European Review for Medical and Pharmacological Sciences 2022; 26: (2 Suppl): 112-123

Efficacy and safety of a hypertonic seawater nasal irrigation solution containing algal and herbal natural ingredients in patients with COVID-19

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Abstract. – OBJECTIVE: The objective of this study was to evaluate the efficacy and safety of using a hypertonic seawater nasal irrigation solution comprising natural ingredients (*HSS-Plus*) with the aim of reducing viral load and ameliorating nasal symptoms in cases of COVID-19.

PATIENTS AND METHODS: This single-center, prospective, single-arm, low-intervention study evaluated daily use of *HSS-Plus* in patients admitted to the Sotiria Hospital, Athens, Greece for a period of up to 10 days or until hospital discharge. Viral load measurements in nasopharyngeal swabs were performed on days 0 (baseline), 3 and 6, and on the final day of participation (day 10 ± 2; Hospital discharge). In addition, study participants were asked to rate the severity of nasal and other symptoms using Visual Analog Scales (VAS) at the same time points. At the final day, the patients also assessed the perceived use benefit of *HSS-Plus*.

RESULTS: 47 patients were enrolled in the study; 93.6% had a decrease in viral load of at least > 0.5 $\log_{10}$ on day 10 ($p<0.001$). Compared to values before nasal irrigation, viral load in nasopharyngeal swabs increased immediately after nasal lavage on days 3 ($p=0.037$) and 6 ($p=0.010$), indicating efficient removal of viral particles from the nasal cavity. Mean VAS symptoms' total score was reduced from 27.57 ± 15.63 at baseline to 6.73 ± 6.59 after 10 days ($p<0.001$). Similar reductions were also evident for individual symptoms at all time points ($p<0.005$). No adverse events were reported in the study.

CONCLUSIONS: *HSS-Plus* nasal irrigation is an effective and safe method for reducing viral load and providing symptom relief in patients with COVID-19.

Key Words:
COVID-19, Saline Nasal Spray, Algal extracts, Herbal ingredients, Nasal viral load.

Introduction

In the midst of a pandemic due to severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2), and in the absence of a vaccine and other prophylactic measures or therapeutic tests, the use of effective alternative practices to limit the spread and reduce morbidity is highly needed. Given the increased presence of SARS-CoV-2 in the nasal cavity and nasopharynx[1], numerous studies have proposed using nasal irrigation against SARS-CoV-2 as an effective method to flush the portals of entry of virus into the respiratory system[1,2]. This hypothesis was initially driven by results obtained in the ELVIS clinical study by Ramalingam et al[3] who have shown that nasal lavage and gargling with hypertonic solutions reduced viral shedding and symptom intensity in patients with upper respiratory tract infections including, among others, individuals infected with Coronavirus COV-229E and other Coronaviruses strains[3]. An overall reduction in the interval of morbidity from coronavirus by two and a half days was subsequently calculated by the same authors in a meta-analysis study[4] conducted based on the data of the same patient pool.

Confirming the above results, analysis of nasal swabs from COVID-19 patients who performed nasal irrigation with hypertonic solutions with or without 1% surfactant showed that patients who performed nasal irrigations had 7-9 days reduction in nasal congestion and headache symptom duration compared with patients who did not perform nasal washes. In addition, between-group differences were recorded in cough and fatigue symptoms[5]. Nasal irrigations practiced a few times daily wash off SARS-CoV-2 by reducing

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nasopharyngeal viral load[6-8]. This was also reported in hospitalized subjects receiving hypertonic nasal irrigations thrice within six hours[9]. Interestingly, when COVID-19 patients practiced nasal irrigation with hypertonic solutions supplemented with steroids, hyposmia and anosmia were improved and the duration to symptom resolution was shortened[10]. Similar results regarding improved sino-nasal symptom relief, resolution of hyposmia, hypogeusia or cacosmia due to COVID-19, restored normal sensory function, and reduced disease duration were recorded in patients receiving nasal lavages with saline solutions[11].

Based on these observations, nasal rinsing as a measure of protection of healthcare personnel and the general public, and to control virus spread in infants, children, and adults with COVID-19, is advocated by the Turkish Scientific Committee's COVID-19 guidelines[12] as well as by the French Association of Pediatric Otorhinolaryngology (AFOP) with the French Society of Otorhinolaryngology (SFORL), and the French ENT scientific societies[13,14].

