Effect of bromhexine in hospitalized patients with COVID-19

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ABSTRACT

Background Bromhexine is a potent inhibitor of transmembrane serine protease 2 and appears to have an antiviral effect in controlling influenza and parainfluenza infection; however, its efficacy in COVID-19 is controversial.

Methods A group of hospitalized patients with confirmed COVID-19 pneumonia were randomized using 1:1 allocation to either standard treatment lopinavir/ritonavir and interferon beta-1a or bromhexine 8 mg four times a day in addition to standard therapy. The primary outcome was clinical improvement within 28 days, and the secondary outcome measures were time to hospital discharge, all-cause mortality, duration of mechanical ventilation, the temporal trend in 2019-nCoV reverse transcription-polymerase chain reaction positivity and the frequency of adverse drug events within 28 days from the start of medication.

Results A total of 111 patients were enrolled in this randomized clinical trial and data from 100 patients (48 patients in the treatment arm and 52 patients in the control arm) were analyzed. There was no significant difference in the primary outcome of this study, which was clinical improvement. There was no significant difference in the average time to hospital discharge between the two arms. There were also no differences observed in the mean intensive care unit stay, frequency of intermittent mandatory ventilation, duration of supplemental oxygenation or risk of death by day 28 noted between the two arms.

Conclusion Bromhexine is not an effective treatment for hospitalized patients with COVID-19. The potential prevention benefits of bromhexine in asymptomatic postexposure or with mild infection managed in the community remain to be determined.

Significance of this study

What is already known about this subject?

- The COVID-19 pandemic remains one of the major public health issues, despite preventive measures being implemented worldwide.
- Bromhexine is a potent inhibitor of transmembrane serine protease 2 and has an antiviral effect.
- One study has shown the clinical benefit of this inexpensive medicine in patients with COVID-19 pneumonia.

What are the new findings?

- Bromhexine is not an effective treatment in hospitalized patients with COVID-19.
- Presence of renal disease is the strongest predictor of mortality in hospitalized patients with COVID-19 in our multivariate analysis.
- Bromhexine as a transmembrane serine protease 2 inhibitor could not reduce duration of hospitalization.
- Bromhexine as a transmembrane serine protease 2 inhibitor could not reduce the need for mechanical ventilation compared to the control.

How might these results change the focus of research or clinical practice?

- The potential prevention benefits of bromhexine in asymptomatic postexposure patients or those with mild infection managed out of medical centers remain to be determined.

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INTRODUCTION

The COVID-19 pandemic remains one of the major public health issues, despite preventive measures such as wearing mask and social distancing, being implemented worldwide.[1] The search for finding the effective treatment to prevent or treat the viral infection is ongoing but, so far, has had limited success.

Bromhexine is an inexpensive and widely available medication with a low side-effect profile and has been used as mucolytic in different respiratory conditions since 1963.[2] Bromhexine is a potent inhibitor of transmembrane serine protease 2 (TMPRSS2) and seems to have an antiviral effect. It has been shown that the presence of TMPRSS2 is very essential for influenza virus infection and propagation. Bromhexine has been shown to be effective in controlling influenza infection by blocking the cleavage of the surface glycoprotein hemagglutinin of the influenza virus.[3 4]

Researchers have proposed that bromhexine may be an effective option to reduce primary transmission, viral load, dissemination and

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secondary replication of SARS-CoV-2.[5–8] COVID-19, like SARS-CoV, binds to human ACE 2 via its spike glycoprotein (S-protein) expressed on its envelope for entering the target cell. S protein is composed of one amino-terminal (S1) and one carboxy-terminal (S2). Cleavage at the S1–S2 junction by protease (TMPRSS2) is essential to prime the virus spikes and activate membrane fusion. It has also been proposed that bromhexine, by blocking non-endosomal pathways via serine protease 2 (TMPRSS2), theoretically blocks the priming of the spikes and virus entry into the host cell.[9 10]

In a small open-label trial, the clinical benefit of bromhexine administration in patients with SARS-CoV-2 pneumonia has been reported.[11] This study tested whether bromhexine hydrochloride was an effective medication to improve clinical outcomes and mortality in hospitalized patients with COVID-19.

Materials and methods

This clinical trial was designed as a randomized, single center, open-label study. From 156 patients who were screened, in Masih Daneshvari Hospital, a tertiary and referral center for COVID-19, 111 patients with a diagnosis of COVID-19 pneumonia were enrolled. The study began on May 6, 2020, and enrollment of patients was completed on June 20, 2020. Written informed consent from all the study subjects was obtained.

