Ozone gas applied through nebulization as adjuvant treatment for lung respiratory diseases due to COVID-19 infections: a prospective randomized trial

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Abstract

The objective of this study was to provide lung disinfection by nebulizing ozone gas with distilled water and olive oil for patients who have clinical symptoms due to coronavirus disease 2019 (COVID-19). The study attempted to reduce the viral load of COVID-19 in the lungs of patients, to provide a faster response to medical treatment. Between August 2020 and September 2020, 30 patients who met the study criteria were prospectively evaluated. There were 2 groups with 15 patients in each group: patients in control group were not treated with ozone and only received standard COVID-19 treatment; patients in ozone group received lung disinfection technique with ozone and standard COVID-19 treatment. A statistically significant difference was found in the length of stay in hospital, change in C-reactive protein, polymerase chain reaction results after 5 days, and computed tomography scores between two groups. There was no statistically significant difference in D-dimer, urea, lactate dehydrogenase, lymphocyte, leukocyte, and platelet between two groups. According to the data, we think that the lung disinfection technique applied with ozone inhalation reduces the rate of pneumonia in COVID-19 patients and makes the patients respond faster to the treatment and become negative according to the polymerase chain reaction tests. The study was approved by the Ethical Committee of the Istanbul Medipol University Clinical Trials (approval No. 0011) on July 2, 2020.

Key words: coronavirus; COVID-19; lung disinfection; medical ozone gas; ozone gas; ozone inhalation; ozone therapy; pulmonary disease; viral pneumonia

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INTRODUCTION

Coronavirus are causing an epidemic that threatens global health, which has a very fast and serious respiratory contagion that causes severe acute respiratory syndrome in the lung.¹ Currently there is no definitive treatment for the new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The use of steroids in the stage of lung inflammation suggests that it reduces parenchymal involvement and destruction.² Hydroxychloroquine has been used as tumor necrosis factor and interleukin 6 suppressor since the beginning of the epidemic. However, there are still doubts in the literature about its use due to its serious side effects.³ Many antiviral agents such as remdesivir, lopinavir, and ritonavir are the drugs recommended for the treatment of coronavirus-induced diseases.

Ozone therapy has been applied in the treatment of coronavirus disease 2019 (COVID-19), generally using major auto-hemotherapy technique.⁴ These treatments have also been reported by the World Health Organization.⁵ When ozone is inhaled by living things at the concentration present in the atmosphere, it chemically reacts with many biological molecules in the respiratory system, causing a number of adverse pathologies in the lungs. It reacts with the surfactant in the lung and disrupts the lung epithelization. Thus, it causes serious lung parenchymal damage.⁶ This pathological finding usually occurs at high ozone concentration. It has antiviral and antibacterial properties without damaging the parenchyma at low ozone concentration. Especially this effect is used in most places for air disinfection today.

After contamination of COVID-19, infection causes severe morbidity and mortality in the patient with pulmonary phase and systemic hyper inflammation stages.⁷ In the pulmonary phase, both contagiousness and symptoms increase significantly. Lung disinfection technique with ozone inhalation in the pulmonary phase can provide a faster recovery in these patients.

In order to benefit from the therapeutic effect of ozone gas in an optimum way and to avoid potentially harmful effects on living things, daily ozone exposure determined by the U.S. Food and Drug Administration was taken as a basis. According to this, it has been noted that industrial workers’ ozone exposure of 0.10 parts per million (0.2 mg/m³) of 8 hours does not affect the current threshold limit value (American State Conference of Industrial Hygienists).⁸⁹ Ozone, distilled water and olive oil, with the help of a specially designed new nebulizer device, is sent to the lungs
by cold steam inhalation, isolation of the lung from the virus and providing comfortable breathing to support the recovery of patients in a short time. It can also be used as a prophylactic against all viral and bacterial pneumonias in the lungs. Especially in viral epidemic, we aim to reduce the viral load in the respiratory system, thus reducing the contagiousness of the coronavirus and accelerating the recovery of patients.

**Subjects and Methods**

**Ethical approval**

This prospective randomized study was approved by the Ethical Committee of the İstanbul Medipol University Clinical Trials (approval No. 0011) on July 2, 2020 and written informed consent was obtained from all the participants. This study follows the CONSolidated Standards Of Reporting Trials (CONSORT) statement (Additional file 1).