Recent data have shown that SARS-CoV-2 initiates infection of the human upper respiratory tract cells through interaction with surface polysaccharides (proteoglycans) of heparan sulphate (HS)[15]. Based on these results, it was suggested that sulphated polysaccharides structurally similar to HS could act as molecular decoys and mechanistically interfere with SARS-CoV-2 binding, ultimately reducing virus infectivity[16]. Indeed, fucoidans, a family of sulphated polysaccharides derived from marine algae and bacteria which are similar in structure to HS, proved to be effective in *in vitro* assays[15-18]. Results from other groups have shown that *Undaria pinnatifida* and *Fucus vesiculosus* derived fucoidans reduce SARS-CoV-2 infection at very low concentrations[18]. It was also shown that fucoidan could deactivate viral particles prior to contamination[19]. Finally, sulphated polysaccharides derived from other algae species such as spirulina, inhibited SARS-CoV-2 entry and infection[20] indicating that algal ingredients could also be used in the fight against COVID-19.

A hypertonic nasal irrigation solution comprising sulphated polysaccharides from *Undaria pinnatifida* and *Spirulina platensis* algae as well as other herbal ingredients (*HSS-Plus*) was recently tested in patients with ENT disorders, including COVID-19 patients. Enhanced nasal cleansing and improved symptom management were reported by patients practicing nasal irrigations with this product. Product users were also satisfied with the efficacy and safety profile observed[21]. In this exploratory study, we sought to further test the properties of *HSS-Plus* in hospitalized COVID-19 patients in Greece and report the obtained clinical results.

In this study, we sought to determine the ability of a hypertonic seawater solution containing algal and herbal natural ingredients to decrease nasopharyngeal viral load and ameliorate rhinological symptoms in hospitalized patients with-SARS-CoV-2 infection at a COVID-19 referral hospital.

Materials and Methods

Study Design

The study was a prospective, low-intervention, single arm observational study aiming to collect real-world data on the efficacy and safety of nasal irrigation in patients with respiratory infection due to the SARS-CoV-2 coronavirus. The study was conducted from June 2021 to March 2022 at Sotiria Hospital (reference hospital for COVID-19) in Athens, Greece. It is conformed with the requirements of the Declaration of Helsinki. Ethical approval was granted by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) and informed consent was obtained in accordance with the local legislation applicable.

Patient Population

The study enrolled 47 adult male and female patients admitted to the hospital with a positive diagnosis of COVID-19 confirmed by a laboratory test result and symptoms indicative of respiratory tract infection. All patients were required to perform nasal irrigations and consent to periodical nasopharyngeal swabs. Patients below 18 years, participating in a different study or using other nasal washes or nasal medication were excluded from the study.

Nasal Irrigation

All enrolled patients received standard medication prescribed for their condition with no exception. In addition, patients received nasal irrigation with a hypertonic (2.3% NaCl) seawater solution containing brown algae (*Undaria pinnatifida*) and blue-green algae (*Spirulina platensis*) as well as essential oils of *Eucalyptus globulus* and *Mentha*

spicata, and *Thymus vulgaris* extract (*HSS-Plus*: Sinomarin® Plus Algae Cold &Flu Relief, Gerolymatos International SA, Krioneri, Greece). The product is a medical device employing continuous flow diffusion and was administered to patients under health personnel supervision in agreement with its instructions of use. Patients received irrigation for a total of 10 days or until hospital discharge.

Study Assessments

Table I presents the study workflow. The following demographic data were collected at baseline: age, sex, body mass and smoking status (number of pack-years). The presence of comorbidities, such as hypertension, diabetes mellitus, chronic obstructive pulmonary disease (COPD) or cardiac disorders, and drug history, if appropriate, were noted. Finally, SARS-CoV-2 vaccination status was also recorded. Patients were considered "fully vaccinated" if they had received two doses of the Moderna or Pfizer vaccines or one dose of the Johnson and Johnson vaccine. Assessments took place on baseline (day 0) and on days 3, 6 and 10 (±2) or until hospital discharge.

Nasopharyngeal swabs were collected at baseline (day 0; baseline titer prior to nasal irrigation), on days 3 and 6, prior to (following at least 8 hours after the last nasal irrigation; steady state) and following nasal irrigations. The samples taken on day 10 or on patient's exit from the hospital (day 10 ± 2) were the final ones used for the analysis and then patients were discharged from the study.

On days 0, 3, 6 and 10 (±2) patients filled a VAS questionnaire.

If a patient missed an assessment, their participation in the study was not affected and their data was taken into account in the final evaluation of the clinical data.

Virological Assessments

Following nasopharyngeal swabbing, collected samples were stored in sterile solution and transported in cooler boxes to the Laboratory of Histology-Embryology located within the Department of Medicine, School of Health Sciences of the University of Athens (a government approved laboratory for SARS-CoV-2 PCR testing). SARS-CoV-2 viral load was measured using quantitative real-time polymerase chain reaction (qPCR). The results of these molecular analyses were entered into the patients' case report forms used in the study.

Table I. Study workflow.

