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Combined Application of Dimethyl Sulfoxide and Ethanol Nasal Spray during COVID-19 Pandemic May Protect Healthcare Workers: A Randomized Controlled Trial

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Abstract

Background: Coronavirus pandemic has affected a large population worldwide. Currently, the standard care for individuals who are exposed is supportive care, symptomatic management, and isolation.

Objectives: This study aimed to evaluate the effects of the combined use of ethanol and dimethyl sulfoxide (DMSO) as a nasal spray in preventing Coronavirus disease 2019 (COVID-19).

Methods: This randomized controlled trial was conducted on volunteer healthcare workers of medical centers who were at the forefront of the fight against COVID-19 in Shahroud, Iran**.** In total, 232 participants were randomly assigned to intervention and control groups to receive DMSO/ethanol or routine care, respectively. The subjects were followed for four weeks to determine the incidence of COVID-19 infection in each group based on the RT-PCR test. Finally, absolute risk difference and relative risk were calculated to evaluate the effect of DMSO on COVID-19 prevention.

Results: The results showed that the incidence rates of COVID-19 were 0.07 and 0.008 in the control and intervention groups, respectively. The relative risk was obtained at 0.12 (0.02-0.97) according to the incidence rate in the two groups.

Conclusion: Combined administration of DMSO and ethanol by healthcare providers can considerably prevent COVID-19.

Keywords: COVID-19, DMSO, Ethanol, Healthcare workers, RCT

1. Background

Severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) is a positive-sense single-stranded RNA virus that causes Coronavirus disease 2019 (COVID-19). The coronavirus has affected a large population worldwide since the beginning of the pandemic in December 2019 in Wuhan, China. Following that, it has resulted in 549.01 million infections and 6.3 million death until July 3, 2022 (1).

The virus has become a major health challenge to humans, health care systems, and the global economy (2, 3) for several reasons including (i) the rate, extent, and variety of transmission routes, (ii) the confusing variety of clinical symptoms, and (iii) the unfamiliar and unusual response of the human immune system to COVID-19. Therefore, the current pandemic has become one of the top priorities of all countries (4).

Due to the absence of the virus in the bloodstream, available antiviral drugs are not effective (5, 6). The production of a potent and stable vaccine against the virus is also questionable because of the instability of the virus genome, which leads to continuous changes in the protein structures of the virus (7-9). In addition, due to the technology of production and storage requirements, they are very expensive and their availability is limited, especially in developing countries. Therefore, in order to control the disease, preventive methods that are available in all geographical areas are required (10, 11).

SARS-CoV-2 tends to infect the nasal and pharyngeal cavities (upper respiratory tract) for colonization and proliferation for the first few days after entering the body. Accordingly, the delivery of effective therapeutics for the prevention and treatment of the disease can be done easily (7-9). The virus only owns the genome and proteins synthesized in the host cell and obtains its phospholipid bilayer from the host cell membrane. Since the virus envelope contains only phospholipids and has no cholesterol, it is very unstable and is a suitable target to destroy the viral particles (12). Thus, hygroscopic and lipolytic compounds seem to be able to disrupt

the SARS-CoV-2 envelope (13, 14). Such compounds denature protein structure by absorbing excess water and include (i) organic solvents, such as ethanol, ether, chloroform, and dimethyl sulfoxide (DMSO), as well as (ii) ionic and non-ionic detergents, such as Tween 80, Triton X100, and SDS (15).

SARS-CoV-2 spike protein plays a crucial role in binding to the angiotensin receptor and entering the lung cell, which disrupts this process, and the virus's particles cannot continue their life cycle (16, 17). Studies show that ethanol and DMSO have the least toxicity in human cells and rats (18, 19). Therefore, the use of a solution containing 3% DMSO and 20% ethanol as an intranasal inhalation spray may destroy the virus's structure, break the virus transmission chain, and reduce the pathogenicity of the virus.

2. Objectives

The present study aims to evaluate the effects of a solution of 20% ethanol, 3% DMSO, and 0.1% menthol as a nasal spray on the prevention and reduction of the viral load in the lungs of patients and those in close contact.

3. Methods

3.1. Design

This single-center, two-armed 1:1, and randomized controlled trial was conducted to examine the effects of DMSO-ethanol nasal spray on the prevention of COVID-19.

3.2. Participants

The study population was healthy personnel of medical centers in Shahroud (northeast of Iran), who had no history of infection with COVID-19 and volunteered to participate in this trial study. In total, 232 participants were recruited using the convenience sampling method in 2020 from a general referral teaching hospital in Shahroud, Iran (Figure 1). At the beginning of the study, serum IgG and IgM antibodies were tested using a rapid test kit to confirm that volunteers were not infected with COVID-19.

