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Short Communication

Effect of Ammonium Chloride in addition to standard of care in outpatients and hospitalized COVID-19 patients: A randomized clinical trial

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Objectives: The COVID-19 pandemic has called an urgent need for drug repurposing to improve the outcome of the disease. Quaternary ammonium compounds have been demonstrated to have antiviral effects and may be of use against SARS-CoV-2 infections.

Design: In this double-blind, single-center study, we enrolled patients with positive PCR test and/or CT findings for COVID-19. The participants of each group were randomly assigned to Diphenhydramine Compound (Diphenhydramine + Ammonium Chloride) plus standard of care or to Diphenhydramine alone and standard of care groups. The primary outcome was all-cause mortality within 30 days of randomization. Secondary outcomes include viral burden, clinical status, assessed by a 5-point ordinal scale, and length of stay in hospitalized patients.

Results: A total of 120 patients were included in the trial, 60 of which were assigned to the Ammonium Chloride group. The primary endpoint was not statistically different between the two groups (HR: 3.02 (95% CI, 0.57–16.06; p = 0.195)). Recovery time and viral burden were significantly lower in the Ammonium Chloride group, corresponding to an odds ratios of 1.8 (95% CI, 1.15–2.83; p = 0.01) and 7.90 (95% CI, 1.62–14.17; p = 0.014), respectively.

Conclusion: The findings of this study advocate the careful addition of Ammonium Chloride to standard of care for COVID-19 patients.

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Introduction

Coronavirus disease 2019 (COVID-19) is an acute respiratory illness caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first identified in December 2019 (Helmy et al., 2020), and has resulted in the death of 1.3 million people (WHO, 2020). Based on preclinical studies, Quaternary ammonium compounds such as Ammonium Chloride have been reported to
have broad-spectrum antiviral activities and may hold therapeutic value against COVID-19 (Kargar Kheirabad and Nourozi, 2020; Baker et al., 2020).

Currently, there is no evidence on the safety or efficacy of the addition of Ammonium Chloride to standard treatment regimen in COVID-19. This trial was designed to examine whether adding the compound would improve the outcome of the infection in COVID-19 patients.

Materials and methods

This double-blind, placebo-controlled, single-center, randomized clinical trial was conducted in Imam Ali Hospital, Alborz province, Iran. Eligible participants were selected from a pool of adults aged between 18 and 70 years who were diagnosed with COVID-19 by positive RT-PCR test or with suspected history, symptoms (cough and dyspnea) and high-resolution computed tomography (HRCT) scan findings accompanied by active clinical disease. Patients with a history of coexisting respiratory diseases (asthma, chronic obstructive pulmonary disease, interstitial lung diseases, etc.) were excluded (Supplementary Figure 1).

The baseline data was acquired using paper-based questionnaires that included symptoms upon admission, history of illnesses, major coexisting illnesses, and demographic data.

Randomization and treatment

Using a four-unit permuted block randomization, the patients were assigned (1:1) to either Diphenhydramine and Ammonium Chloride (10 ml oral syrup three times a day for 7 days) plus standard of care or Diphenhydramine (10 ml oral syrup three times a day for 7 days) plus standard of care alone. For double blinding, the Medicine boxes were either labeled as A (Ammonium Chloride) or B (Control).

Procedures

At the time of the trial, Diphenhydramine was part of the standard care in outpatients and hospitalized COVID-19 patients to provide symptomatic alleviation of the disease in this hospital. Therefore, we opted to use already formulated Diphenhydramine compound (12.5 mg Diphenhydramine +125 mg Ammonium Chloride/5 ml syrup) for our intervention group and Diphenhydramine (12.5 mg Diphenhydramine/5 ml syrup) as placebo for control group. The standard of care in the hospital also included concomitant Atazanavir (300 mg PO bid in hospitalized patients) and Methylprednisolone (1 g/day for 3 days in patients admitted to ICU) based on hospital recommendations. Laboratory tests were obtained following admission into the hospital. HRCT-scan
imaging and RT-PCR tests were performed on the day of admission as baseline measurements and a second assessment was performed at day 7 after randomization to evaluate clinical improvements.

Outcomes

The primary outcome was all-cause mortality within 30 days after randomization for all patients. The secondary outcomes included hospitalization duration in discharged patients, clinical status at day 7 following randomization and viral burden. The scores on the ordinal clinical status scale are fully demonstrated in Table 1.

Statistical analysis

The study sample size for each treatment arm was calculated to be 60, in order for the trial to have 80% power at a two-sided P value of 0.05 to detect a clinically relevant reduction in mortality with an assumed mortality rate of 10.9% and 2% in intervention and control groups (Self et al., 2020).

The statistical plan and analysis of all outcomes and the effect of Ammonium Chloride on CT findings and ICU admission and the use of invasive mechanical ventilation as standalone endpoints have been described in Table 2.

Results

Between August 22 and October 22, 2020, 120 patients were included in the trial, with 60 patients receiving Ammonium Chloride. 45 patients in the Ammonium Chloride group and 44 patients in the control group comprised the hospitalized patients, respectively. Baseline characteristics of the participants are available in Supplementary Table 1.

Outcomes

The Kaplan–Meier curve for the time to death as primary endpoint is shown in Supplementary Figure 2, corresponding to a hazard ratio of 3.02 (95% confidence interval [CI], 0.57–16.06; p = 0.195). The between-group differences in mortality did not vary considerably according to baseline severity.

Time to discharge from hospital was significantly lower in the Ammonium Chloride group (Supplementary Figure 3) with an adjusted odds ratio of 1.8 (95% CI, 1.15–2.83; p = 0.01).

Patients receiving placebo were associated with higher clinical scores in the scale compared to those receiving Ammonium Chloride. Odds ratio for scoring higher values in the Ammonium Chloride group was 0.35 (95% CI, 0.17–0.7; p = 0.003) in the ordinal clinical scale.

The analysis of viral burden demonstrated a significant decrease in viral load for the Ammonium Chloride group compared to control group, corresponding to an odds ratio of 7.90 (95% CI, 1.62–14.17; p = 0.014).

Discussion

The findings of this trial suggest that the addition of Ammonium Chloride to standard of care was not superior to standard of care alone in reducing mortality rate in COVID-19 patients. Patients receiving Ammonium Chloride had shorter time to recovery and were more likely to have reduced viral load and clinical improvement.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical approval

The trial was approved by ethics committee at Alborz University of Medical Sciences (Approval No. 3762-6).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2021.04.043.

References

Baker N, Williams AJ, Tropaia A, Ekins S. Repurposing quaternary ammonium compounds as potential treatments for COVID-19. Pharm Res 2020;37(6):104.

Helmy YA, Fawzy M, Elaswad A, Sobieh A, Kenney SP, Shehata AA. The COVID-19 pandemic: a comprehensive review of taxonomy, genetics, epidemiology, diagnosis, treatment, and control. J Clin Med 2020;9(4):1225.

Kargar Kheirabad A, Nourozi M. Ammonium chloride as a potential candidate for the treatment and controlling of Covid-19. Iran J Virol 2020;14(1):42–4.

Self WH, Semler MW, Leither LM, Casey JD, Angus DC, Brower RG, et al. Effect of hydroxychloroquine on clinical status at 14 days in hospitalized patients with COVID-19: a randomized clinical trial. JAMA 2020;324(21):2163–76.

WHO. WHO COVID-19 weekly epidemiological update. 17 November 2020.