Variable	Day 0 (Baseline)	Day 3	Day 6	Day 10 (± 2)
Signed Consent Form	X			
Eligibility criteria met	X			
Age at admission, sex, weight	X			
Medical History/Co-morbidities	X			
Smoking history	X			
Concomitant treatment	X	X	X	X
Initial SARS-CoV-2 viral load result[1]	X			
Result of viral load assessment (Before irrigation)[2]		X	X	
Result of viral load assessment (After irrigation)[3]		X	X	
Final SARS-CoV-2 viral load result[4]				X
VAS Questionnaire	X	X	X	X
Adverse events	X	X	X	X

[1]The initial sample to perform a PCR viral load assessment test on the day of inclusion in the study (Day 0) was collected after the consent form was signed and before any irrigation occurred.
[2]The sample to perform a viral load assessment test by PCR prior to irrigation was collected before any irrigation occurred, with a preferred collection time of between 8-10 am.
[3]The sample to perform a viral load assessment test by PCR post irrigation was collected immediately after nasal irrigation provided the product had been used in accordance with the manufacturer's instructions for use.
[4]The final sample for performing PCR viral load assessment testing on Day 10 was collected just before trial completion.

VAS Assessments

Participants were evaluated for new clinical symptoms, worsening or improvement of existing symptoms using validated psychometric 10 cm Visual Analogue Scale (VAS) with markings of 0 = light and 10 = intense severity of rhinitis-related symptoms such as nasal congestion, rhinorrhea, cough, sore throat, change in olfaction and in taste perception, fatigue, emotional state, and shivering. Overall, VAS questionnaires are easy to apply, do not require administration by specialized personnel, and provide a reliable tool for assessing disease severity[22,23].

Study Objectives

The primary objective of the study was to define the percentage of patients with a reduction in viral load of at least >0.5 $\log_{10}$ at the end of the study. Secondary objectives were to define the percentage of patients with a reduction in viral load of at least >0.5 $\log_{10}$ during the study and the difference in viral load before and after nasal rinses on day 3 and day 6. Additional secondary objectives were the assessment of symptom scores using VAS on days 0, 3, 6 and 10 and the frequency of any adverse effects and their severity.

Statistical Analysis

Sample size calculation was performed using the ICC Sample. Size package library[24] within the R-CRAN software (R Core Team 2020, Vienna, Austria)[25]. The expected percentage of patient responders at 10 days of treatment with *HSS-Plus* was estimated to be approximately equal to 90% while the corresponding percentage expected in the absence of *HSS-Plus* treatment was considered to be 65%. Response was defined as a reduction in viral load of at least >0.5 $\log_{10}$. Therefore, to compare the response rate of the study patients against historical data at a significance level of α=0.05 and power =90%, the minimum sample size calculated to reject the null hypothesis at 10 days was at least 21 patients using binomial distribution control. However, to increase statistical power, 25 individuals were originally planned for inclusion in the study taking into account a 15% drop-out rate. This population was finally expanded to 47 patients due to high availability of patients at the clinical center that expressed interest to participate in the study. The occurrence of any side effects was recorded each day for the duration of the study. Even where a particular data point was missing due to failure to obtain relevant samples, the participant involved remained in the study and such data as were available were incorporated into the overall analysis of results.

For the primary endpoint analysis, the percentage of patients with a viral load reduction of at least >0.5 $\log_{10}$ at the end of the study was assessed at the full analysis set (FAS) using a binomial test with the following statistical hypothesis: H0: $p=p0$, H1: $p\neq p0$, where $p0=0.65$. The test was two-sided and at the level of statistical significance α=0.05. The corresponding 95% confidence interval of the response rate was given. Logistic regression analysis was performed with the response to treatment on day 10 as the dependent variable, and age, smoking, gender, and co-morbidities (hypertension, diabetes, COPD, cardiac disease if present at >25% frequency) as the independent variables.

For the secondary endpoint analysis, the percentage of patients with a reduction in viral load of at least >0.5 $\log_{10}$ on day 3 and day 6 of the study was assessed using 95% confidence intervals in the FAS. The absolute difference between viral load measurements at the time point prior to each nasal irrigation was statistically analyzed using repeated-measures mixed models with the change in viral load from day 0 as the dependent variable at each measurement (logarithmic to base 10). In this model, the number of visits, age, smoking, gender, as well as coexisting diseases (hypertension, diabetes, COPD, cardiac disease if present at >25% frequency) were the fixed factors. The patients were fitted at random distribution. The change between each visit was given by the least squares estimate of the mean followed by the corresponding confidence interval (CI). The change in VAS scores from day 0 to each study day was assessed with repeated measures mixed models with dependent variable the viral load change (log scale) on days 3, 6 and 10 and independent variables in order to assess the course of symptoms over time in the FAS. The change in viral load on days 3 and 6 of treatment relative to pre- and post-nasal irrigation values was assessed by paired *t*-test on log-ranked values. Data are indicated as mean values ± Standard Deviation (SD). A *p*-value not higher than 0.05 was considered statistically significant.