Patients were randomized at a 1:1 ratio to receive either oral bromhexine in addition to standard therapy or standard therapy alone. Subjects who were enrolled received a trial number. Every single trial number was randomized to either arm of the study through computer randomization. The study was randomized, controlled, and open-labeled, and the trial was monitored by the data monitoring committee. Trial recruitment stopped after the target study population had been reached and was closed when all of the patients had completed their follow-up visit.

Inclusion criteria

The inclusion criteria were as follows: hospital admission, 18 years old or greater at the time of signing the informed consent, chest imaging and clinical symptoms consistent with COVID-19 pneumonia, laboratory (reverse transcription polymerase chain reaction (RT-PCR)) confirmed infection with 2019-nCoV, willingness to participate in the study, and no concurrent participation in other clinical trials.

Exclusion criteria

The following exclusion criteria were used: pregnancy or lactation, severe liver disease (eg, aspartate aminotransferase (AST)>5 times upper limit), undergoing dialysis or transferred to another hospital within 72 hours and a history of allergy to bromhexine.

Standard arm

Patients received treatment based on the hospital COVID-19 treatment protocol and best practice guidelines in place at that time. (lopinavir/ritonavir) (Kaletra) 400/100 two times per day for 7 days or discharge from hospital and interferon (IFN) beta-1a (Rebif) 44 μg subcutaneous every other day for five doses in addition to supportive and symptomatic therapy.

Treatment arm

The treatment arm received oral bromhexine hydrochloride 8 mg four times a day for 2 weeks in addition of standard therapy.

Outcome measures

The primary outcome was clinical improvement within 28 days. Clinical improvement was defined as the time (in days) from initiation of the study treatment (active or placebo) until a decline of two categories on a clinical status scale occurred. The six-category ordinal scale of clinical status which ranged from hospital discharge to death and is itemized as follows: (1) hospital discharge or meeting discharge criteria (discharge criteria are defined as clinical recovery, ie, fever, respiratory rate, oxygen saturation returning to normal, and cough relief); (2) non-intensive care unit (ICU) hospitalization, not requiring supplemental oxygen; (3) non-ICU hospitalization, requiring supplemental oxygen (but not noninvasive ventilation/high-flow nasal cannula); (4) ICU/non-ICU hospitalization, requiring noninvasive ventilation/high-flow nasal cannula therapy; (5) ICU hospitalization, requiring invasive mechanical ventilation; and (6) death.

The criteria for ICU admission were worsening of respiratory distress assessed by the physician, hemodynamic instability requiring vasopressors, and oxygen desaturation of <85% that was not responsive to low-flow oxygen therapy.

Secondary outcome measures included time to hospital discharge, all-cause mortality, duration of mechanical ventilation, time to 2019-nCoV RT-PCR negativity and frequency of serious adverse drug events, within 28 days from the start of medication.

Statistical analysis

Data were analyzed using SAS V.9.4. The distribution of the demographic and clinical characteristics of the sample was summarized by treatment status. Number and percent were reported for binary outcomes. Means and SD were calculated for continuous outcomes such as time to hospital discharge. Associations between the treatment status and patient characteristics were tested for statistical significance using χ^2 or Fisher's exact test, as appropriate, for categorical variables, and two-sample t-tests for continuous variables. An alpha of 0.05 was used for all significance testing.

Study subjects were tested for COVID-19 using a polymerase chain reaction (PCR) test on days 1, 7, and 28. The prevalence of PCR test positivity was plotted by time. A longitudinal data analysis using generalized estimating equations was attempted. However, there was an error in the estimation routine when fitting the generalized estimating equations logistic regression model and the convergence was questionable. Standard errors could not be generated.

Kaplan-Meier curves were created for time to improvement. Wilcoxon tests (rather than log-rank tests) were performed to determine if the survival curves for the treatment groups differed from one another in the population. The assumption of proportional hazards was violated for multiple predictors for both outcomes, time to improvement and time to death. Given these violations, HRs from Cox (proportional hazards) regression models were not calculated. Instead, ORs for improvement and mortality were calculated from logistic regression models and reported with 95% CIs and p values.

