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ORIGINAL RESEARCH

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Effects of hypertonic alkaline nasal irrigation on COVID-19

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Abstract

Objective: The causative agent of COVID-19 is a novel member of coronaviridaes, SARS-CoV-2. It has been reported that the spike (S) protein of SARS-CoV-2 is responsible of infectivity. The S protein is demonstrated to be inactivated under environmental condition, such as hypertonicity and alkaline pH. The aim of the study was to investigate the effect of hypertonic alkaline nasal irrigation (HANI) on SARS-CoV-2.

Methods: Sixty patients divided into two groups. The patients in Group 1 used hydroxychloroquine (HCQ), and the patients in Group 2 used HCQ and HANI. Nasopharyngeal samples were collected at the beginning, on 3rd and 7th day of the PCR test positivity. The nasopharyngeal viral load (NVL) changes analyzed with quantitative PCR.

Results: NVL decrease in weekly period was statistically significant for both groups, when the difference between NVL day 0 and 3rd in Group 1 and NVL difference between day 0 and 3rd in Group 2 were compared. The difference between Groups 1 and 2 in terms of NVL change was statistically significant ($P < 0.05$).

Conclusion: We demonstrated a significant decrease in nasopharyngeal SARS-CoV-2 load with HANI solution and suggest that HANI may be promising modality for the COVID-19 treatment.

Level of evidence: IB

KEYWORDS
alkaline hypertonic solutions, COVID-19, nasal lavage, nasal sprays, quantitative PCR

1 | INTRODUCTION

COVID-19, emerged in China at December 2019 and affected the entire world, was declared as a pandemic by the World Health Organization (WHO).[1] As of May 20, 2021, 165 664 116 cases and

This manuscript is original and it, or any part of it, has not been previously published; nor is it under consideration for publication elsewhere.

3 434 058 deaths related to COVID-19 were reported.[2] The causative agent of COVID-19 is a member of the family *Coronaviridae*, named SARS-CoV-2. SARS-CoV-2 is a pleomorphic, enveloped, single-stranded RNA virus.[3,4] SARS-CoV-2, like SARS-CoV, has four structural proteins: Spike (S), nucleocapsid (N), membrane "matrix" (M), and envelope (E) proteins.[5-7]

COVID-19 is transmitted by inhalation and/or to contact of infected droplets to the mucosa.[8,9] Dominant infection site of SARS-CoV-2 in the respiratory tract is nose.[10,11] SARS-CoV-2 is primarily replicated in nasal cells, which supports the hypothesis that viral replication develops in the proximal airways and these infective particles are transported to the distal via micro-aspirates.[10] S protein binds to the angiotensin-converting enzyme-2 (ACE-2) receptor and initiates intracellular entry. Binding of the S protein with ACE-2 receptor provides proteolytic cleavage and exposes the fusion protein, which is thought to be responsible for the onset of viral infectivity.[12,13]

The S protein is affected by the environment changes such as pH and tonicity. In vitro pH level changes affect SARS-CoV-2, the structural proteins of the virus changes conformationally, and its infectivity.[8] Another study demonstrated the inactivation of SARS-CoV-2, in vitro, under hypertonic conditions.[14]

Nasal irrigation (NI) is a routine part of the treatment of rhinosinusitis and allergic rhinitis and nasal postoperative care. Sprays used for NI provide removal of debris, mucus, and purulent secretion and regulate mucociliary clearance (MCC).[15,16] Hypertonic alkaline-buffered fluids also been improving MCC.[17]

In light of this information, the aim of this study was to investigate the possible positive in vivo effects of hypertonic alkaline nasal irrigation (HANI) prepared based on pure Lake Van water, the Earth's largest soda lake in Turkey, with high salt (23 g/L) and pH (about 10) levels,[18] on nasopharyngeal SARS-CoV-2 viral load (NVL).

2 | MATERIAL AND METHODS

This study is a clinic-randomized controlled trial. It was approved by the Istanbul University-Cerrahpasa Ethics Committee (83045809-604.01.02).