Results

There were 47 patients enrolled in the study, of which 38 (80.8%) completed the entire study. The mean age of the participants was 50.1 ± 13.7 years old. There were 36 males (76.6%) and 11 females

Table II. Summary of demographics and baseline characteristics at day 0.

	Total N=47		Total N=47
Age (years)		***Pack-years***	
n	47	n	18
Mean	50.06±13.68	Mean	35.44±126.17
Gender. n (%)		***Vaccination. n (%)***	
Male	36 (76.60%)	Yes	16 (34.04%)
Female	11 (23.40%)	No	31 (65.96%)
Weight (kg)		***Hypertension. n (%)***	
n	47	Yes. n (%)	11 (23.40%)
Mean	84.60±14.65	No. n (%)	36 (76.60%)
Smoking. n (%)		***Diabetes. n (%)***	
Yes	23 (48.94%)	Yes. n (%)	6 (12.77%)
No	24 (51.06%)	No. n (%)	41 (87.23%)
Years of smoking		***COPD. n (%)***	
n	10	Yes. n (%)	2 (4.26%)
Mean	22.10±8.60	No. n (%)	45 (95.74%)
Number of cigarettes per day		***Cardiac disease. n (%)***	
n	10	Yes. n (%)	2 (4.26%)
Mean	30.35±16.33	No. n (%)	45 (95.74%)
		Other comorbidities. n (%)	
		Yes. n (%)	23 (48.94%)
		No. n (%)	24 (51.06%)

SD = Standard Deviation;
COPD = Chronic Obstructive Pulmonary Disease.

(23.4%). Sixteen participants were fully vaccinated against COVID-19 (34.0%). Patient demographic details and their co-morbidities (if present) as recorded upon enrollment are provided in Table II.

The total viral load (log_{10}/mL) of SARS-CoV-2 from day 0 to day 10 in the total population and in vaccinated and un-vaccinated sub-groups is shown in Table III. Table IV shows the corresponding changes in viral load *per* study day. Overall, the viral load (log_{10}) decreased by 1.397 ± 0.255 (p<0.001) on day 3, 2.145 ± 0.259 (p<0.001) on day 6 and finally 2.591 ± 0.276 (p<0.001) on

Table III. Total viral load (log_{10}/mL) per day of study. Viral load (log scale) = log_{10} (Viral Load+1). Day 0: values on samples collected at study enrollment. Days 3 and 6: values on samples collected before irrigation. Day 10: values on samples collected just before trial completion.

	Viral load log_{10}/mL			
	Day 0	Day 3	Day 6	Day 10
n	44	41	40	31
Mean	4.84	3.87	3.29	2.29
SD	1.77	1.57	1.60	1.42
Vaccinated				
n	16	13	15	11
Mean	4.90	3.70	2.87	1.63
SD	1.89	1.81	1.71	1.26
Unvaccinated				
n	28	28	25	20
Mean	4.81	3.94	3.54	2.65
SD	1.74	1.48	1.51	1.40

Table IV. Change in SARS-CoV-2 viral load (log_{10}) per study day in hospitalized patients with COVID-19 performing nasal irrigation with *HSS-Plus*.Repeated-measures mixed model with dependent variable viral load change (log scale) at days 3, 6 and 10 and independent variable days of study and vaccination status.

Study Day	Reduction of viral load (log_{10})	SD	95% CI		*p*-value
Day 3					
Total population	-1.397	0.255	-1.89	-0.89	<0.001
Vaccinated	-1.72	0.409	-2.52	-0.92	<0.001
Unvaccinated	-1.21	0.310	-1.81	-0.60	<0.001
Day 6					
Total population	-2.145	0.259	-2.65	-1.64	<0.001
Vaccinated	-2.84	0.399	-3.63	-2.06	<0.001
Unvaccinated	-1.72	0.321	-2.35	-1.09	<0.001
Day 10					
Total population	-2.591	0.276	-3.13	-2.05	<0.001
Vaccinated	-3.25	0.440	-4.12	-2.39	<0.001
Unvaccinated	-2.21	0.337	-2.86	-1.55	<0.001

SD=Standard Deviation; CI=Confidence interval.

day 10 for the total population. Similar drops in viral load were recorded for the vaccinated sub-group on day 3 (p<0.001) and on day 6 (p<0.001) as well as for the un-vaccinated sub-group on day 3 (p<0.001) and on day 6 (p<0.001) (Table IV, Figure 1). Compared to values before nasal irrigation, the measured viral load collected in naso-pharyngeal swabs immediately after nasal lavage increased on day 3 log_{10} = -0.368, CI%: (-0.712 -) (-2.331), p=0.037 and on day 6 log_{10} = -0.538, CI: (-0.941 -) (-0.135), p=0.010 in the total population, indicating efficient removal of viral particles from the nasal cavity. Similar change trends were observed in the two sub-groups; however, the differences were not statistically significant (Table V).