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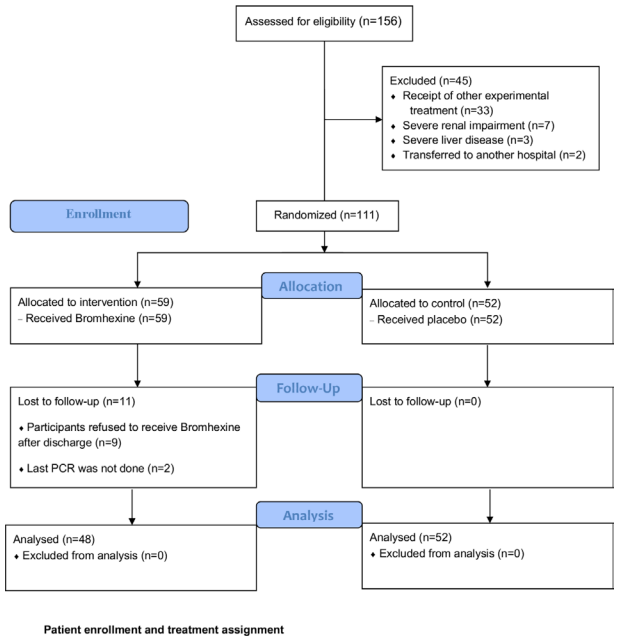

Figure 1 CONSORT flow diagram. Patient enrollment and treatment assignment.

Given the imbalanced distribution of several factors of clinical significance between the two study arms, ORs were adjusted for obesity (defined as a Body Mass Index (BMI) of ≥30 kg/m^2), smoking, and renal disease (defined as an estimated glomerular filtration rate between 16 and 60 mL/min). Sparse data bias was a possibility, given the small number of patients who did not improve and the small number of deaths. To minimize the risk of triggering sparse data bias, Firth's penalized maximum likelihood estimation was used when estimating the unadjusted and adjusted ORs for both death and improvement.[12]

RESULTS

A total of 156 patients with proven COVID-19 pneumonia were screened. Forty-five of them were excluded (33 patients were enrolled in another experimental trial, 7 were on hemodialysis, 3 had severe liver disease and 2 were transferred to another hospital). A total of 111 patients were enrolled in this randomized clinical trial. They were assigned to either the treatment with bromhexine group or the standard treatment group in a 1:1 ratio with 59 patients in the treatment arm and 52 patients in the standard/control arm. Eleven patients were lost to follow-up in the treatment arm. No attrition occurred in the control arm. Data from the total of 100 patients (48 patients in the treatment arm and 52 patients in the control arm) were analyzed (figure 1).

The distributions of most of the demographic and disease characteristics were similar in the treatment and standard groups (table 1).

The mean age±SD was 50.7±16.4 years among the treated arm and 53.1±15.2 in the standard arm. In terms of gender, the percentage of men in both the treatment and standard groups was approximately 46%. There was a significant difference (p<0.0001) in the mean BMI between the treatment group (26.2±1.8) and the standard treatment group (33.2±4.5). The distribution of other comorbidities such as asthma, hypertension, diabetes, chronic obstructive pulmonary disease, cancer and cerebrovascular accident were almost identical between the study arms.

Table 1 Demographic and clinical characteristics of the 100 study subjects*

Characteristics	Bromhexine (n=48)	Placebo (n=52)	P value
Age (years), mean (SD)	50.7 (16.4)	53.1 (15.2)	0.44
Male, n (%)	22 (45.8)	24 (46.2)	0.97
Married	40 (83.3)	46 (88.5)	0.46
BMI, mean (SD)	26.2 (1.8)	33.2 (4.5)	<0.0001
Obese (BMI≥30 kg/m^2), n (%)	0 (0.0)	47 (90.4)	<0.0001
Smoker, n (%)	4 (8.3)	9 (17.3)	0.18
Traveled, n (%)	7 (14.6)	10 (19.2)	0.54
Exposure to a COVID-19 case prior to infection, n (%)	8 (16.7)	16 (30.8)	0.10
Blood group, n (%)			0.91
A+	10 (20.8)	11 (21.2)	
A–	1 (2.1)	4 (7.7)	
B+	11 (22.9)	8 (15.4)	
B–	2 (4.2)	2 (3.9)	
O+	16 (33.3)	18 (34.6)	
O–	1 (2.1)	2 (3.9)	
AB+	4 (8.3)	3 (5.8)	
AB–	3 (6.3)	4 (7.7)	
Comorbidities, n (%)			
Asthma	3 (6.3)	3 (5.8)	1.0
Autoimmune disease	4 (8.3)	1 (1.9)	0.19
Cancer	3 (6.3)	3 (5.8)	1.0
Cerebrovascular accident	1 (2.1)	1 (1.9)	1.0
Chronic obstructive pulmonary disease	3 (6.3)	4 (7.7)	1.0
Coronary heart disease	6 (12.5)	3 (5.8)	0.31
Diabetes	16 (33.3)	17 (32.7)	0.95
Hypertension	20 (41.7)	19 (36.5)	0.60
Liver diagnosis	2 (4.2)	1 (1.9)	0.61
Renal diagnosis	4 (8.3)	1 (1.9)	0.19

*Data are reported as mean and SD for continuous variables and number (n) and percent for categorical variables.
BMI, Body Mass Index.