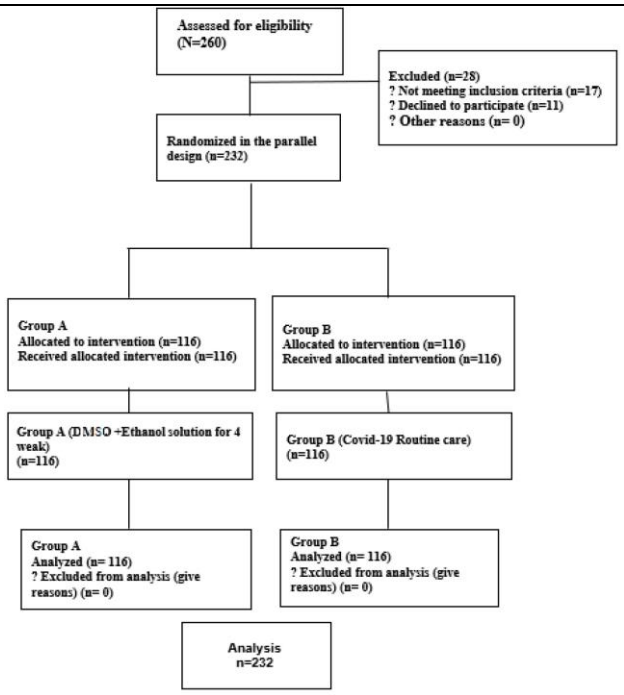

Figure 1. The process of the study according to the CONSORT flow diagram

3.3. Inclusion and exclusion criteria

The inclusion criteria included all the healthy personnel of the medical centers that were volunteers to participate in the study. On the other hand, those with a history of seizures or mental illness, allergy to any ingredient included in the spray, and acute febrile disease on the day of enrolment, as well as the cases who received any blood products in the past four months and being unable to comply with the study schedule were excluded from the study.

3.4. Intervention

The random allocation sequence was created online (www.sealedenvelope.com/simple-randomiser/v1/lists) to randomly determine the composition of blocks (size of each block 4). Subsequently, the participants in the study were randomly allocated to two groups receiving routine care+DMSO combination spray with ethanol and routine care alone (Figure 1). For concealment purposes, the random allocation order was placed in closed opaque envelopes. Due to the nature of the study, it was not possible to blind the

participants; however, the data collector and the data analyzer were blinded. Enrollment and allocation of participants to study groups were done by a nurse who was trained in this field. In the next stage, a 20-cc spray containing a combination of DMSO and ethanol was given to the individuals assigned to the intervention group. It is worth mentioning that the instruction was taught by the nurse. Individuals assigned to the intervention group were advised to spray a puff of DMSO spray into each nostril every 8 hours for four weeks in addition to routine daily care. On the other hand, the control group was recommended to continue daily routine care (Figure 1).

3.5. Outcomes measures

The outcome of the present study was COVID-19 infection. To determine the incidence of COVID-19 in each group, the subjects were followed for four weeks. During the follow-up, RT-PCR (COVITECH, Iran, master mix for COVID RT-PCR) was performed to confirm or rule out the disease in individuals with clinical symptoms of COVID-19. Furthermore, at the end of the fourth week, all participants in the study underwent RT-PCR to investigate any asymptomatic infections.

3.6. Sample size

Considering the power of 80%, α=0.05, and effect size of 0.1, the minimum sample size was estimated to be 93 individuals in each group. Regarding the sample attrition, the final sample size was determined to be 116 individuals in each group.

3.7. Ethical considerations

The process of conducting the study, the possible benefits and harms of the intervention, the right to withdraw from the study at the desired time, and the confidentiality of the information obtained were explained to the eligible individuals, and written informed consent was obtained from all participants in two copies. The research protocol was approved by the Medical Ethics Committee affiliated with the Shahroud University of Medical Sciences, Shahroud, Iran (decree code: IR.SHMU.REC.1399.091). The research protocol was registered at the IRCT website under the number:IRCT20200727048217N.

3.8. Statistical analysis

In this study, in order to evaluate the effect of using the combination of DMSO with ethanol, absolute risk difference and relative risk were calculated. In calculating the absolute risk difference, the group that received routine care was considered the exposed group. STATA (version 16) was used for the data analysis. Descriptive statistics including mean and standard deviation for quantitative variables, as well as frequency and percentage for qualitative variables were applied to describe these variables. Statistical tests were two-tailed, and P-value<0.05 was considered statistically significant.

