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Comparison of Effectiveness of Chlorine Dioxide Mouthwash and Chlorhexidine Gluconate Mouthwash in Reduction of Oral Viral Load in Patients with COVID-19

Sanket Keshav Avhad[1], Mrinalini Bhanushali[1], Sanpreet Singh Sachdev[2], Siddhesh Sandip Save[3], Dheeraj Kalra[4], Kamala DN[5]

[1]Assistant Medical Officer, Dedicated COVID Health Centre, G/North Ward, Dadar, [2]Post Graduate, Department of Oral Pathology and Microbiology, Government Dental College and Hospital, Mumbai, [3]Post Graduate, Department of Orthodontics, MGM Dental College and Hospital, Navi Mumbai, [4]Professor (Acad) and Head of Department, Department of Public Health Dentistry, Government Dental College and Hospital, Mumbai, [5]Medical Advisor-Oral Care, Group Pharmaceuticals Limited, India

Abstract

Background: Air-borne droplets constitute the main route of transmission of COVID-19. Considering the exponential increase in number of cases, it has become the need of the hour to develop additional measures to limit the spread of infection.

Materials and Methods: 40 patients were provided with Chlorhexidine gluconate (0.2%) mouthwash and Chlorine dioxide (0.1%) mouthwash to rinse and gargle thrice a day for one week. The qualitative COVID antigen test confirmed by Qualitative PCR on an oropharyngeal swab collected from the patients was compared for both the groups at baseline and post-intervention levels.

Results: There was an improvement in symptoms such as cough, sore throat and bad breath in both the groups. The number of cases demonstrating reduction in intensity of symptoms as well as testing qualitatively negative for COVID-19 antigen were found to be greater in the group that was provided with Chlorine dioxide mouthwash.

Conclusion: Regular use of Chlorine dioxide could effectively reduce the symptoms and oral viral load, thereby subsequently reducing the symptoms and risk of transmission of COVID-19. Use of Chlorine dioxide mouthwash may be recommended as a part of health policies and preprocedural protocols.

Keywords: *Pandemic; Airborne spread; Oropharyngeal; Virucidal mouthrinses; Health policy*

Introduction

The cases of COVID-19 have been increasing ever since its inception and considering its alarming level of spread, it was characterized as a pandemic by WHO on 11th March, 2020 [1]. The virus is abundantly present in the oropharyngeal region wherein especially high load has been reported in the saliva [2-3]. Airborne droplets generated during sneezing, coughing, breathing, and talking comprise the most common route of infection [4]. The aerosols generated by patient may permit COVID-19 to remain suspended in the air for about 3-4 hours and can spread up to a distance of 1 meter [5].

Therefore, in order to limit the physical spread of the outbreak, various measures such as social distancing, lockdown, wearing masks and practicing sanitization are implemented. However, despite numerous efforts, the number of cases globally are exponentially increasing

Corresponding Author:
Dr. Sanpreet Singh Sachdev
Phone: 9029012515
Email: sunpreetss@yahoo.in
Mailing address: A-1, 201, Bldg No.122, Bhakti Heights, Tilak Nagar, Mumbai – 400089

day by day. It is, thus, imperative to find adjunctive measures that could aid in limiting the spread of the disease.

Chlorhexidine gluconate when used as a mouthwash has been proven effective in reducing oral viral load in 0.12% and 0.2% concentrations according to various scientific reports [6,7]. Yet another less commonly utilized mouthwash i.e. chlorine dioxide (0.1%) has been proven effective in reduction of viral load [8]. Its safety as a mouthwash for oral rinse and gargle has also been documented in scientific literature [9,10].

If the oropharyngeal viral load is substantially lowered by rinsing and gargling by a mouthwash having virucidal activity, it could play a pivotal role in curbing the number of cases during the pandemic. In this context, we have attempted to assess the effectiveness of chlorhexidine gluconate mouthwash and chlorine dioxide mouthwash in reducing the oral viral load of COVID-19 positive patients so as to reduce the risk of transmission.

Materials and Methods

Study sample and Grouping:

A sample size of 40 patients was determined for the pilot study. Patients confirmed as positive for COVID-19 antigen by means of Qualitative PCR test with age ranging from 19 to 49 years were included in the study. A detailed case history including their present symptoms were recorded at the time of selecting patients. Patients having medical history or family history of systemic disorders; or of allergic reaction to either components of the mouthwash; inadequate records or disambiguous history; those with severe symptoms in need of immediate critical care; non-compliant and not willing to participate in the study were excluded from the study.

Informed consent was obtained from the cases deemed as eligible for the trial and a randomly generated code was provided. On the basis of code generated, the participants were divided into two groups:

Control Group *(n=20)* à provided with Chlorhexidine gluconate (0.2%) mouthwash

Study Group *(n=20)* à provided with Chlorine dioxide (0.1%) mouthwash

Clinical Protocol:

Both the investigator and the participant were blinded with respect to the mouthwash provided. The participants were provided with either chlorhexidine gluconate (0.2%) mouthwash (Guard OR, Group Pharmaceuticals Ltd., India) or with chlorine dioxide (0.1%) mouthwash (Freshclor, Group Pharmaceuticals Ltd., India) depending on the group they were assigned to. Instructions to rinse and gargle with 10 ml of undiluted mouthwash, thrice a day – before brushing in the morning and after meals during afternoon and night for seven days were given and regular daily follow up was carried out.

