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A Prospective Study of AFree Oral Spray as an Adjuvant Therapy for Mild and Moderate COVID-19 in Community Health Stations: Clinical Progression and Viral Clearance Outcomes

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Abstract. *Background/Aim: The aim of this study was to evaluate the safety and efficacy of AFree oral spray, in combination with Standard of Care, in treating mild to moderate COVID-19 patients. This was an open-label, single-blinded, and controlled randomized clinical trial. Patients and Methods: The study involved 1,252 patients, who were randomly assigned to either the control or study group, with 626 patients in each group. Patients in the control group were treated with Standard of Care recommended by the Ministry of Health of Vietnam, while patients in the study group received AFree oral spray in addition to Standard of Care for a period of 10 days. The clinical progression and outcomes of both groups were compared. Results: The results showed that the proportion of patients with clinical symptoms on the 5th, 7th and 10th days were significantly lower in the study group (45.05%, 3.19% and 0%, respectively) compared to the control group (86.10%, 67.73% and 22.84%, respectively). Additionally, the rate of Real-time PCR test positivity for COVID-19 was significantly lower in the study group compared to the control group on the 4th, 7th, and 10th days (82.75% vs. 98.72%, 9.27% vs. 92.97%, and 1.12% vs. 50.48%, respectively). Furthermore, no side effects or complications related to AFree oral spray were recorded in the study group. Conclusion: The use of AFree oral spray resulted in significant improvements in clinical symptoms, recovery time, and viral clearance in COVID-19 patients with mild to moderate symptoms. This therapy has been shown to be safe and can be used as an adjuvant treatment for COVID-19 as well as other respiratory viral infections.*

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COVID-19 is a highly contagious disease that can cause a wide range of clinical symptoms, ranging from mild to severe. In the worst cases, it can lead to the development of respiratory distress syndrome, sepsis, septic shock, and even death. Unfortunately, there is currently no readily available, inexpensive, and non-toxic antiviral medication that can be widely used in communities with limited economic resources (1). Although some antiviral drugs have been approved and recommended for COVID-19 treatment, they are expensive and have the potential to cause severe adverse effects, particularly in patients with pre-existing health conditions such as heart disease, diabetes, and kidney disease (2-4).

The primary mode of entry for SARS CoV-2 is through the nasopharynx mucosal membrane. Therefore, an effective strategy to reduce the incidence of infection and disease severity is to suppress the colonization of the virus. Several studies and meta-analyses have emphasized the importance of maintaining good oral hygiene and using chemical antiseptic (disinfectant) agents to prevent respiratory disorders and nosocomial infections, including COVID-19 (5-7).

Zinc and iodine are essential micronutrients that play a crucial role in various physiological processes. Both elements have been shown to possess antiviral properties and can inhibit the replication of respiratory and other viral infections (8). Several clinical and experimental studies have investigated the efficacy of zinc and iodine in treating viral infections, including influenza, rhinovirus, and coronavirus. Recently, researchers have explored the potential use of zinc iodide in combination with dimethyl sulfoxide (DMSO) or other zinc ionophores as a viable preventive and therapeutic solution for COVID-19 and other viral infections (9, 10). Studies have shown that zinc ions can interfere with the replication of coronaviruses by inhibiting viral RNA polymerase and reducing viral particle production (11).

AFree is a registered oral sanitizer and disinfectant in Vietnam that contains zinc iodide and DMSO as its key ingredients. We conducted a prospective, controlled, randomized, open-label, and single-blinded parallel-group clinical trial to evaluate the efficacy of AFree oral spray in combination with Standard of Care on COVID-19 patients with mild and moderate clinical symptoms in community health stations. The objective of this trial was to assess whether the use of AFree oral spray as an adjunct therapy to the Standard of Care could improve the clinical outcomes of COVID-19 patients, reduce the severity of symptoms, and shorten the recovery time. The findings of this trial could have important implications for the management and treatment of COVID-19 patients, especially in areas with limited resources and access to expensive antiviral medications.

Patients and Methods

AFree is an oral spray that has been registered in Vietnam as a medical device under registration number 2100000725/PCBA-HN. AFree was developed in the Nimni-Cordoba Tissue Engineering and Drug Discovery Laboratory at the Keck School of Medicine of the University of Southern California in collaboration with Thai Minh Pharmaceutical Company. AFree has undergone acute and chronic toxicity testing, skin and mucosal irritation testing, and thyroid toxicity testing at the Pharmacology Department of the National Institute of Drug Quality Control of Vietnam. The results of these tests demonstrated an excellent safety profile and tolerability. Key ingredients in AFree include zinc iodide, ginger extract, propolis extract, natural fruit flavor, xylitol, DMSO and pure water.