2.1 | Populations, inclusion, and exclusion criteria

This study was conducted on patients who applied to Cerrahpasa Medical Faculty Hospital Between July 2020 and September 2020.

Patients, who applied to the hospital on the day the symptoms started, were diagnosed with COVID-19 and with no pulmonary involvement in thorax computer tomography (CT) imaging or limited lung involvement in CT (with the involvement of less than 25% of the lungs quantitatively in thorax CT imaging, without symptoms such as dyspnea, tachypnea, and with sO2 > 95% without oxygen support) were included.

Patients under 18 years of age, pregnant and lactating patients, patients with additional neurological or psychiatric diseases, patients with known cancer who have received or are receiving chemotherapy, radiotherapy or chemoradiotherapy, patients with chronic hepatitis and/or chronic kidney disease, patients allergic to hydroxychloroquine (HCQ) and its derivatives, or with allergic reactions during treatment, and patients who had intolerable side effects due to HCQ treatment were excluded from the study.

According to these criteria, 60 patients whose oro-nasopharyngeal swab qualitative PCR tests were positive for COVID-19 included. Informed consent forms were obtained from patients.

2.2 | Randomization and blinding

The participants were randomized in two equal groups via a computer program. The clinic and laboratory staff are blind to randomization status. The laboratory analysis was done blindly. The study outcomes were evaluated blindly.

2.3 | Sample size and sampling technique

In our study, we planned to use parametric tests to obtain more statistically significant results. At least 30 patients were planned to be included in each group, since the minimum sample size for parametric tests was 30.[19] Sixty patients were separated in two groups as a control group (Group1) and a study group (Group 2). In addition, a posthoc test analysis was planned to calculate the sampling power of valid equations.

2.4 | Procedures and data collection

The patients in Group 1 were given only COVID-19 treatment and in Group 2 were given a HANI solution (Na^{+}: 7747 mg/L, K^{+}: 508 mg/L, Mg^{2+}: 94.8 mg/L, Ca^{2+}: 5-10 mg/L, CL^{-}:5450 mg/L, CO_3^{-2}: 3331 mg/L, SO_4^{-2}: 2344 mg/L, HCO_3^{-1}: 2191 mg/L, PO_4^{-3}: 0.52 mg/L), in addition to COVID-19 treatment. COVID-19 treatment was standardized for all patients and was containing 400 mg (200 mg twice a day) HCQ (Plaquenil, Sanofi, France), which was the protocol of the Turkish Ministry of Health at the time of the study. The HANI spray was prescribed as one puff through each nostril four times a day. The patients were informed about how to use the spray. At the end of the study, patients were questioned about possible side effects of HANI.

2.5 | Collection of swab samples and viral RNA isolation

Nasopharyngeal swab samples were collected from patients whose RT-qPCR test resulted positive for COVID-19, on the first day of diagnosis (day 0), the 3rd and 7th days. RT-qPCR kit (Bio Speedy, Turkey) targeting RNA-dependent RNA polymerase (RdRp) gene was used for the qualitative detection of SARS-CoV-2 (for initial diagnosis). Six hours after NI, the swab samples were taken first from the

oropharynx and then from the nasopharynx with the same swab stick by an otorhinolaryngologist at the clinic and initially stored at −20°C. Then, the samples were transferred to the and viral RNAs were isolated by using a RTA Viral RNA Isolation Kit (RTA, Turkey) in the first 24 hours and the samples were stored −80°C.

2.6 | Real-time PCR

The PCR master mixture was prepared using a SentiFAST Probe No-ROX One Step Kit (Bio Line, USA) and primer-probe mix targeting N1 and N2 gene regions selected from WHO and USA-CDC sources. The PCR was performed on Rotor-Gene Q System (Qiagene, USA) where the PCR program was as follows: Initial step, one cycle at 45°C for 600 seconds, one cycle at 95°C for 120 seconds, 45 cycles at 95°C for 10 seconds, and 55°C for 30 seconds. TaqMan probes are labeled at the 5′-end with the reporter molecule 6-carboxyfluorescein (FAM). Copies/μL values of the standards prepared by serial dilution of the synthetic gene with a certain number of copies (200 000 /μL) are shown in Figure 1.