4. Results

The mean±SD of the participant's age was obtained at 37.18±8.69 years in this study. Moreover, the majority of the cases were female (62.3%), and the sample drop-out at the follow-up was zero. Other characteristics of the participants are listed in Table 1.

Based on the results of PCR, out of 116 recipients of the DMSO-ethanol combination, 1(0.8%) case developed COVID-19 during the follow-up; however, out of 116 people assigned to the control group, 8 (6.8%) cases were diagnosed with COVID-19. The incidence rates of COVID-19 in the control and intervention groups were 0.07 and 0.008, respectively. According to the incidence rate in the two groups, the relative risk was estimated at 0.12 (0.02-0.97). This means that the combination of DMSO with ethanol reduces the risk of COVID-19 by up to 88% (Attributable Fraction Exposer) (Table 2).

Table 1. Characteristics of the study participants

Variable	Intervention group	Control group	P-value
Age (year)	38.28±9.96	36.01±6.96	0.16
Work experience (year)	3.82±1.66	4.03±1.67	0.34
Gender			0.32
Male	48 (40.7)	40 (34.5)	
Female	68 (59.3)	76 (65.5)	
Place of residence			0.54
Rural	111 (96.4)	5 (3.6)	
Urban	113 (98.0)	3 (2.0)	

Table 2. Outcomes of DMSO + ethanol solution therapy against COVID-19

Intervention	Outcome n (%)		Risk	Risk ratio	Absolut risk difference	Attributable fraction exposer
	Confirmed COVID-19	Non confirmed COVID-19				
DMSO + ethanol solution	1(0.88)	115(99.12)	0.008	0.12(0.02-0.97)	0.06(0.01-0.11)	0.87 (0.06-0.79)
Routine care	8(2.89)	108(93.1)	0.07			

5. Discussion

The present study showed that the use of DMSO in combination with ethanol significantly reduced the risk of COVID-19. According to the results, the risk of COVID-19 was about 8-fold higher in those who used routine care than in those who used DMSO spray. Due to the structure of the virus and the action mechanism of the combined DMSO with ethanol, the reduction in the risk of COVID-19 in people taking this spray can be justified.

Most viruses including coronavirus species have an envelope, wherein all functionally important virus surface proteins are implanted and secured from dismantling or falling off the virus coat. The virus lipid bilayer is completely derived from host cells, and the virus's genome has nothing to do with its generation. Therefore, it will not be affected by the extreme mutability of the virus. The physicochemical characteristics of the viral lipid envelope not only help the virus to maintain its spherical structure but also play a pivotal role as a concrete foundation for the viral surface proteins (20). It is of extreme importance to know that the lipid envelopes of these virus species are highly sensitive to desiccation imposed by hygroscopic chemical agents and lipid-solving substances. Thus, it is utterly simple to conceptualize that even trivial and hardly noticeable changes in the lipid membrane's ultra-structural properties could deeply affect the virus infectivity and virulence (21). Considering that most viruses, including coronavirus species, are wrapped up by an envelope, which is completely derived from the hosting cell, the virus's genome has nothing to do with its generation (20). Therefore, the induction of noticeable dryness at the surface of the coronavirus envelope and the nasal cavity epithelial cell membrane as well may cause the process of virus-epithelial cell attachment less.

DMSO is an aprotic polarity solvent that effectively solubilizes a wide variety of organic and inorganic chemicals including lipids with an unquestionable safety profile even at high molar concentrations. It looks as if even low concentrations of DMSO are yet of drastic effect on inducing dehydration and desiccation of lipid membranes. DMSO desiccates and weakens the lipid bio-membrane of cells and microorganisms (22-26). In a comprehensive and professionally designed biochemical study regarding the DMSO inducing dehydration near lipid membrane surfaces C-Y, Cheng concludes that DMSO "sprayed" on lipid surfaces even at low molar concentrations induces profound dehydration of lipid membranes, leading to marked physicochemical changes. He states that the physicochemical effects of DMSO on lipid surfaces are complex and significant at a broad range of DMSO concentrations (27). DMSO is known to directly interact with phospholipid bilayers as well. Molecular dynamics simulations of DMSO- dipalmitoyl phosphatidylcholine systems demonstrate that DMSO modulates the mechanical properties of lipid bilayers, reducing the area compressibility, thickness, solidarity, stability, and bending moduli. These cumulative effects make the lipid layer loose and floppy (28-34). DMSO also causes significant changes in the phospholipid bilayer of cultured skin fibroblast cells and disturbs the quality of the membrane lipid matrix. Numerous biophysical studies clearly demonstrate that the DMSO can induce phospholipid bilayer thinning and create pores through membrane lipid structures. These findings lend support and shed light on the facts behind the antimicrobial and antiviral effects of DMSO. It has been shown that topical DMSO blocks the transcription-replication process of the lipid enveloped, dsDNA containing herpes simplex virus, introducing itself as a direct antiviral agent (35). DMSO has been shown to potentiate the antiviral effects of all disinfectants. It is also among the low toxicity solvents exerting strong free radical scavenger activity. Impressive research by L. Costa reveals that DMSO decreases cell proliferation, as well as TNF-a, IFNs, and IL-2 production in the cultures of peripheral blood lymphocytes. This study signifies the strong anti-inflammatory effects and cytokine storm-preventing capabilities of DMSO (36).