Primary clinical outcome

There was no significant difference in the primary outcome of this study, which was time to clinical improvement. The median time to improvement in the bromhexine arm was 7 days, while that in the control arm it was 6 days. The p value from the Wilcoxon test for equality of the survival curves in the population was 0.61 (figure 2).

The unadjusted OR for clinical improvement comparing patients in the bromhexine arm with those in the standard treatment arm was 0.92. After adjusting for obesity, smoking, and renal disease, this OR was 4.15 (95% CI 0.13 to 138.25, p=0.43) (table 2).

Patients with no renal disease had 25 times the odds of improving compared to patients with renal disease (1/0.04=25): adjusted OR=0.04, 95% CI 0.004 to 0.42, p=0.007.

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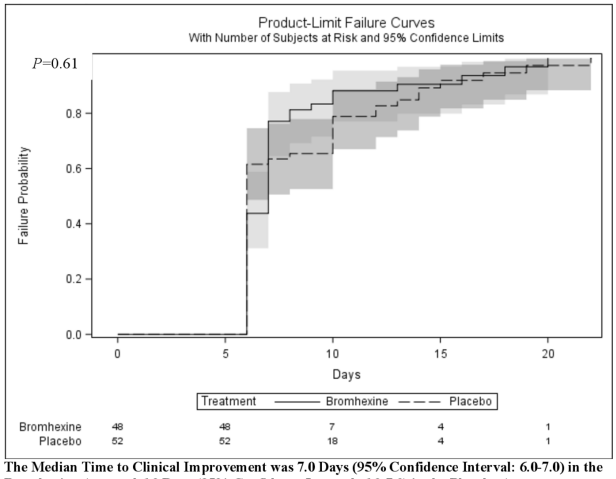

Figure 2 Kaplan-Meier estimates of cumulative clinical improvement. The median time to clinical improvement was 7.0 days (95% CI 6.0 to 7.0) in the bromhexine arm and 6.0 days (95% CI 6.0 to 7.0) in the placebo arm.

Unadjusted and adjusted ORs for death are listed in table 3.

The unadjusted OR for death comparing patients in the bromhexine group with those in the control arm was 1.09. After adjustment for obesity, smoking, and renal disease, the bromhexine OR was 0.24. Given this result, it appears, at first, that there was a 76% reduction in the odds of dying (bromhexine vs standard treatment); however, this result was not statistically significant: 95% CI:0.007 to 8.03, p=0.43. In a similar fashion, the obesity OR was also affected by strong joint confounding by the remaining three variables found in the multiple logistic regression model. The confounding was severe enough to reverse the direction of the association: unadjusted obesity, OR=1.13, and adjusted obesity, OR=0.48. Neither of the obesity ORs were statistically significant. In contrast, both the unadjusted and adjusted ORs for mortality for the presence of renal disease were above 1 and statistically significant: adjusted renal disease, OR=24.98, 95% CI 2.40 to 259.71, p=0.007.

Secondary clinical outcomes

There was no significant difference in the mean (average) time to hospital discharge between the two arms. There were also no differences observed in the mean ICU stay, frequency of intermittent mandatory ventilation, duration of supplemental oxygenation and risk of death by day 28 noted between the two arms. The prevalence of the use of high-flow nasal oxygenation in the bromhexine group was significantly higher than the prevalence in the standard arm (56.3% vs 23.1%, p=0.001) (table 4).

The temporal trend in the probability of being PCR positive was assessed. On days 1, 7, and 28, 100%, 60.4%, and 0%, respectively, of the patients in the bromhexine arm were PCR positive. Among patients in the standard arm, the prevalence of PCR positivity on days 1, 7, and 28 were 100%, 34.6%, and 0%, respectively.

Adverse events

No major adverse events were noted.

DISCUSSION

This open-label, randomized, single-center, controlled trial determined that bromhexine was not an effective treatment in hospitalized patients with COVID-19. Our data do not show a benefit of bromhexine in regard to clinical improvement, ICU admissions, the need for mechanical ventilation, or all-cause mortality.

