6	85.7
Fever	685	54.2	51.4	56.9	3	42.9
Dyspnea	427	33.8	31.2	36.4	5	71.4
Nasal congestion	397	31.4	28.9	34	3	42.9
Headache	360	28.5	26	31	2	28.6
Chills	259	20.5	18.3	22.8	0	0
Muscle pain	207	16.4	14.4	18.5	2	28.6
Joint pain	223	17.6	15.6	19.8	1	14.3
Chest pain	141	11.2	9.5	13	1	14.3
Diarrhea	103	8.1	6.8	9.8	1	14.3
Nausea	82	6.5	5.2	8	0	0
Anosmia	65	5.1	4	6.5	1	14.3
Ageusia	49	3.9	2.9	5.1	1	14.3
Vomiting	39	3.1	2.3	4.2	0	0
Back pain	26	2.1	1.4	3	0	0
Unspecified pain	21	1.7	1.1	2.5	1	14.3
Abdominal pain	11	0.9	0.5	1.6	0	0
Irritability	8	0.6	0.3	1.3	0	0
Eye redness	4	0.3	0.1	0.8	0	0
Decreased appetite	3	0.2	0.1	0.7	0	0
Dizziness	1	0.1	0	0.6	0	0
Skin rash	1	0.1	0	0.6	0	0

Table 4
Odds ratio for death in patients with suspected COVID-19 who were treated with hydroxychloroquine + azithromycin. N = 904.

Variables	Univariated				Multivariated			
	OR	p	95% CI		OR	p	95% CI	
Age	**1.06**	**0.021**	**1.01**	**1.11**	1.06	0.087	0.99	1.13
Sex (female)	0.07	0.062	0	1.15	0.11	0.131	0.01	1.93
Risk factors (at least one)	2.18	0.275	0.54	8.87	1.16	0.855	0.24	5.54
SpO2 (first measurement)	**0.87**	**0.005**	**0.79**	**0.96**	0.93	0.423	0.78	1.11
Time from symptom onset to treatment **(days)**	**1.16**	**0.002**	**1.06**	**1.27**	**1.18**	**0.005**	**1.05**	**1.32**
Temperature (°C)	1.19	0.703	0.49	2.89	1.49	0.471	0.51	4.38

Table 5
Comparison of mortality between Peruvian COVID-19 patients and those treated at the Tahuantinsuyo Bajo CMI with hydroxychloroquine + azithromycin.

Age (women)	Deaths (Tahuantinsuyo)	Cases	Deaths (%)	95% IC	
0–19	0	24	0.00%	0	0
20–39	0	239	0.00%	0	0
40–59	**0**	**277**	**0.00%**	0	0
60–79	**0**	**86**	**0.00%**	0	0
80+	0	8	0.00%	0	0
Total (Women)	0	634	0.00%	0	0
Age (men)	Deaths (Tahuantinsuyo)	Cases	Deaths (%)	95% IC	
0–19	0	15	0.00%	–	–
20–39	2	198	1.00%	0.25%	3.93%
40–59	**2**	**311**	**0.64%**	**0.16%**	**2.53%**
60–79	**1**	**90**	**1.10%**	**0.15%**	**7.49%**
80+	2	10	16.67%	3.92%	49.48%
Total (Men)	7	624	1.12%	0.30%	1.95%
Total (all patients)	7	1265	0.60%	0.30%	1.20%

Table 6
Distribution according to days of symptom onset to treatment of COVID-19 patients and deaths of the CMI Tahuantinsuyo Bajo who were treated with hydroxychloroquine + azithromycin.