The synthetic gene (GenBank: NC_045512.2) copy/μL value was calculated with the formula [(amount × 6.022 × 1023)/(length × 1 × 109 × 650)]. Samples with known copies/μL values obtained by serial dilution from the synthetic gene of 200 000 copies/μL were included in the study. In the PCR study, standards were run in sample conditions and the values were recorded in the software of the device. The formulation created by the device according to the recorded standard values was applied on the samples and the copy/μL values were obtained.

2.7 | Statistical analysis

Normal distribution of data was analyzed with the Kolmogorov-Smirnov test and Levene's tests were used to assess homogeneity. The Mann-Whitney *U* test and the Friedman test were used (for continuous variables) and Chi-square test (for categorical variables) were used to comparing the groups. The statistically significant level was accepted as a *P* value < 0.05.

The SPSS 21.0 was used for statistical analysis Normal distribution of data were analyzed with the Kolmogorov-Smirnov test and Levene's tests were used to assess homogeneity. The comparisons of two independent groups were made using the Mann-Whitney *U* test. The dependent group analyzes were performed using the Friedman test. The subgroup analyzes were performed using the Wilcoxon analysis and interpreted with the Bonferroni correction, statistical significance level of alpha was accepted as $P_{adj} < 0.017$. The rates in independent groups were compared using the Chi-square test. The significance level of alpha was accepted as $P < 0.05$ for other tests. The calculation of the sample power was performed by using the G * Power software version 3.1.[20]

3 | RESULTS

The demographic data of patients are shown in Table 1. Three patients in Group 1 were hospitalized (10%) due to development of respiratory distress, dyspnea, tachypnea, and a decrease in blood oxygen saturation in room air (sO_2 < 95%) (Table 1).

Patients' blood samples that collected on day 0 were analyzed. In Group 1, the mean values of WBC, NEU, Lypm, PLT, AST, ALT, CK, CRP, d-dimer, fibrinogen, and ferritin were 5450 × 10^3/μL, 2900 × 10^3/μL, 1800 × 10^3/μL, 227 × 10^3/μL, 18 IU/L, 15.5 IU/L, 76.3 mg/dL, 183.5 mg/dL, 0.295 mg/L, 377 mg/dL, and 125 ng/mL, respectively. In Group 2, the mean values of WBC, NEU, Lymp, PLT, AST, ALT, CK, CRP, d-dimer, fibrinogen, and ferritin were 5300 × 10^3/μL, 3200 × 10^3/μL, 1600 × 10^3/μL, 228 × 10^3/μL, 21 IU/L, 18 IU/L, 80, 4 mg/dL, 184 mg/L, 372 mg/dL, and 119 ng/mL, respectively. There was no statistically significant difference between the two groups in terms of the initial biochemical values ($P > 0.05$).

The symptom distribution of the patients in the groups on day 0 was evaluated, it was determined that 26 patients (86.7%) in Group 1 had at least one symptom, whereas 25 (83.3%) patients in Group 2 had at least one symptom; and there was no statistically significant difference in initial symptoms between the groups ($P < 0.05$). The most common symptom in both groups was fatigue, and it was detected in 21 (70%) patients in Group 1, and