The goal of randomization is to balance the distribution of known and unknown confounders between the two arms of the study, thereby reducing the possibility of confounding by these factors. At times, the desired balancing is not achieved with random allocation. In our study, the patients in the standard arm had a higher prevalence of obesity (90.4%) compared with those in the bromhexine arm (0.0%): p<0.0001.

It has been observed that obese patients have higher mortality in COVID-19 infection than non-obese patients.[13 14] Clinicians, no doubt, would like to know if the effect of bromhexine on the risk of mortality varies by obesity status. Is it possible that among obese patients, treatment with bromhexine has a different effect on mortality than in patients who are not obese? We are unable to answer this question via a stratified analysis, given that none of our bromhexine patients were obese. Future similar studies should be powered in such a fashion as to be able to assess if there is an interaction between bromhexine and obesity when mortality is the outcome.

Regarding the mortality, our data showed that the presence of renal disease was strongly correlated in a positive fashion with the outcome of death in both the unadjusted and adjusted analyses (table 3). The adjusted OR for clinical improvement comparing patients with renal disease to those without renal disease is 0.04. ORs possess the reciprocal

Table 2 Unadjusted and adjusted ORs* for clinical improvement in 100 patients with COVID-19: 96 improved vs 4 did not improve

	Unadjusted			Adjusted†		
Risk factor (sample size)	OR	95% CI	P value	OR	95% CI	P value
Bromhexine (n=48) vs standard treatment (n=52)	0.92	0.15 to 5.66	0.93	4.15	0.13 to 138.25	0.43
Obese‡ (n=47) vs not obese (n=53)	0.88	0.14 to 5.43	0.89	2.11	0.08 to 56.39	0.66
Renal disease (n=5) vs no renal disease (n=95)	0.04	0.004 to 0.33	0.003	0.04	0.004 to 0.42	0.007
Smoker (n=13) vs non-smoker (n=87)	1.46	0.07 to 31.67	0.81	0.99	0.05 to 18.14	0.99

*ORs were calculated from logistic regression models that used Firth's penalized maximum likelihood estimation.
†Each OR is adjusted for the remaining variables that are found in the table.
‡Defined as a Body Mass Index of ≥30 kg/m^2.

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Table 3 Unadjusted and adjusted ORs* for time to death in 100 patients with COVID-19: 4 died vs 96 survived

	Unadjusted			Adjusted†		
Risk factor (sample size)	OR	95% CI	P value	OR	95% CI	P value
Bromhexine (n=48) vs standard treatment (n=52)	1.09	0.18 to 6.67	0.93	0.24	0.007 to 8.03	0.43
Obese‡ (n=47) vs not obese (n=53)	1.13	0.18 to 6.96	0.89	0.48	0.02 to 12.71	0.66
Renal disease (n=5) vs no renal disease (n=95)	26.72	3.02 to 236.50	0.003	24.98	2.40 to 259.71	0.007
Smoker (n=13) vs non-smoker (n=87)	0.69	0.03 to 14.96	0.81	1.02	0.06 to 18.67	0.99

*ORs were calculated from logistic regression models that used Firth's penalized maximum likelihood estimation.
†Each OR is adjusted for the remaining variables that are found in the table.
‡Defined as a Body Mass Index of ≥30 kg/m^2.

property, and therefore the adjusted OR for clinical improvement comparing patients free of renal disease to those who have renal disease is 1/0.04 or 25. This adjusted OR of 25 is statistically significant (p=0.007) and indicates the presence of a very strong relationship between this risk factor and the outcome of clinical improvement. Similarly, the adjusted OR for mortality for the presence of renal disease is approximately 25 and statistically significant. Our finding is in agreement with other reports that indicated renal disease is a strong predictor of mortality in COVID-19 infection.[15 16]

It has also been reported that people with the blood group of O and Rh− have lower risk of COVID-19 infection.[17] None of the four decedents in our study were Rh− but three of them were O positive.