**Subjects**

Patients who were treated in Dr. Feriha Öz Emergency Hospital for coronavirus were examined prospectively between August and September 2020.

**Diagnostic criteria of COVID-19:** Common symptoms of COVID-19 are respiratory symptoms, fever, cough, dyspnea, headache, sore throat, runny nose, muscle and joint pain, extreme weakness, emerging loss of sense of smell and taste, and diarrhea. Polymerase chain reaction (PCR) test was routinely applied in patients with such symptoms.

**Inclusion criteria:** Patients with a positive PCR test regardless of sex between the ages of 18–80 years.

**Exclusion criteria:** Patients with glucose-6-phosphate dehydrogenase deficiency (favism allergy), previous lung cancer or suspected lung mass in lung tomography, and patients with previous surgery in their lungs were excluded from the study. The patients did not complete treatment.

Out of 72 patients, 30 patients who met the study criteria who followed the patients until the end of the study and examined their clinical and laboratory parameters.

Patients were divided into control (n = 15) and ozone (n = 15) groups. All the patients were treated with routine COVID-19 treatment (Table 1).

In our study, a device that has never been produced before, and that is not available in the market has been designed and the prototype of this device has been used. The device enables the nebulization of ozone gas with distilled water and olive oil, allowing it to be delivered directly to humans by cold steam inhalation (Figure 1A). The olive oil mechanism added to the device is designed to prevent the irritating effect of ozone and to transfer it to a wider surface area with distilled water, providing a soft and moist (Figure 1B), thus comfortable breathing, and applying it to patients without any side effects, inflammation or pain (Figure 1C).

Ozone treatment protocol: consists of three sessions applied for 10 minutes at intervals of 5 minutes daily for 5 days. In each session; 0.2 ppm ozone gas was given as cold vapor using lung disinfection technique inhalation device. In clinical practice, ozone gas is connected from the ozone generator equipped with an oxygen tube to a glass reservoir with olive oil inlet from the bottom and exit from the top 0.2 ppm ozone gas coming from the lower inlet passes through the olive oil and is connected to the mask via a silicone hose attached to the outlet at the top of the glass chamber. The mask is connected to the nebulizer drug reservoir to which 5 mL of distilled water has been added with its hose. In this way, cold water vapor and 0.2 ppm olive oil ozone gas are given directly to the patient in the mask. In this way, ozone gas at a concentration of 0.2 ppm/second is applied directly for one session for 10 minutes. The purpose of the study in this way is to ensure that the ozone gas obtained is spread to a wider area homogeneously in the lung and respiratory system with water vapor and to eliminate its irritating feature with the effect of olive oil. With this application, it is aimed to enable patients to take ozone gas at a dose of 0.2 ppm without any inflammation or inflammation in the lungs, to reduce their symptoms and to relieve the respiratory system.

| Drug name            | Daily dose, way of giving | Treatment time (d) |
|----------------------|--------------------------|-------------------|
| Hydroxychloroquine   | 2× 200 mg tablet, oral   | 5                 |
| Favipiravir          | Loading: 2× 1600 mg tablets, oral | 1               |
|                      | Maintenance: 2× 600 mg tablets, oral | 4               |

Note: COVID-19: Coronavirus disease 2019.
Measurement
Patients diagnosed with COVID-19, before starting the study and after the study was applied for 5 days; hemogram, C-reactive protein (CRP), D-dimer, urea, lactate dehydrogenase, PCR, chest X-ray or thoracic computed tomography (CT) tests were performed.

Thorax CT scans were made with a 64-channel CT (GE, Boston, MA, USA). Images were taken during a single breath-holding, with the hands raised and the hand in the supine position. Tube voltage is standard 100 kV, current 110 mA, pitch 1.375, section thickness 5 mm. CT scoring system has been developed to more clearly express the severity and prevalence of COVID-19 pneumonia. Opacities in each segment were scored according to how much of the segment was visually covered. Each segment involved was scored as 0, 1, and 2 (0 point if there is no opacity in the segment parenchyma, 1 point if the opacities cover 0–50% of the segment, and 2 points if the opacities cover more than 50%).