Table I. *Patient underlying diseases and vaccination.*

	Study group		Control group	
Underlying disease	No	%	No	%
Pneumonia	504	80.51	540	86.26
Diabetes	187	29.87	165	26.36
COPD	144	23.00	107	17.09
Cancer	50	7.99	29	4.63
Chronic kidney disease	36	5.75	15	2.40
Obesity/overweight	79	12.62	64	10.22
Cardiology	130	20.77	94	15.02
Neurology	51	8.15	15	2.40
Asthma	108	17.25	80	12.78
Hypertension	166	26.52	159	25.40
Liver diseases	51	8.15	15	2.40
Other diseases	87	13.90	21	3.35
Vaccination				
1 shot	137	21.88	159	25.40
2 shots	360	57.51	411	65.65
No vaccination	129	20.61	56	8.95

Table II. *Symptoms at hospitalization.*

	Study group		Control group	
Reason for hospitalization	No	%	No	%
Cough	611	97.60	526	84.03
Fever	563	89.94	582	92.97
Fatigue	612	97.76	561	89.62
Shore throat	612	97.76	571	91.21
Decrease/loss of taste	244	38.98	180	28.75
Chest pain	410	65.50	417	66.61
Headache	389	62.14	331	52.88
Numbness in limbs	287	45.85	287	45.85
Breathing difficulty	368	58.79	359	57.35
Tachypnea	216	34.50	207	33.07
Muscle pain	424	67.73	187	29.87
Runny nose	396	63.26	417	66.61
Decrease/loss of sense of smell	249	39.78	237	37.86
Dizziness	208	33.23	108	17.25
Vomiting/nausea	215	34.35	72	11.50
Shortness of breath	159	25.40	130	20.77
Diarrhea	273	43.61	280	44.73
Other	129	20.61	36	5.75
Patient classification				
Mild	65	10.38	87	13.90
Moderate	561	89.62	539	86.10

Study design. The present study was a prospective, controlled, randomized, parallel-group clinical trial that utilized a single-blinded open-label design. The study enrolled COVID-19 patients with mild to moderate symptoms in Binh Duong and Dong Nai provinces of Vietnam.

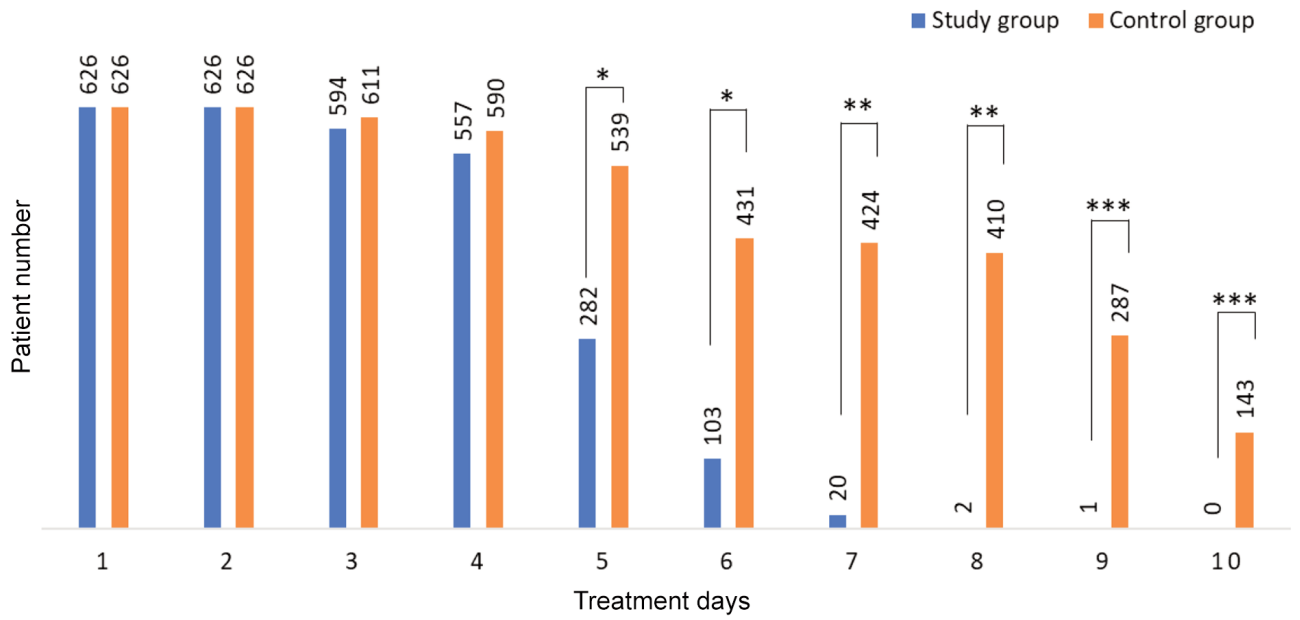

Figure 1. *Number of patients with symptoms by treatment days, *p<0.05, **p<0.01, ***p<0.001.*