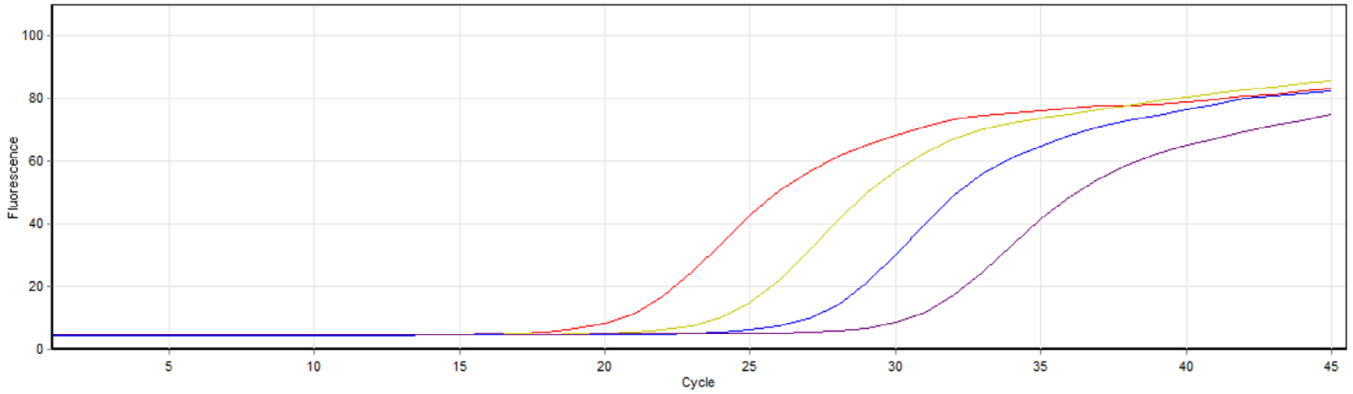

FIGURE 1 Results of standard curves by real-time PCR: *red line*: Standard 1:20 000 copy/μL; *yellow line*: Standard 2: 2000 copy/μL; *blue line*: Standard 3: 200 copy/μL; *purple line*: Standard 4: 20 copy/μL

TABLE 1 Demographic data of patients in Group 1 and Group 2

		Group 1 n (%)	Group 2 n (%)	P*
Sex	Male	18 (60.0%)	18 (60.0%)	1.000
	Female	12 (40.0%)	12 (40.0%)	
	Age mean ± SD (min-max)	34.3 ± 8.7 (19-54)	36.2 ± 12.0 (20-74)	0.762
	BMI mean ± SD (min-max)	26.9 ± 5.0 (18.8-42.2)	27.0 ± 4.5 (18-39)	0.842
Smoking		8 (26.7%)	5 (16.7%)	0.347
Chronic diseases		6 (20.0%)	5 (16.7%)	0.739
In-patient treatment		3 (10.0%)	0 (0.0%)	0.237

Abbreviation: BMI, body mass index.
*Chi-square test.

TABLE 2 Examination of symptoms

	Group 1	Group 2	P
Fatigue	21 (70.0%)	16 (53.3%)	0.184
Miyalgia	17 (56.7%)	14 (46.7%)	0.438
Fever	10 (33.3%)	4 (13.3%)	0.067
Cough	6 (20.0%)	8 (26.7%)	0.542
Sputum	1 (3.3%)	1 (3.3%)	1.000
Headache	17 (63.3%)	14 (36.7%)	0.438
Farenx_Ache	9 (30.0%)	7 (23.3%)	0.559
Diarrhea	4 (13.3%)	4 (13.3%)	1.000
Nausea	4 (13.3%)	2 (6.7%)	0.671
Vomiting	0 (0.0%)	0 (0.0%)	—
Runny nose	6 (20.0%)	4 (13.3%)	0.488
Nasal congestion	6 (20.0%)	7 (23.3%)	0.754
Anorexia	8 (26.7%)	5 (16.7%)	0.347
Lack taste	11 (36.7%)	5 (16.7%)	0.080
Anosmia	12 (40.0%)	5 (16.7%)	**0**.045*

*Chi-square test $P < 0.05$.