Statistical analysis
Number Cruncher Statistical System software (NCSS version 5.0, LLC, Kaysville, UT, USA) was used for statistical analysis. Descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, maximum) were used while evaluating the study data. The suitability of the quantitative data to normal distribution was tested by Kolmogorov-Smirnov, Shapiro-Wilk test and graphical evaluations. In the comparison of the normally distributed age variable between two groups, Student’s t-test was used. Mann-Whitney U test was used for comparing variables not showing normal distribution between two groups. Pearson chi-square test and Fisher Exact test were used to compare qualitative data. Wilcoxon Signed Ranks test was used for in-group comparisons before and after the application. Significance was assessed at least at the P < 0.05 level.

RESULTS
Inpatient clinic with 30 patients, 43% of them were female and 57% were male. The ages of the cases ranged from 21 to 80 years, with a mean of 51.56 ± 16.91 years. Chronic disease (e.g., hypertension, diabetes mellitus, peripheral arterial disease, rheumatological diseases, Chronic obstructive pulmonary disease) is seen in 43% of the cases. The length of stay varies between 12 and 25 days, with an average of 19.96 ± 2.74 days. There is no statistically significant difference in the rates of chronic disease in the cases according to the groups (P > 0.05). The length of stay of the ozone group cases was lower than the control group (P < 0.001; Table 2).

There was no statistically significant difference in the CRP measurements of the cases before and after the application according to the groups (P > 0.05). The change in post-application CRP measurements of the ozone group cases compared with the baseline was not statistically significant (P > 0.05). When the change in CRP measurements after treatment compared with the baseline was examined, a decrease was detected in 75% of the cases in the ozone group, which was statistically significantly higher than that (25%) in the control group (P < 0.05; Table 3). Before the application, PCR was found to be positive in all cases in both groups. While the rate of positive cases which turned negative was 93% in the ozone group, and 20% in the control group, and the rate of negativity was statistically significantly higher in the ozone group (P < 0.01; Table 3). A statistically significant difference was found between the groups in terms of CT severity scores (P < 0.05). Biochemistry results of the patients in the control group and ozone group before and after treatment are listed in Table 4.

DISCUSSION
As we all know, no drug or vaccine studies have been completed so far for the full treatment of the COVID-19 virus, a type of coronavirus that is extremely dangerous and deadly, causing a pandemic both in Turkey and in the world. According to the current data of the World Health Organization, more than

| Table 2: Evaluation of demographic characteristics |
|-----------------------------------------------|
|                               | Ozone (n=15) | Control (n=15) | P-value |
|-----------------------------------------------|
| Gender                                      |              |                |        |
| Female                                      | 13 (43)      | 6 (40)         | 0.713  |
| Male                                        | 17 (57)      | 9 (60)         |        |
| Chronic illness                             |              |                |        |
| Available                                   | 17 (57)      | 8 (53)         | 0.713  |
| Absent                                      | 13 (43)      | 7 (47)         |        |
| Length of stay (d)                          | 12–25 (20)   | 12–23 (19)     | 0.001  |
|                                            | 19.97±2.75   | 18.73±2.91     |        |
| Note: Data in age and length of stay are expressed as minimum-maximum (median), mean ± SD, and were analyzed by Student’s t-test. Data in gender and chronic illness are expressed as number (percentage), and were analyzed by Pearson Chi-square test. |

| Table 3: Evaluation of PCR and CRP results before and after ozone treatment in coronavirus disease 2019 (COVID-19) patients |
|---------------------------------------------------------|
| CRP (mg/dL)                                 | Ozone (n=15) | Control (n=15) | P-value |
|-----------------------------------------------|--------------|----------------|---------|
| Before treatment                              |              |                |         |
| Decreased                                    | 12 (75)      | 5 (33)         | 0.028   |
| Increased                                   | 3 (25)       | 10 (67)        |         |
| PCR                                          |              |                |         |
| Before treatment                              |              |                |         |
| Positive                                    | 15 (100)     | 15 (100)       | –       |
| Negative                                    | 14 (93)      | 3 (20)         | 0.001   |
| After treatment                              |              |                |         |
| Positive                                    | 1 (7)        | 12 (80)        |         |

Note: Data in CPR are expressed as minimum-maximum (median), mean ± SD, and were analyzed by Student’s t-test (intragroup) and Pearson Chi-square test (intragroup). Data in CRP exchange and PCR are expressed as number (percentage), and were analyzed by Mann-Whitney U-test. CRP: C-reactive protein; PCR: polymerase chain reaction.