Overall, 94% of patients had a decrease in viral load of at least > 0.5 log_{10} on day 10 of the study (CI: 79% - 99%, p<0.001). The corresponding percentage was 100% in vaccinated patients (CI: 72% - 100%, p=0.011) versus 90% in un-vaccinated patients (CI: 68% - 99%, p=0.018). The proportion of patient responders at 10 days of treatment with *HSS-Plus* was not significantly different on day 3 (p=0.100) and day 6 (p=0.407) (Table VI).

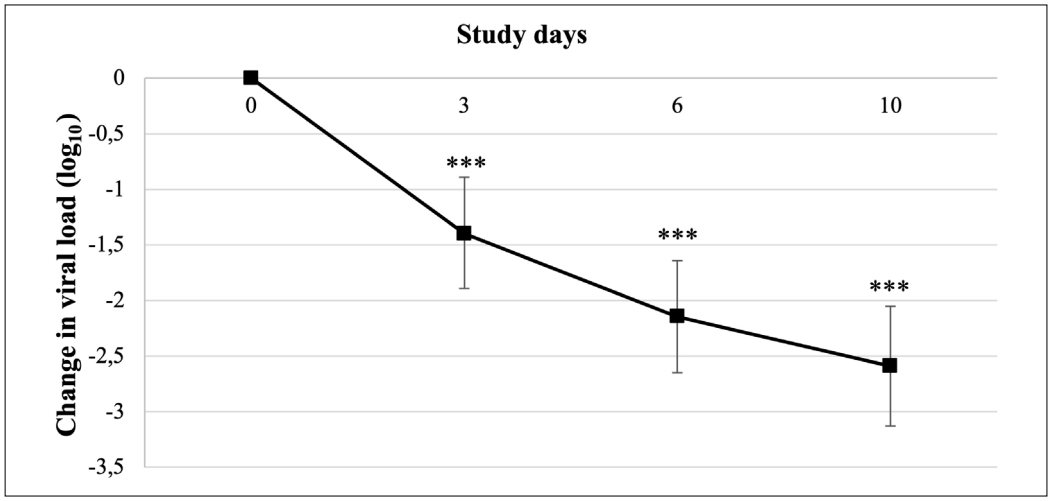

Figure 1. Change in SARS-CoV-2 viral load per study day in hospitalized patients with COVID-19 performing nasal irrigation with *HSS-Plus*. Repeated-measures mixed model with change in viral load (log_{10}) at day 3, 6, and 10 as dependent variable, and study days and vaccination status as independent variables. ***p < 0.001

Table V. Change in SARS-CoV-2 viral load before and after nasal irrigation with *HSS-Plus* at day 3 and day 6. Change in viral load = log (viral load) before - log (viral load) after nasal irrigation.

	Change in viral load (log_{10})	SD	95% CI		*p*-value
Day 3					
Total population	-0.368	0.170	-0.712	-2.331	0.037
Vaccinated	-0.365	0.311	-1.042	0.312	0.263
Unvaccinated	-0.369	0.208	-0.795	0.057	0.087
Day 6					
Total population	-0.538	0.199	-0.941	-0.135	0.010
Vaccinated	-0.719	0.363	-1,497	0.059	0.067
Unvaccinated	-0.430	0.236	-0.918	0.058	0.081

In patients performing nasal irrigation with *HSS-Plus* the severity of symptoms, i.e., nasal congestion, rhinorrhea, cough, sore throat, change in smell perception, change in taste perception, fatigue, emotional state, and shivering was reduced along the study course. Mean VAS symptoms' total score was 27.57 ± 15.63 at the beginning of the study (baseline), dropping to 6.73 ± 6.59 after 10 days (Table VII). VAS changes of all symptoms significantly decreased in all individuals (total population and both sub-groups) performing nasal irrigation at all time points (Table VIII). The decreases were more profound in the un-vaccinated subjects (-11.39 ± 2.108, $p<0.001$) only in day 3 compared to the fully vaccinated ones (-5.62 ± 2.484, $p=0.038$).

The change in each nasal symptom (log_{10} scale) in hospitalized patients with COVID-19 performing nasal irrigation with *HSS-Plus* per study day is presented in Table IX. Regarding the total population, rhinorrhea and shivering were significantly decreased on day 6 ($p<0.001$ for rhinorrhea; $p = 0.002$ for shivering) but not on day 3. Improvement in nasal congestion was already noticed since the third day ($p=0.013$) and kept improving during the course of the study. Nasal olfaction ($p=0.003$) and taste ($p=0.017$) had also been recovered since day 3 after study initiation and were further enhanced with nasal irrigation in days 6 and 10 (Table IX).