Time of illness	Deaths per day	Cases per day	%
1	0	83	0
2	0	124	0
3	0	153	0
4	2	157	1.27
5	0	143	0
6	1	106	0.94
7	1	139	0.72
8	1	81	1.23
9	0	35	0
10	0	39	0
11	0	44	0
12	1	29	3.45
13	0	17	0
14	0	8	0
15	0	10	0
16	0	12	0
17	0	2	0
18	0	1	0
19	0	4	0
20	0	6	0
21	0	0	0
22	0	1	0
23	0	1	0
24	0	0	0
25	0	0	0
26	0	2	0
27	0	1	0
28	0	1	0
29	0	0	0
30	0	2	0
31	1	1	100

(0.72%) reported having sought care within the first 20 days of symptom onset (Table 6). The proportion of patients seen within the first 72 h was different than the proportion of patients that arrived with longer duration of illness [28.06% (95% CI:25.65–30.61%) vs 71.94%, (95% CI: 69.39–74.35%), $p < 0.0001$] (**Fig. 1**). The percentage of case fatality increased progressively with the number of days after treatment initiation ($p = 0.0039$), reaching 0.89% (95% CI: 0.1–6.12%) among those who received treatment from day 10–12 of illness (**Fig. 2**). Importantly, no patients had cardiovascular side effects or had to be hospitalized for any effect attributable to the use of HCQ/AZT, but there were 0.79% (10/1265) who had their scheme suspended due to side effects, being nausea the most frequent present in 5 patients (Table 1).

4. Discussion

Of the 1265 COVID-19 patients treated at Tahuantinsuyo Bajo with HCQ-AZIT, 0.6% died (**Table 1**). This outcome is consistent with the first European study that used the same treatment regimen for 1061 patients. The case-fatality rate in this healthcare center was 0.75% [28]. The mortality rate among the 1265 patients treated with this specific regimen was six times lower than the national average [29]. As of September 1st, 2020, the updated case counts show that 74687 out of 694314 COVID-19 Peruvian patients died (case-fatality rate of 10.8%), being 13.2% (49121/372685) for men and 7.9% (25566/321629) for women [30]. Unfortunately, as of August 25th, Peru has one of the highest CFR of COVID-19 related deaths and ranks on seventh position with 6086 deaths per million inhabitants.

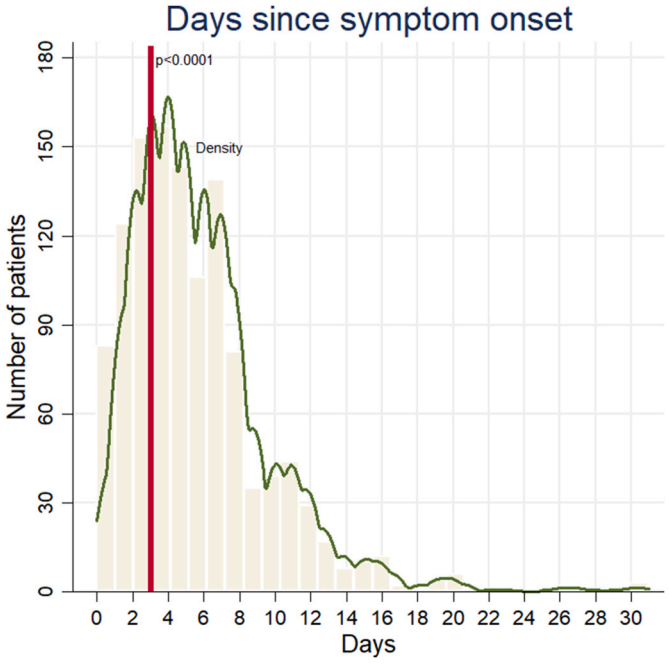

Fig. 1. Presentation by symptom onset to treatment of COVID-19 patients at Tahuantinsuyo Bajo.

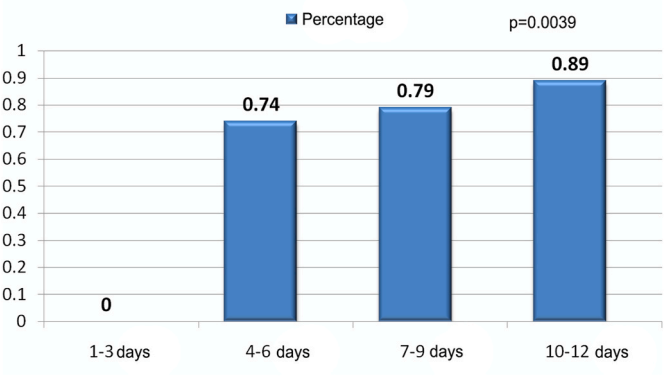

Fig. 2. Deaths by days from symptom onset to treatment (p = 0.0039).