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Bacterial and viral infections cause a decrease in body system resistance, severe illness, and damage to other vital organs in the body due to the side effects of the drugs used in the treatment process. It is possible to treat existing diseases more quickly and easily without any side effects, by killing even these deadly viruses and bacteria. Bacteria and viruses can only be killed by disinfection methods.

Remondino et al. used the maximum use dose of ozone for disinfection, released by the U.S. Food and Drug Administration in order to treat lung respiratory diseases (for 8 hours, 5 days a week, 0.1 ppm ambient air application). A clinical study was conducted on 40 randomly selected patients and the therapeutic effect was examined. Ozone is a highly reactive gas composed of three oxygen atoms. It is a compound that occurs naturally in the upper atmosphere and lower atmosphere of the Earth or can be produced man-made. Stratospheric ozone occurs naturally through the interaction of solar ultraviolet radiation with molecular oxygen. The “ozone layer” approximately 10 to 50 km above the Earth’s surface reduces the amount of harmful ultraviolet radiation reaching the Earth’s surface.

In addition, studies on ozone have shown that ozone has an antiviral, antibacterial and disinfectant effect depending on the concentration, environment and application method. Ozone therapy refers to the process of applying ozone gas to the body to treat a disease or wound. It can be used to treat medical conditions by stimulating the immune system. It has been stated in studies that it can also be used to disinfect and treat the disease. Medical ozone has been used to disinfect medical supplies and treat different conditions for more than 150 years. For example, ozone therapy can stop its spread if you have an infection in your body.

Ozone therapy shows its effect by destroying bacteria, viruses, yeast, fungi and unicellular animals. It also helps cleanse the infected cells. When the body escapes from these infected cells, it produces new and healthy cells. Ozone therapy can be used to treat the disease. Medical ozone has been used to disinfect medical supplies and treat different conditions for more than 150 years. For example, ozone therapy can stop its spread if you have an infection in your body.

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Despite the existence of anatomical differences between human, nonhuman primate and canine lungs and common experimental rodent lungs, the anatomical lesion of ozone inhalation occurs at the functionally equivalent site of the region between the conductive airway and the respiratory zone. The ciliary cells of the upper respiratory tract and the type 1 cells of the centriacinar region are most affected. Type 2 cell proliferation is the hallmark of ozone toxicity. A study with ozone has noted various biochemical and physiological changes in various animal species and humans. In a scientific study conducted in 2017, a drug intended to be used for ozone therapy may have a therapeutic effect on human diseases such as chronic obstructive pulmonary disease and cystic fibrosis (NCT00551811). Ozone therapy is currently being studied in patients with knee arthritis and other inflammatory diseases, but results are not yet available. Patients with back pain from herniated discs can also benefit from ozone therapy.

### Table 4: Biochemical variables before and after ozone treatment in patients with coronavirus disease 2019

|                      | Ozone (n=15) | Control (n=15) | P-value |
|----------------------|-------------|----------------|---------|
| **Lymphocyte (×10^3/mm³)** |             |                |         |
| Before treatment     | 1.53±0.65   | 1.23±0.6       | 0.152   |
| After treatment      | 1.6 (0.3-2.4) | 1.3 (0.4-3)   |         |
| Difference           | 0.04±0.53   | 0.11±0.5       | 0.494   |
| **Leukocyte (×10^3/mm³)** |             |                |         |
| Before treatment     | 5.11±1.19   | 5.26±1.83      | 0.590   |
| After treatment      | 5.5 (2.6-7.4)| 4.6 (2.9-8.7) |         |
| Difference           | 0.14±1.08   | 1.28±4.21      | 0.619   |
| **Urea (mM)**        |             |                |         |
| Before treatment     | 13.87±5.78  | 13.2±5.47      | 0.707   |
| After treatment      | 12 (5-27)   | 12 (7-31)      |         |
| Difference           | 0.13±3.72   | 2.07±5.44      | 0.416   |
| **D-dimer (mg/L)**   |             |                |         |
| Before treatment     | 0.47±0.22   | 0.92±0.78      | 0.056   |
| After treatment      | 0.5 (0.1-0.9)| 0.7 (0.2-3.4) |         |
| Difference           | 0.08±0.47   | -0.13±0.93     | 0.710   |
| **LDH (U/L)**        |             |                |         |
| Before treatment     | 237.6±71.93 | 254.43±90.57   | 0.616   |
| After treatment      | 222 (135-358)| 234 (163-466) |         |
| Difference           | -3.6±84.39  | 11.79±81.1     | 0.419   |
| **PLT (×10^3/mm³)**  |             |                |         |
| Before treatment     | 180.73±50.35| 199.53±71.65   | 0.455   |
| After treatment      | 169 (115-285)| 190 (116-400) |         |
| Difference           | 30.87±41.65 | 6.93±76.46     | 0.395   |