Change in symptoms was perceived at different time points in vaccinated and un-vaccinated patients. Changes in smell and taste impairment were perceived by day 3 only in the un-vaccinated sub-group ($p=0.005$ for both) and in Day 6 in the vaccinated one ($p=0.002$ for change in smell perception; $p=0.027$ for change in taste perception). Improvements in sore throat, fatigue, emotional taste, and shivering were statistically noted in day 3 only in un-vaccinated patients. Rhinorrhea was significantly reduced in days 6 and 10 in all groups ($p<0.01$). The only symptom that did not statistically change with nasal irrigation during the course of study in vaccinated individuals was shivering ($p>0.100$ at all study time points). On the contrary, the un-vaccinated individuals had experienced an improvement in shivering since day 3 ($p=0.041$).

No patients performing nasal irrigation with *HSS-Plus* have experienced adverse events.

Table VI. Proportion of patients with viral load decrease> 0.5 log_{10} at each study day.

	95% CI	*p*-value
Proportion of responders at day 3		
53%	36% - 69%	0.100
Proportion of responders at day 6		
72%	55% - 85%	0.407
Proportion of responders at day 10		
94%	79% - 99%	<0.001
Proportion of vaccinated responders at day 10		
100%	72% - 100%	0.011
Proportion of un-vaccinated responders at day 10		
90%	68% - 99%	0.018

p-value is comparing to 65%, see Materials and Methods.

Table VII. Symptoms score according to VAS scale per study day in hospitalized patients with COVID-19 performing nasal irrigation with *HSS-Plus*.

	Day 0 47	Day 3 47	Day 6 47	Day 10 38
Nasal congestion				
n	47	47	45	37
Mean±SD	3.34±2.94	2.45±1.85	1.33±1.41	0.92±1.21
Rhinorrhea				
n	47	47	45	36
Mean±SD	1.91±2.12	1.60±1.64	0.78±1.10	0.53±0.94
Cough				
n	47	47	45	37
Mean±SD	3.68±2.46	2.13±1.3	0.89±9.6	0.54±0.9
Sore throat				
n	47	47	45	37
Mean±SD	1.11±1.9	0.60±1.28	0.36±0.61	0.16±0.44
Change in smell perception				
n	47	47	45	37
Mean±SD	4.51±3.99	3.11±3.48	1.62±2.69	1.03±1.98
Change in taste perception				
n	47	47	45	38
Mean±SD	3.83±3.78	2.74±3.97	1.40±2.61	1.05±2.07
Fatigue				
n	47	47	45	37
Mean±SD	4.66±2.88	2.55±2.19	1.47±1.78	0.92±1.09
Emotional state				
n	47	47	45	37
Mean±SD	3.23±2.96	2.40±2.184	1.56±1.63	1.27±1.69
Chills				
n	47	47	45	37
Mean±SD	1.30±2.72	0.57±0.16	0.13±0.50	0.05±0.23
Total score				
n	47	47	45	37
Mean±SD	27.57±15.63	18.15±12.44	9.53±8.30	6.73±6.59

SD=Standard Deviation.

Table VIII. Change of VAS score for all symptoms from day 0 to day 10 after *HSS-Plus* use in total population and according their COVID-19 vaccination status.

Study day	VAS scale change for all symptoms	SD	95% CI		*p*-value
Day 3	-9.43	1.649	-12.66	-6.19	<0.001
Day 6	-17.61	1.663	-20.87	-14.35	<0.001
Day 10	-20.29	1.732	-23.68	-16.89	<0.001
Vaccinated					
Day 3	-5.62	2.484	-10.49	-0.76	0.038
Day 6	-17.32	2.532	-22.28	-12.35	<0.001
Day 10	-21.35	2.719	-26.68	-16.02	<0.001
Unvaccinated					
Day 3	-11.39	2.108	-15.52	-7.26	<0.001
Day 6	-17.80	2.117	-21.95	-13.65	<0.001
Day 10	-19.84	2.175	-24.10	-15.57	<0.001

SD=Standard Deviation, CI=Confidence interval.

Table IX. Change in nasal symptoms (log scale) in hospitalized patients (vaccinated and unvaccinated) with COVID-19 performing nasal irrigation with *HSS-Plus* per study day. Nasal symptoms were rated by patients in VAS score. Mixed repeated measures model with VAS change at 3, 6 and 10 days as dependent variable and study days as independent variable.