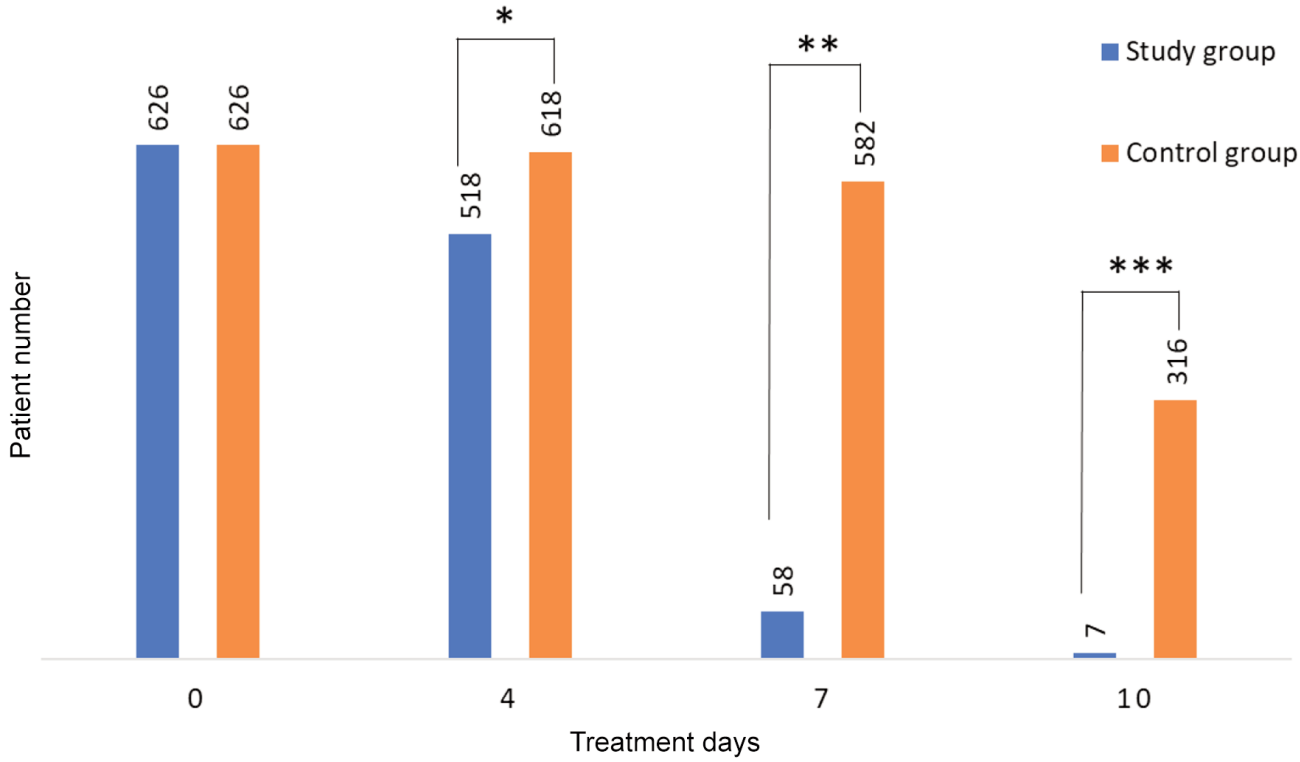

Figure 2. *Real-time PCR positive patient numbers according to treatment duration. *p<0.05, ** p<0.01, ***p<0.001.*

Table III. *Chest x-ray results*.

	Study group		Control group	
Chest x-ray	No	%	No	%
Pneumonia	561	89.62	539	86.10
Normal	65	10.38	87	13.90
		$p>0.05$		

Statistical analysis. The data of the study was evaluated using SPSS 20.0 statistics 25.0 software for Windows (IBM, Armonk, NY, USA). Outcomes were compared using a paired *t*-test and chi-square test for a significant value of $p<0.05$.