4.1. Time of symptom onset to treatment before HCQ/AZIT

The first clinical manifestations at the onset of COVID-19 are thought to be attributable to viral replication, and it is generally assumed that the disease then moves into the inflammatory phase if the immune system fails to mount an adequate response. Thus, in patients who present late after the onset of the infection, the use of antiviral agents alone will not suffice to stop the ensuing cytokine storm, lung destruction and respiratory distress [31]. This could explain why HCQ has a better clinical efficiency when given earlier in the disease [32]. In the present study, treatment with HCQ-AZIT began on average 5.9 days after symptom onset, and there was no mortality among patients that received this drug combination during the first three days of symptom onset **(Fig. 2).**

This is consistent with the known relationship between early antiviral therapy and survival in influenza [6]. In a study of 657 influenza A/H1N1-2009 patients, those who received oseltamivir as an antiviral therapy within 48 h of symptom onset had lower ICU mortality and consumption of ICU resources and showed protection (OR 0.44, p = 0.02) [33], while in another research study those receiving treatment later had greater morbidity and mortality (OR 2.20, 95% CI: 1.47–3.57) [34]. While it has been observed that the interval for treatment onset was 2 days in community cases, 4 for those hospitalized and 6 in those admitted to the ICU (p < 0.001) [35]. Our study is most consistent with a study in which the use of HCQ before the fifth day of diagnosis was the only protective factor for prolonging viral shedding in patients with COVID-19 (OR = 0.111, p = 0.001) [7]. This conclusion is also consistent with the observation that HCQ had a protective effect in 67 patients who took this drug before hospitalization (p < 0.001), while there was no apparent protection among the 558 patients that received these drugs when they were already hospitalized [36].

Our results also corroborate other studies in which COVID-19 patients were treated from the first days of the disease. Among 141 HCQ-AZIT patients that were treated on average four days after symptoms onset only four (2.8%) were hospitalized, which was significantly less (p < 0.001) compared to the 58 hospitalized patients out of 377 (15.4%) (odds ratio 0.16, 95% CI 0.06–0.5) that were untreated [37]. Moreover, among 100 nursing home residents there was less mortality among those who received HCQ-AZIT on the first day of symptom onset (OR = 22. 6; p = 0.004) [38]. In a cohort of 46 patients in Wuhan that had SatO2 >93%, those treated with CQ and HCQ had a shorter clinical recovery time and viral RNA negativity [39]. In 57 patients with early treatment onset, 41% at the first day of symptoms, matched for age, sex and BMI in three groups, those who received HCQ-AZIT (p = 0.0002) or AZIT (p = 0.0149) recovered faster than those who did not use these drugs [40].

The early use of HCQ, within 5 days of diagnosis, was a protective factor associated with disease aggravation (95% CI: 0.040–0.575, p = 0.006). Clinical improvement by 20 days was significantly different between patients with HCQ used early and those with HCQ not used (p = 0.016, 95% CI: 1.052–1.647). The median time to clinical improvement was 6 days in the HCQ used early group, compared with 9 days in the without HCQ used group and 8 days in the with HCQ not used early group (p < 0.001). HCQ used early was associated with earlier PCR conversion in both throat swabs (HR = 1.558, p = 0.001) and stool swabs (HR = 1.400, p = 0.028) [41].

In a cohort of 28,759 Iranian patients with COVID-19, 7295 (25.37%) with mild symptoms consented to receive and use HCQ within the first 3–7 days of diagnosis. HCQ reduced the odds of hospitalization by 38%, because it was required in 7.17% and 11.1% of patients who received and did not receive HCQ, respectively. A total of 314 patients died of COVID-19 complications, 27 (0.37%) and 287 (1.34%) in those who receive and did not receive HCQ, respectively, indicating a 73% mortality risk reduction on logistic regression model. The effect of HCQ on the outcome measures was maintained after adjusting for confounding factors and comorbidities. This effect remained significant whether patients were diagnosed based on positive RT-PCR or otherwise [42].