Score type and time point (change from baseline)	Day 3		Day 6		Day 10	
	VAS scale change ± SD	*p*-value	VAS scale change ± SD	*p*-value	VAS scale change ± SD	*p*-value
Nasal congestion						
Total population	-0.89±0.343	0.013	-1.97±0.344	<0.001	-2.20±0.353	<0.001
Vaccinated	-0.94±0.640	0.173	-2.32±0.644	0.003	-2.71±0.662	0.001
Unvaccinated	-0.87±0.396	0.037	-1.78±0.398	<0.001	-1.95±0.407	<0.001
Rhinorrhea						
Total population	-0.32±0.249	0.214	-1.11±0.250	<0.001	-1.24±0.259	<0.001
Vaccinated	-0.44±0.333	0.218	-1.48±0.338	<0.001	-1.61±0.361	<0.001
Unvaccinated	-0.26±0.332	0.450	-0.93±0.334	0.010	-1.05±0.342	0.004
Cough						
Total population	-1.55±0.302	<0.001	-2.76±0.304	<0.001	-3.07±0.313	<0.001
Vaccinated	-1.25±0.537	0.037	-3.25±0.542	<0.001	-3.62±0.561	<0.001
Unvaccinated	-1.71±0.356	<0.001	-2.52±0.357	<0.001	-2.81±0.365	<0.001
Sore throat						
Total population	-0.51±0.232	0.034	-0.71±0.234	0.004	-0.97±0.242	<0.001
Vaccinated	-0.50±0.433	0.278	-1.22±0.438	0.014	-1.04±0.455	0.038
Unvaccinated	-0.52±0.265	0.063	-0.46±0.267	0.097	-0.93±0.274	0.002
Change in smell perception						
Total population	-1.40±0.452	0.003	-2.80±0.455	<0.001	-3.37±0.471	<0.001
Vaccinated	-1.00±0.806	0.244	-3.07±0.814	0.002	-4.01±0.845	<0.001
Unvaccinated	-1.61±0.539	0.005	-2.66±0.542	<0.001	-3.06±0.559	<0.001
Change in taste perception						
Total population	-1.09±0.437	0.017	-2.26±0.440	<0.001	-2.86±0.456	<0.001
Vaccinated	-0.25±0.824	0.772	-2.05±0.833	0.027	-2.91±0.870	0.004
Unvaccinated	-1.52±0.500	0.005	-2.37±0.503	<0.001	-2.85±0.519	<0.001
Fatigue						
Total population	-2.11±0.370	<0.001	-3.17±0.372	<0.001	-3.75±0.385	<0.001
Vaccinated	-1.19±0.621	0.079	-2.38±0.629	0.001	-3.22±0.659	<0.001
Unvaccinated	-2.58±0.443	<0.001	-3.58±0.445	<0.001	-4.04±0.458	<0.001
Emotional state						
Total population	-0.83±0.326	0.014	-0.83±0.328	<0.001	-1.95±0.339	<0.001
Vaccinated	0.00±0.574	>0.999	-0.66±0.580	0.284	-1.34±0.604	0.043
Unvaccinated	-1.26±0.371	0.002	-2.13±0.373	<0.001	-2.27±0.386	<0.001
Shivering						
Total population	-0.72±0.367	0.057	-1.18±0.369	0.002	-1.17±0.377	0.003
Vaccinated	-0.06±0.480	0.901	-0.84±0.489	0.107	-0.87±0.525	0.121
Unvaccinated	-1.06±0.492	0.041	-1.36±0.493	0.010	-1.27±0.497	0.017
Total score						
Total population	-9.43±1.649	<0.001	-17.61±1.663	<0.001	-20.29±1.732	<0.001
Vaccinated	-5.62±2.484	0.038	-17.32±2.532	<0.001	-21.35±2.719	<0.001
Unvaccinated	-11.39±2.108	<0.001	-17.80±2.117	<0.001	-19.84±2.175	<0.001

SD=Standard Deviation

Discussion

Use of nasal lavage has been proposed as a safe method that could be used to reduce the local SARS-CoV-2 viral load and associated symptoms[11,13,14] as based on clinical studies in patients with COVID-19 or other upper respiratory tract infections (URTIs) showing reduction of viral shedding upon irrigation use versus no irrigation[3-5]. Sulphated polysaccharides, such as fucoidan, inhibit the binding of SARS-CoV-2[16,18,26] while polysaccharides derived from blue-green algae (e.g., Spirulina) have also been recommended for use in patients with COVID-19[19,27].

In a previous study conducted in patients with ENT disorders, including COVID-19 patients, the use of *HSS-Plus* helped reduce nasal congestion and increase symptom-free days[11]. In this study, we sought to extend initial observations by performing an exploratory study aiming to test *HSS-Plus* in hospitalized COVID-19 patients. Specifically, we sought to determine whether patients receiving nasal irrigation for a period of up to 10 days could benefit from a decrease in nasopharyngeal SARS-CoV-2 viral load and an improvement of their nasal and other symptoms.