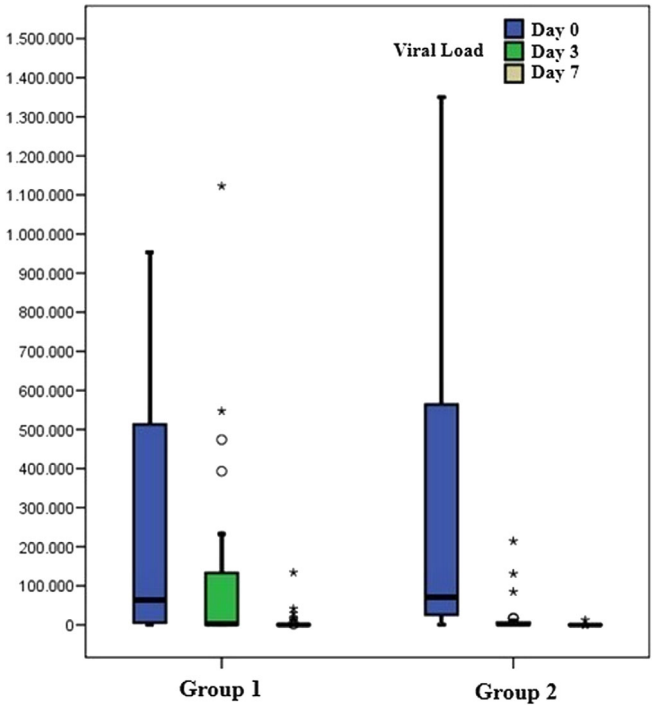

FIGURE 2 Viral load changes in groups

16 (53.3%) patients in Group 2. The rate of self-perceived smell disorder in the patients in Group 1 was significantly more common than the patients in group ($P = 0.039$, $P = 0.045$, respectively) (Table 2).

There were not any adverse events reported in regards to using the nasal spray such as epistaxis, smell loss, and burning. There was no statistically significant difference in the evaluations of the patients in the groups according to the initial (0th), 3rd day, and 7th day virus density ($P = 0.243$; $P = 0.165$; $P = 0.067$, respectively). During the study period, the decreases in the NVL observed in the groups were statistically significant ($P < 0.001$). In Group 1, the decrease in NVL in the first 3 days was not statistically significant ($P_{adj} = 0.027$). The first 3-day NVL decrease in Group 2 patients was found to be statistically significantly higher than Group 1 ($P_{adj} = 0.019$). There was no statistically significant difference in the groups according to the 7-day NVL decrease ($P_{adj} = 0.152$). No significant difference was found in other statistical evaluations (Figure 2) (Tables 3 and 4).

Three patients (10%) in Group 1 had to be hospitalized due to deterioration in their condition. The mean NVL of outpatients (27 patients) of Group 1 on day 0, 3rd, and 7th were 76 400, 2686, and 50.02/μL while the mean NVL of inpatients (3 patients) of Group 1 on day 0, 3rd, and 7th were 50 100, 111 000, and 23/μL according to this, there was no statistically significant difference. However, the increase of NVL on third day was thought to be valuable.

The posthoc test, which was used to determine the sampling power of the present study, was performed with a 5% error probability according to the sample size used. The sample power ($1 - \beta$ err prob) was 0.8.

	Group 1 Median (IQR)	Group 2 Median (IQR)	P
Viral load density			
Day 0	63 250 (4375-570 250)	70 850 (23562-632 250)	0.243
Day 3	3058 (181.8-145 750)	1747 (196.5-6060)	0.165
Day 7	39.51 (8.9-1094)	15 (1-44)	0.067
P_{adj}	<0.001*	<0.001*	
Viral load differences			
Day 0 vs Day 3	8112.65 (569.375-384 500)	62 880.5 (22 961.25-629 262.5)	0.019**
Day 0 vs Day 7	42 580.35 (1368.25-565 650)	70 820.5 (23 524.03-632 230.6)	0.152

TABLE 3 The viral load density on Days 0, 3, 7, and viral load differences between Day 0–3 and Day 0–7 in Group 1 and Group 2

*P_{adj}: The Friedman *t* test $P < 0.017$. **P: The Mann-Whitney *U* test $P < 0.05$.

TABLE 4 Subgroup analyses of the change in virus loads within groups

	Group 1 P	Group 2 P
Day 0 vs Day 3	0.027	<0.001*
Day 0 vs Day 7	<0.001*	<0.001*
Day 3 vs Day 7	<0.001*	<0.001*

*The Wilcoxon test (the Bonferroni correction $P < 0.017$).