Inclusion criteria. To be eligible for inclusion in this study, a patient must meet all of the following criteria. First, they must have a confirmed SARS-CoV-2 infection through a positive RT-PCR test. Additionally, patients who were diagnosed with COVID-19 through a positive rapid test or positive RT-PCR within 72 hours were eligible. Finally, patients (or their legally acceptable representatives, in the case that they are under 18 years old) must understand, agree to, and follow the study's instructions. Meeting these criteria was essential for ensuring that the study participants are appropriately selected and can contribute to the research in a meaningful way.

Exclusion criteria. Patients who met any of the following criteria were not enrolled in this study: if the physicians deemed that trial involvement would not be in the best interest of the patient or if any condition arises that could potentially compromise the safety of the patient while following the protocol. Furthermore, patients who were currently enrolled or had previously participated in another clinical trial for an experimental treatment for COVID-19 were also excluded from this study.

Severity classification. Patients were classified into two groups, mild and moderate, based on their clinical symptoms and findings. Mild COVID-19 patients had non-specific clinical symptoms such as fever, dry cough, sore throat, nasal congestion, fatigue, headache, muscle aches, numbness of the tongue, and loss of taste and smell. They did not exhibit signs of pneumonia or hypoxia, with a breathing rate of ≤20/min and SpO2 levels of ≥96% with air breathing. Additionally, their chest X-rays were normal. Patients with moderate COVID-19 exhibited the same symptoms as the mild group, but they also had pneumonia diagnosed based on clinical or imaging findings such as chest X-ray, ultrasound, or CT if there was interstitial pneumonia or complications. Furthermore, these patients had clinical symptoms of pneumonia, such as fever, cough, shortness of breath, and rapid breathing of more than 20 breaths/minute, but no signs of severe pneumonia with SpO2 levels of ≥93% with air breathing.

Research ethics. All patients and their legal representatives have voluntarily agreed to participate in this clinical trial, as recommended by their physicians. Patients had the right to withdraw from the study at any time without providing any reason. A previous study involved the use of AFree oral spray to treat 200 COVID-19 patients with moderate and severe conditions at a field hospital established by the Public Security Department in Ho Chi Minh City, under the study protocol 0016/QD-IMP. The current study is an extension of the previous trial and will include a larger population of patients treated at community health stations in various provinces.

Intervention. Patients in the experimental group received AFree oral spray 5 to 10 times per day, with 5 pushes administered each time depending on the severity of their symptoms (5 times for mild symptoms and 10 times for moderate symptoms). This treatment was given for a period of 10 days, in combination with the Standard of Care for Covid-19 and other treatments for any comorbidities. The control group only received the Standard of Care without AFree oral spray.

Results

Patient characteristics. In our randomized study, 1,252 eligible COVID-19 patients were assigned to either the study group, which received AFree oral spray, or the control group, which did not. The two groups were well-matched, with 626 patients in each group and similar age and sex distributions. Patient age ranged from 6 to 108 years, with a mean age of 36.7 years in the study group and 33.5 years in the control group ($p>0.05$). The sex distribution was 49.1% female and 50.9% male in the treatment group and 48.5% female and 51.5% mail in the control group.

As indicated in Table I, no significant differences were observed between the study group and control group in relation to underlying diseases and COVID-19 immunization status. However, it is worth noting that the study group had a higher proportion of patients with tachypnea, cancer, chronic kidney diseases, neurological abnormalities, asthma, and liver diseases, as well as a larger number of unvaccinated patients. Despite these differences, the random assignment of patients to the study and control groups ensures that the comparison between the groups is unbiased, enabling us to draw reliable conclusions regarding the efficacy of the intervention being studied.

According to Table II, there was no significant difference in clinical symptoms upon hospitalization and the proportion of mild and moderate subgroups between the two groups of patients. Most of the patients had pneumonia and the frequencies of pneumonia, documented in chest x-ray examination, were similar in both groups (Table III). Patients in both groups received similar treatment except for the study group that was given AFree oral spray (Table IV).

Treatment results. The data depicted in Figure 1 indicate that the study group exhibited a significantly faster decrease in the number of patients with residual symptoms compared to the control group, by the 5th day of treatment. Additionally, there was a faster improvement COVID-19 related symptoms of patients in the study group, particularly from the 7th day of therapy onward.