Our results show that the vast majority of COVID-19 patients (93.6%) presented with a viral load reduction of at least >0.5 $\log_{10}$ at the end of study (day 10). The percentage of viral load reduction was 100% and 90% in vaccinated and un-vaccinated patients, respectively. Although the contribution of nasal irrigation in the reduction of the viral load cannot be concluded due to the absence of control group in our study, the results do suggest a reduction in steady state viral levels in participating patients that correlates with symptom improvement. These results agree with other publications[8] measuring steady state viral load levels in patients during clinical study course.

When comparing the measured viral loads before and after nasal irrigation, the results showed that the viral load measured in nasopharyngeal swabs increased following nasal lavage on both day 3 and on day 6 (Table V). Although this result appears paradoxical *versus* the results obtained by analyzing samples collected during the course of the study, it is noted that measured values correspond to the number of viral particles collected immediately after nasal wash, not steady-state levels. Considering that the aim of performing nasal lavage is the rapid removal of viruses based on the mechanical force of the solution (during spraying), it is expected that the spray would dislodge viral particles from the nasal mucosa; these particles would actually enrich the nasopharyngeal swab sample collected immediately after irrigation, as observed in our measurements. Therefore, the apparent rise in viral load following nasal lavage which we report in this study most likely reflects a transient viral dislodgement during spraying rather than being a true increase in viral load.

Analyzing VAS scores of different symptoms, we report clear reductions of severity of total symptom scores and individual symptoms, namely nasal congestion, rhinorrhea, cough, sore throat, changes in smell and taste perception, fatigue, emotional state and shivering in our study (Tables VII-IX). This reduction was independent of vaccination status against COVID-19 and correlated with the observed clinical improvement of patients. Interestingly, symptom improvement was evident and olfactory dysfunction had resolved already at day 3. Although the impact of nasal irrigation remains to be elucidated in the current setting, yet this period appears to be shorter than the reported periods to resolve such symptoms in studies by other investigators i.e., 8 days[28], more than 15 days[29,30] or one month[31,32] in the general population or hospitalized patients not practicing nasal irrigation. The same observation seems to apply for other reported symptoms, clearly these observations merit further investigation in randomized controlled studies.

In addition to hypertonic seawater of 2.3% NaCl, *HSS-Plus* also contains algal extracts rich in sulphated polysaccharides similar in structure to HS. As reported in a series of studies, these may act as molecular "decoys" preventing the virus binding to cells[15-18]. In the current study, it is difficult to conclude whether such ingredients may have exerted a biological action or whether changes in viral load seen before and after irrigation (Table V) are purely related to the sprayed hypertonic solutions. Additional clinical studies would be needed to approve or disprove this hypothesis.

Limitations

The study has several limitations. First, it is a single-centered, non-randomized study with a small sample size. Second, it lacks a control group not performing nasal irrigation. Third, the patient cohort included only hospitalized patients and probably mild-to-moderate cases of infections might have been missed. Finally, the precise timing of COVID-19 infection and vaccination were not available. Based on these parameters, our findings should be treated with caution as further confirmation in larger multicenter prospective randomized studies is needed.

Conclusions

Our observations suggest that hypertonic irrigation solutions comprising algal and herbal ingredients have a good safety profile. Although a correlation of use of these solutions with the observed reduction of viral load and improvement of symptoms in our patient group cannot be made, our results do suggest that irrigation can be used to dislodge viral particles from the nasal cavity. This property is in agreement with the reported use of irrigation solutions against other respiratory viruses and could be exploited further during the COVID-19 pandemic.

Conflict of Interest

Konstantinos Alevizopoulos is the Scientific Director and Stella Georgiou an employee of Gerolymatos International S.A. Elias Kainis was primary investigator of the clinical study. The authors declare that they have no financial interests.

Funding

This research received no external funding. *HSS-Plus* was provided by Gerolymatos International SA who did not interfere with the management of the study, data collection, processing and reporting.

Authors' Contributions

Conceptualization: K. Alevizopoulos; Methodology: K. Alevizopoulos, E. Kainis, M. Gangadi; Data collection, processing and analysis: M. Gangadi, E. Moschotzopoulou, TA; Data interpretation: S. Georgiou, M. Gangadi, K. Alevizopoulos; Literature review: S. Georgiou, K. Alevizopoulos; Manuscript preparation, editing and review: S. Georgiou, K. Alevizopoulos. All the authors approved the present version for publication.

Ethics Approval

The Ethics Committee approval was obtained from the Scientific Board of the Chest Diseases Department of the Sotiria General Hospital (June 3rd, 2021; No. 14922), followed by the approval of the Hospital's Board of Directors (29.6.2021).

Informed Consent

Informed consent was obtained in accordance with the local legislation applicable.

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