4 | DISCUSSION

COVID-19 has affected the whole world in the past year. In the medical field, the most effort in the past year has been invested in the treatment and prevention of this disease that affects the health care system in an unprecedented way. In this study, we showed that the use of HANI reduces NVL in the early period of the disease, as the first in the literature.

In COVID-19, most patients exhibit mild clinical symptoms. Mild symptoms are influenza-like fever, cough, fatigue, muscle pain, and taste, and smell disorders. Progressive pneumonia and/or ARDS can be seen in severe cases.[21,22] In our study, the most common symptom in both groups was fatigue, similar to the literature.

The time between the onset of COVID-19 symptoms has been reported to be between 2 and 14 days (average of 5.2 days) after viral exposure.[8] There are two hypotheses regarding the spread of SARS-CoV-2 infection: Inhalation of infected droplets into the lung[23,24] or the presence of a viral inoculum in the nose, which is the first site of infection, and the aspiration of micro-aspirates from the naso-oropharynx into the lungs.[11,25] In viral cultures taken 24, 48, and 96 hours after SARS-CoV-2 infection, higher viral replication rate was detected in the samples taken from nose than the viral load obtained in the epithelium of the large airways. These findings suggested that the primary infection site is the nasal cavity.[10] S protein is the part that binds to ACE-2 receptor and initiates intracellular entry.[12,13] It was also determined that the distribution of ACE-2 expression in the respiratory tract was diverse and that it was most intensively found in the nasal region. This suggests that the nasal region is more frequently affected in the early stages of COVID-19. The nasal cavity is exposed to environmental factors in high and variable doses and develops varying degrees of innate immunity. This situation is thought to be responsible for different clinical responses to COVID-19.[10,26] Any procedure in the nose that relieves infection and viral load will prevent the disease from progressing and spreading to the lungs and the whole body.

NI provides mechanical cleaning and helps to remove contaminants such as mucus, cell debris, pathogens, allergens, and particles. While increasing MCC, it reduces the contact of particles present in the inhaled air with the mucosa.[15,16] Ramalingam et al reported that the need for drug use, domestic transmission, and the spread of the viruses, that infect the upper respiratory tract, were reduced with NI and mouth washing performed with hypertonic solution.[27] The viral replication of SARS-CoV-2 was inhibited with 210 mM NaCl by 90% and completely inhibited with 260 mM NaCl solutions, in vitro.[14] High salt concentration in the extracellular space creates a hyperosmotic medium. It has been suggested that this will inhibit the entry of the SARS-CoV-2 into cell.[13]Previous studies reported that the SARS-CoV-2 is affected by pH changes and hypertonic medium in vitro.[14,28] It has been reported that the S protein of the SARS-CoV exhibits cell fusion at neutral pH and was affected by the medium, such as pH and tonicity, and undergo conformational changes.[28]

We prepared a solution to achieve the effects of both hypertonic and alkaline solutions reported previously in the literature. The HANI solution used in the study was prepared by based on the water of lake Van. It contains bicarbonate, sulfate, sodium, chloride, potassium, and its pH is 9.3. The HANI solution creates a hyperosmolar medium in the nasal region. In our hypothesis, the interaction of the S protein with ACE-2 will be impaired, as a result of the conformational changes, due to a hypertonic and alkaline environment and the unbinding virus is mechanically removed from the nasopharynx due to NI and MCC which increases with NI.