Table IV. *Symptoms at hospitalization.*

	Study group		Control group	
Treatment	No	%	No	%
Symptomatic treatment	626	100.00	626	100.00
Underlying disease	462	73.80	446	71.25
Antibiotic	539	86.10	576	92.01
Vitamin, supplementation	626	100.00	626	100.00
Corticosteroid	626	100.00	572	91.37
Antivirus, monoclonal antibody agents	0	0	0	0
Oxygen by nasal cannula	102	16.29	122	19.49
Oxygen by mask	71	11.34	65	10.38
AFree spray	626	100.00	0	0

According to Figure 2, the study group exhibited a significantly faster decline in the number of RT-PCR-positive patients compared to the control group, with a highly statistically significant difference on the 7th day of observation (Figure 2).

Discussion

Viruses such as SARS-CoV-2 are known to undergo frequent mutations, resulting in the emergence of different strains or variants that may reduce the effectiveness of antiviral drugs. In light of this, it is important to promote the research and development of holistic preventive and therapeutic strategies that focus on enhancing the innate and adaptive immune defense mechanisms of the human body to fight against COVID-19 (12).

This approach is particularly important in the context of a scenario where the virus may become endemic and seasonally recurrent. In this case, boosting the body's immune response can potentially reduce the severity and frequency of infections. By prioritizing the development of immune-based interventions, we may be better equipped to effectively manage the ongoing COVID-19 pandemic and prepare for future outbreaks.

Upon entering the body, SARS-CoV-2 initially infects the upper respiratory tract, where it colonizes and proliferates during the first few days. Our study findings strongly suggest that effective therapeutics for preventing and treating COVID-19 could be delivered *via* oral and pharyngeal cavities using AFree oral spray.

AFree contains active ingredients, including zinc iodide, that have been proven to effectively eliminate SARS-CoV-2 in the oral and pharyngeal areas (6, 8, 11, 13). These ingredients can also activate innate immune defense mechanisms locally and systemically, leading to a significant reduction in viral load in the respiratory system (8-11). Using AFree oral spray can be a safe and effective way to administer treatments to the upper respiratory tract, where they can directly target the virus and activate the body's immune defenses. This approach may offer a promising strategy for combatting COVID-19 and reducing the impact of the ongoing pandemic.

Incorporating DMSO into the AFree oral spray can improve its anti-inflammatory, antibacterial, antifungal, analgesic, target-delivering, and transmembrane potentiating properties, thereby increasing its therapeutic benefits for COVID-19 patients both locally and systemically (9, 14, 15).

Patients were randomized based on their clinical symptoms, resulting in similar distributions of ages, clinical severity, and sexes between the two groups. Although the study group had more underlying diseases and was less vaccinated, we do not believe this difference favored the clinical effectiveness of AFree oral spray. Asymptomatic patients were not included in our study, as the Ministry of Health of Vietnam recommended, they receive no treatment. Therefore, it is difficult to estimate the clinical effectiveness of AFree for this group. The majority of our patients were classified as moderate, with 87.86% in the study group and 86.10% in the control group. Due to the lack of a placebo for the control group, we were only able to conduct a single-blinded study. However, this did not pose a problem as all patients were aware of whether they received AFree oral spray or not, whereas the doctors conducting the clinical assessments were unaware of the patients' treatment status. Furthermore, laboratory technicians were also not informed of the patients' treatment regimen.

Our study clearly indicates that AFree oral spray is a highly effective therapeutic option against COVID-19. In addition to reducing the severity of symptoms, improving the course of the disease, and accelerating viral clearance rates, early use of AFree oral spray in clinical practice may also lower the number of patients progressing to severe disease and curb transmission in the community.

AFree oral spray consists of well-characterized minerals and pharmaceutical ingredients that have been demonstrated to be safe. This advantageous safety profile is beneficial for the development and application of AFree oral spray as a repurposed therapeutic product against COVID-19 and other respiratory viral infections.

Conclusion

The use of AFree oral spray resulted in significant improvements in clinical symptoms, recovery time, and viral clearance in COVID-19 patients with mild to moderate symptoms. This therapy has been shown to be safe and can be used as an adjuvant treatment for COVID-19 as well as other respiratory viral infections.

Conflicts of Interest

All Authors declare no conflicts of interest in preparing and submitting the manuscript.

Authors' Contributions

DTT: Investigation, data curation, writing – original draft; TNP: Investigation, data curation, writing – review & editing; NHTN: Investigation, data curation; HDT: Conceptualization, visualization, writing – original draft; HQH: Writing – review & editing; BH: Conceptualization, prepared the tables, figure, writing – review & editing; BXH: Methodology, conceptualization, writing-original draft-reviewing an editing. All Authors read and approved the manuscript prior to submission.

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