Among the 1067 outpatients with 5 days of COVID-19 in the propensity matched cohort, three hundred and five (31.4%) patients with no outpatient exposure to HCQ were hospitalized and 21 (21.6%) of patients with exposure to HCQ were hospitalized (p = 0.045), and 47 (4%) patients with no outpatient exposure died compared to 2 (2%) patients with outpatient exposure to HCQ [43]. In a group of Brazilian COVID-19 patients with an average delay from the start of symptoms to ER visit of 4.6 days the use of HCQ had a significant protective effect of 55% (OR 95% 0.45 (0.25–0.80), p = 0.0065) for hospitalizations [44].

In Saudi Arabia there were 5541 study participants, almost 33% (n = 1817) received HCQ in addition to SC while 67.2% (n = 3724) received the SC only, with significant fewer hospital admissions in the HCQ group compared to the SC (171 (9.36%) vs. 617 (16.6%), p < 0.001). This corresponded to a relative risk reduction in hospital admission of 43%. The rate of ICU admissions and mortality rate were also lower in the HCQ compared to the SC (0.77 vs. 1.5 (p = 0.022), and 0.39 vs. 1.45 (p < 0.001), respectively) [45].

So, all the studies with HCQ since the first week of symptoms in COVID-19 patients, including this, demonstrate protection for hospitalization and/or CFR. And with other drugs also used in COVID-19 for second indication, because their action over SARS-CoV-2 with excellent selectivity index, as ivermectin [46], colchicine [47], fluvoxamine [48], they have nice results when patients received them since the first symptoms days.

4.2. Sex

Although 50.1% of our patients were female, none of them died **(Table 5)**. The sexual dimorphism in the evolution of COVID-19 may be hormonally based. Women produce higher levels of estrogens which is known to cause a more potent innate, cellular and humoral response, which is associated with a greater number of regulatory T cells and immunoglobulins [49]. Progesterone has higher levels in women and it is a Sigma R1/R2 active drug with antiviral action to SARS-CoV-2 [25]. Moreover, the immune cells of females exhibit a 10-fold higher expression of TLR [50]. Another factor could be the presence of two X chromosomes which confers a stronger innate and adaptive immune response to viral infections in women [51]. By contrast, males could have a higher susceptibility to SARS-CoV-2 as they express more RCT2 [52], which activity increases after ovariectomy and is reduced after orchiectomy [53], human bronchial epithelial cells treated with 17β-estradiol express lower levels of RCT2 mRNA [54], and the serine protease gene TMPRSS2, required for virus entry [55], increases after exposure to androgens [56].

4.3. Age

With HCQ/AZIT treatment, mortality in male patients did not exceed

1% in the group younger than 80 years of age, while 16.67% in those older than 80 years (Table 5). Multiple factors contribute to the well-established age difference. In the elderly there is mild chronic inflammation, in which ACE2 expression, as well as autophagy and mitophagy are altered. There is also excess production of reactive oxygen species and senescent adipocyte activity, immunosenescence, as well as vitamin D deficiency. These and many other known factors will compromise the inflammatory response associated with cytokine storm in patients with severe COVID-19 resulting in an increased mortality among the elderly [57].

4.4. Comorbidities

The four most prevalent comorbidities of COVID-19 are hypertension, diabetes, cardiovascular and respiratory diseases, all of which are closely associated with obesity which is a major factor in the severity of morbidity and mortality of COVID-19 [58]. In the present cohort, 17.3% were obese (**Table 2**). This may affect ACE2 receptor expression in adipocytes and virus entry [59]. Moreover, in diabetic patients there is chronic inflammation, and in the elderly, immunological senescence aggravates the evolution of the disease, increasing the vulnerability in patients that do not control their glycemia, which may in part explain why diabetes is the comorbidity with the highest case fatality number [60]. Although the presence of comorbidities confers a much higher mortality risk in COVID-19 [61], their presence in our patients treated with HCQ/AZT was not associated with the case-fatality rate(**Table 4**).