In previous clinical studies, it was reported that COVID-19 disease had a more severe course in men.[29] In order to show the possible positive effect on patients who are likely to have a more severe clinical course, the study groups were formed with a predominance of male patients. In our study, in the first 3 days, the decrease in NVL in the Group 2 was statistically significant ($P < 0.001$) but not in the Group 1 ($P_{adj} > 0.017$). The first 3-day NVL decrease in Group 2 patients was found to be statistically significantly higher than Group 1 ($P < 0.05$). It means that the use of HANI may help in the early viral

response. COVID-19 is a biphasic disease, with a viral response phase in the early period and an inflammatory phase in the later period.[30] There was a statistically significant decrease in NVL in both groups on seventh day. It is noteworthy that this NVL decrease in Group 2 was almost twice as much as that in Group 1. These findings suggest that HANI may have a contribution to viral clearance in upper airways. However, there was no significant difference between in groups regarding NVL decrease between 3rd-7th and 0th-7th days. Previous studies showed that the significant NVL decrease in COVID-19 patients was observed within the first 4 days after the onset of symptoms.[31] Accordingly, the absence of a significant decrease in NVL in the present study, except in the early period, may be due to late inflammatory response.

Although the relationship between the NVL and the severity of the disease is not clear, it suggests that higher exposure to NVL and a slower decrease in the NVL may be related to the severe clinical symptoms of the disease. During the study, three patients in Group 1 had symptoms that required hospitalization. When the NVL of these patients were compared with the NVL of other patients in the same group, it was found that there was an increase in the NVL of the inpatients on third day. Although there was no statistically significant difference, this increase in the NVL strengthens the hypothesis that a relationship may exist between the NVL and the course of the disease. Detection of NVL decreases in the early period in the patient group who received HANI and no hospitalization in the HANI group shows promise for the control of COVID-19.

The present study has some limitations along with its first and unique features in the literature. At the time of this study, HCQ and its effects on SARS-CoV-2 were still in the trial phase, and it was the first drug of choice at the beginning of the pandemic. With the increase in the number of randomized controlled studies, the results of larger patient groups have been published. Subsequent studies have reported that HCQ has no effect in terms of viral clearance, death, or clinical deterioration.[32-35] With the increase of data in the literature, the use of HCQ in COVID-19 has deserted. Due to the fact that present study was the first in the literature, the abandonment of HCQ use during our study, in order not to change homogeneity of groups, and the possibility of transmission during outpatient follow-ups during the COVID-19 period, a limited number of individuals were in this study. The number of patients limited our study, especially the inability to evaluate the clinical effect of NI. However, with the post hoc analysis, the number of participants in our study was found to be statistically sufficient. The fact that HCQ does not affect viral load suggested that NVL decreased significantly in the first week without any effect. However, since HCQ was used in the control group, a synergistic relationship between HANI and HCQ cannot be excluded. Although we predicted this possibility at the beginning of the study, we used HCQ in the control group patients in accordance with the COVID-19 guideline on the grounds that leaving COVID-19 patients without treatment would not be ethically and medically appropriate. In addition, we used hypertonic alkali solution only in spray form in our study. Spray use may also be effective in reducing NVL by increasing MCC. Another limitation is the difference in the frequency of, self-perceived, smell disorder between the groups. Smell disorder, with a higher rate in the control group, is a good prognosis criteria in COVID-19 disease.[36] Therefore, the results we obtained in the group with a worse prognosis are more clinically significant. In addition, since the patients we included in the study were in home isolation, we did not have objective information about their compatibility with drug use. However, there are studies in the literature showing that patients adapt better to the use of their medicines in the period of COVID-19.[37] Therefore, we think patients were followed our instructions. In our study, we used NVL values to determine treatment efficacy and determined these values with RT-qPCR test, similar to other studies in the literature.[38,39] However, the most important limitation of our methodology is that it is not certain whether the values determined by the RT-qPCR test belong to live viruses.[40]

5 | CONCLUSION

A significant decrease in NVL due to HANI and the presence of patients who has severe disease in the group who did not perform NI suggest that HANI may be effective in the treatment of COVID-19. The fact that it reduces NVL significantly in the early stage has the potential to prevent the spread of the disease by reducing intracommunity transmission. The repetition of data that can be obtained with larger numbers is required to support the present findings.

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CONFLICT OF INTEREST

Each of the authors has contributed to, read and approved this manuscript. None of the authors has any conflict of interest.

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