Note: Data are expressed as mean ± SD, minimum-maximum (median), and were analyzed by Mann-Whitney U test. LDH: Lactate dehydrogenase; PLT: platelet.
It is a well-known fact that ozone has toxic effects on lungs at high doses. However, inhalation of low doses of distilled water and olive oil into the lungs does not have a toxic effect. Low-dose ozone improves blood circulation and tissue delivery to ischemic as a result of the concerted effect of nitric oxide. To prevent cytokine storm, ozone also has potent anti-inflammatory properties through the modulation of the nucleotide-binding domain-like receptor protein 3 inflammasome which is recognized to play a crucial role in the initiation and continuance of inflammation in various diseases.

The limited number of cases in the study is due to the lack of power analysis before starting the study. It is difficult to make a safe power analysis because there are as yet no long-term studies on COVID-19. Therefore, we did not need to do power analysis.

According to the data we obtained in our study, we think that the lung disinfection technique applied with ozone inhalation can reduce the rate of pneumonia in COVID-19 patients and make the patients respond faster to the treatment and become negative according to the PCR tests.

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| Section/Topic         | Item No | Checklist item                                                                 | Reported on page No |
|----------------------|---------|---------------------------------------------------------------------------------|---------------------|
| Title and abstract   | 1a      | Identification as a randomised trial in the title                               | 1                   |
|                      | 1b      | Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts) | 1                   |
| Introduction         | 2a      | Scientific background and explanation of rationale                              | 2-4                 |
|                      | 2b      | Specific objectives or hypotheses                                                | 4                   |
| Methods              | 3a      | Description of trial design (such as parallel, factorial) including allocation ratio | 4                   |
|                      | 3b      | Important changes to methods after trial commencement (such as eligibility criteria), with reasons | 4                   |
| Participants         | 4a      | Eligibility criteria for participants                                           | 4-6                 |
|                      | 4b      | Settings and locations where the data were collected                            | 4                   |
| Interventions        | 5       | The interventions for each group with sufficient details to allow replication, including how and when they were actually administered | 6-7                 |
| Outcomes             | 6a      | Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed | 7-10                |
|                      | 6b      | Any changes to trial outcomes after the trial commenced, with reasons           | NA                  |
| Sample size          | 7a      | How sample size was determined                                                   | 11                  |
|                      | 7b      | When applicable, explanation of any interim analyses and stopping guidelines     | NA                  |
| Randomisation:       | 8a      | Method used to generate the random allocation sequence                           | 3                   |
|                      | 8b      | Type of randomisation; details of any restriction (such as blocking and block size) | 3                   |
|                      | 9       | Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned | 3                   |
|                      | 10      | Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions | 3                   |
| Blinding             | 11a     | If done, who was blinded after assignment to interventions (for example, participants, care providers, those | 3                   |
| Section | Item | Description |
|---------|------|-------------|
| Assessing outcomes | 11b | If relevant, description of the similarity of interventions |
| Statistical methods | 12a | Statistical methods used to compare groups for primary and secondary outcomes |
| | 12b | Methods for additional analyses, such as subgroup analyses and adjusted analyses |
| Results | 13a | For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome |
| | 13b | For each group, losses and exclusions after randomisation, together with reasons |
| | 14a | Dates defining the periods of recruitment and follow-up |
| | 14b | Why the trial ended or was stopped |
| | 15 | A table showing baseline demographic and clinical characteristics for each group |
| | 16 | For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups |
| | 17a | For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval) |
| | 17b | For binary outcomes, presentation of both absolute and relative effect sizes is recommended |
| Ancillary analyses | 18 | Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory |
| Harms | 19 | All important harms or unintended effects in each group (for specific guidance see CONSORT for harms) |
| Discussion | 20 | Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses |
| | 21 | Generalisability (external validity, applicability) of the trial findings |
| | 22 | Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence |
| Other information | 23 | Registration number and name of trial registry |
| | 24 | Where the full trial protocol can be accessed, if available |
| | 25 | Sources of funding and other support (such as supply of drugs), role of funders |

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.