The most common use of bromhexine is as a mucolytic cough suppressant in respiratory diseases. Bromhexine has no Food and Drug Administration approval in the USA but has been used all over the world for more than 50 years.[2] This inexpensive medication that blocks (TMPRSS2) might interfere with the process of cell entry of the SARS-CoV-2.[10]

A small, single-center, open-label study showed that bromhexine is an effective medication that reduced the rate of ICU admission, need for mechanical ventilation and mortality in patients who suffer from SARS-CoV-2 pneumonia, and concluded that bromhexine treatment may result in a milder course of the disease.[11] Despite the many similarities between our study and theirs, such as being a single-center and open-label trial, there are some obvious differences. A higher number of patients were enrolled in our study (100 vs 78 patients) and our data did not support the effectiveness of bromhexine in hospitalized patients with COVID-19. One of the other differences between these two similar trials was the standard therapy regimen. Our standard treatment was the combination of lopinavir/ritonavir (Kaletra) and IFN beta-1a (Rebif), while in the other study, the majority were taking hydroxychloroquine (HCQ). In the RECOVERY trial, Kaletra was found to be ineffective in hospitalized patients with COVID-19.[18] Additionally, the results from the Solidarity Trial were disappointing and did not show any benefit of subcutaneous IFN beta-1 (Rebif) in the same population.[19] It is plausible that the combination of HCQ and bromhexine is the key to improvement, but studies need to evaluate and assess this hypothesis.[20] There is one ongoing ClinicalTrials.gov Identifier: NCT04355026 that may find the answer to this question.

Table 4 Outcomes of the 100 study subjects

Characteristic	Bromhexine (n=48)	Placebo (n=52)	P value
Time to hospital discharge, mean (SD)	9.1 (3.1)	9.2 (3.4)	0.82
ICU admission (days), mean (SD)	0.6 (2.1)	0.8 (2.0)	0.67
No ICU admission and no oxygen, n (%)	0 (0.0)	0 (0.0)	–
No ICU admission and yes oxygen, n (%)	43 (89.6)	47 (90.4)	1.0
High-flow nasal cannula oxygen, n (%)	27 (56.3)	12 (23.1)	0.001
Intermittent mandatory ventilation, n (%)	5 (10.4)	5 (9.6)	1.0
Duration of supplemental oxygen, mean (SD)	6.8 (2.2)	7.0 (3.3)	0.71
Deaths by day 28, n (%)	2 (4.2)	2 (3.9)	1.0

*Data are reported as mean and SD for continuous variables and number (n) and percent for categorical variables.
ICU, intensive care unit.

Our data also showed a higher proportion of patients needing high-flow oxygenation support. The interaction of ACE2 receptor and TMPRSS2 with the S-protein in SARS-CoV-2 was studied by Hörnich *et al*. They claimed that SARS-CoV-2 does not require TMPRSS2 on target cells for cell–cell fusion and suggested that bromhexine is ineffective in COVID-19 infection. They also moved one step further and claimed that bromhexine may even moderately enhance fusion. Although their paper has yet to be peer reviewed, they concluded that SARS-CoV-2 fusion with the host cell, as compared with the first SARS virus, depends more on the expression of the ACE2 receptor than protease activation.[21] Possible enhancement of fusion by bromhexine may explain why patients in our Bromhexine group were more than twice as likely to have received high-flow oxygen therapy than patients in our standard treatment arm.

The other counterintuitive finding in our study was the higher percentage of PCR test positivity on day 7 in the bromhexine arm (60.4%) compared with the standard arm (34.6%). While the prevalence of a positive PCR test was 75% higher in the bromhexine group than in the standard treatment group on day 7, we do not advise scrutinizing the results at a single point in time. A proper longitudinal data analysis would incorporate information from all of the time points. We attempted to conduct such an analysis using generalized estimating equations logistic regression; however, an error in the estimation routine was encountered while fitting this model. This error was most likely

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due to the fact that there was insufficient variation in the prevalence of test positivity. Measurement of the outcome at additional time points was most likely required.

Our trial had some limitations. The trial was a single-center, single-country investigation, and hence generalizability may be reduced. Additionally, our study was not blinded. One of the purposes of blinding the study investigators to the treatment allocation is to reduce the chance that the outcomes were assessed differently between the two study arms.[22] However, one of our endpoints was all-cause mortality, an objective rather than a subjective outcome. Finally, given the small number of patients who did not improve and the small number of patients who died, there was a strong possibility that our ORs would be affected by sparse data bias. To reduce the chance of sparse data bias, Firth's penalized likelihood method was used.[12]

CONCLUSION

Our findings indicate that bromhexine is not an effective treatment for hospitalized patients with COVID-19, and renal disease was the strongest predictor of mortality in our multivariate analysis. Our study did not address bromhexine's use as prophylaxis. The potential prevention benefits of bromhexine in asymptomatic postexposure patients or those with mild infection managed out of medical centers remain to be determined.

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