A limitation of the present investigation is the underreporting of information, mainly of comorbidities and symptoms. In order to prevent an increased mortality among healthcare workers, forty percent of healthcare workers in Peru were sent home from the beginning of the pandemic, either because they were elderly with/without risk factors or because they had medical comorbidities. In addition, many of the healthcare workers that continued became ill in the meantime, which could have affected the completeness of the information.

Another limitation of the present investigation is that the diagnosis was primarily based on clinical guidelines [25]. In China, suspected COVID-19 cases are considered on the basis of epidemiological and clinical manifestations [62]. In Perú, the definition of a "suspected COVID-19 case" encompass a group of symptoms classically associated with COVID-19 according to national guidelines, while the definition of a "probable case" includes additional epidemiological or imaging criteria. Specific tests to detect the presence of the SARS-CoV-2 antigens or RT-PCR assays are only for diagnostic confirmation [63]. Of note, most healthcare centers faced difficulties securing enough tests for their assigned population. Of the 1265 patients evaluated in this study, only 134 (10.6%) had a test performed that was positive in 38 cases (28.4%), and both groups shared similar characteristics.

This limitation is typical for many countries including Peru, because the pandemic quickly overwhelmed the local health care systems. Global COVID-19 studies show that the median time from the first symptom onset to hospital admission is 7 days, 5–8 days for dyspnea, 8–9 days for acute respiratory distress syndrome, 10 to 5 days for mechanical ventilation and admission to hospital, and 5 days for mechanical ventilation and admission to the intensive care unit [64]. Any delays in the initiation of treatment would increase the frequency of hospitalization and worsen the outcomes in a healthcare system that experiences severe shortages in all aspects of clinical care. Moreover, during the first week of illness the positivity of RT-PCR is not more than 71% [65] and that of the combined IgM/IgG test is even lower at 39.3% [66]. Thus, relying on the positivity of any one of these tests in order to make the diagnosis of COVID-19 is inadequate because the diagnosis is delayed or never made. Thus, the Peruvian government decided to address this limitation by regulating that the diagnosis should be done on a clinical suspicion basis and the tests were only to confirm the patient as a COVID-19 case [26]. Indeed, as shown in the present study the reliance on the clinical diagnosis was an important prerequisite to ensure not only the early treatment onset but also an increased therapeutic success.

The case-fatality rate with this treatment regimen was 0.6%, which was significantly lower than the national average. There is a large discrepancy between the case-fatality rate reported in this study and the rest of the country. Peruvian government put in its guidelines HCQ-AZT as one of the treatments for these patients [67]. At the same time, the World Health Organization stated no recommendation in favor of any specific treatment at inpatient settings to date, along Peruvian medical societies [68], physicians [69] and mass media [70]. This could have led to the underusage of the national COVID-19 guidelines which suggested diverse early treatment schemes. On another hand, the primary-care physicians at the Tahuantinsuyo Bajo Maternal and Child Center continued to treat patients with COVID-19 with HCQ-AZT, being the first primary care center in Lima that treated them early including patients outside its area of influence. This situation could explain the difference in the results between Tahuantinsuyo Bajo and the rest of the Peruvian territory.

In conclusion, our study showed that case-fatality rate in COVID-19 patients treated on an outpatient basis with HCQ/AZIT was associated with the number of days of illness when treatment was initiated.

CRediT authorship contribution statement

Roberto Alfonso Accinelli: Conceptualization, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Grisel Jesús Ynga-Meléndez:** Investigation, Resources, Data curation, Writing – original draft, Funding acquisition. **Juan Alonso León-Abarca:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Lidia Marianella López:** Methodology, Software, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Juan Carlos Madrid-Cisneros:** Investigation, Writing – original draft, Visualization. **Juan Diego Mendoza-Saldaña:** Investigation, Writing – original draft, Visualization.

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