In addition, a CDC guideline [2008] regarding chemical disinfectants states that ethanol has generally been underrated as a potent antiviral agent inactivating and disinfecting all "enveloped lipophilic viruses". The widely accepted explanation for the antiviral action of ethyl alcohol is the denaturation of viral proteins and phospholipid bilayer envelope. In a recent study published by G. Kampf, 80% ethanol was highly effective at killing all 21 tested enveloped viruses within only 30 seconds (37). Another report reveals that ethanol disinfects the lipid-coated viruses instantly in less than 10 seconds. The desiccation and partial denaturation of the virus lipid coat would simply loosen the embedded proteins across the virus envelope. Therefore, it is a reasonable expectation that even the low concentrations of a desiccating substance (DMSO) in conjunction with a powerful lipid solvent (ethanol) might execute the desired impact on enveloped viruses. Kampf specifies that the powerful antiviral effects of ethanol were not as remarkable in virus species lacking lipid bilayer coat. It obviously indicates that the primary target for the antiviral effects of ethanol is the virus lipid coat (37).

It is known that viruses are obligatory and obsessed inhabitants of nasal cavity mucosa, throat, pharynx, and later in its pathogenesis, the lower respiratory tract epithelial cells, alveolar pneumocytes, and capillary endothelial cells (38). Furthermore, SARS-COV-2 has no noticeable desire to present high viral load viremia (39) and uses

angiotensin-converting enzyme-2 as the major binding site to enter the cells. In addition, the upper and lower respiratory tract harbors the highest concentrations of this protein. Thus, the SARS-CoV-2 must be regarded as a restricted inhabitant of our respiratory tract. Based on these practical reasons, we can constantly intrude and continuously disturb the coronavirus replication milieu in nasal cavities and oropharyngeal area via a nasal spray composed of DMSO and ethanol and causing total turmoil in the breeding and replicating virus herd inside the nasopharynx and throat.

5.1. Strengths and Limitations

Regarding the limitations in this trial, one can refer to the followings. First, this phase-3 trial started before the full analysis of the data from phases 1 and 2. Second, it was not possible to blind the study groups. On the other hand, considering the main strengths of this study, firstly, it can be stated that DMSO and ethanol have very few contraindications as a mouthwash or nasal spray. Second, the administration is cheap, simple, and rapid. Furthermore, it is readily available in healthcare worldwide, and its sensitization is extremely rare. Finally, there was no sample loss at the follow-up.

6. Conclusion

The results of this study showed that the administration of combined DMSO and ethanol by healthcare providers can considerably prevent COVID-19.

6.1. Recommendation for practice

This study highlighted that the use of DMSO and ethanol is an effective way in the prevention of COVID-19 in medical centers. As a result, the findings of this study if replicated by other studies can be used by healthcare providers, such as physicians and nurses, to reduce the risk of COVID-19.

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Footnotes

Conflicts of Interest: The authors have no conflict of interest to declare.

Authors' Contribution: Conceptualization and methodology were conducted by Moslem Jafarisani, Ali Hosseinzadeh, and Mohammad Hassan Emamian.
Data collection, analysis, and interpretation were conducted by Abbas Tavakolian, Vahid Kia, Hossein Sheibani, and Ehsan Binesh.
Drafting the article, revising, and final approval of the manuscript were conducted by Reza Jafari, Hossein Ebrahimi Seyed Mohammad Mirrezaie, Ali Hosseinzadeh, Mohammad Hassan Emamian, and Moslem Jafarisani.

Ethical Approval: The research proposal was approved by the Medical Ethics Committee affiliated with Shahroud University of Medical Sciences, Shahroud, Iran (decree code: IR.SHMU.REC.1399.091). The research protocol was registered at the IRCT website under the number:IRCT20200727048217N.

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