Another swab test was carried out for the patient at the 8th day on which the patient would not use the mouthwash so as to eliminate temporary and mechanical effects of mouthwashes on the test results. Qualitative assessment of symptoms and oral viral load by RT-PCR test from each group were compared to the initial baseline and the results of each group were compared to each other.

Results

The number of cases remaining positive for COVID-19 antigen as tested by RT-PCR test after 1 week of mouthwash protocol have been illustrated in **Figure 1**.

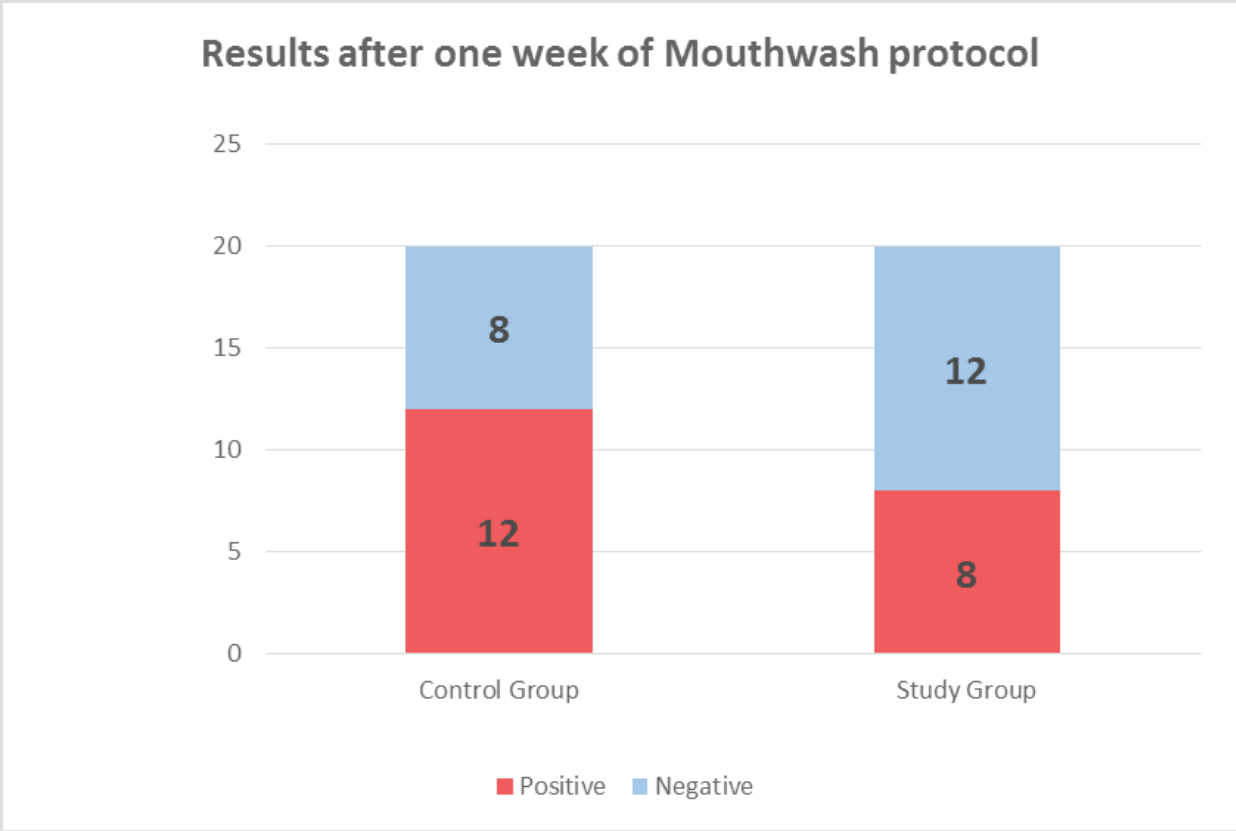

Figure 1 illustrates the resultant number of cases testing positive and negative for COVID-19 antigen by Qualitative PCR test after one week of mouthwash intervention in the control and study groups respectively.

Although the results could not be subjected to statistical analysis owing to the small sample size of the present pilot study, it was found that more number of patients recovered from the disease after one week of Chlorine dioxide mouthwash protocol along with routine therapy. Thus, it could be extrapolated from the results that Chlorine dioxide mouthwash could possibly prove more beneficial in reduction of oral viral load and subsequently limiting the spread of infection through oral droplets.

Furthermore, all the patients having cough, sore throat, bad breath and loss of taste showed rapid improvement in their symptoms. Patients with symptoms such as diarrhoea, cold and headache were more or less unaffected and any improvement could not be entirely attributed to mouthwash.

Discussion

Coronaviruses are a group of positive-sense single-stranded RNA viruses surrounded by a fatty layer, called a "lipid envelope," into which the spike glycoproteins required for infection are inserted. Disruption of the lipid envelope represents a viricidal strategy to target many coronaviruses [11]. Due to its positive charge, Chlorhexidine gluconate reacts with the negatively charged microbial surface, thus penetrating into the cell. It has been proven to effectively reduce the viral concentration of the lipid enveloped viruses [12]. A recent meta-analysis showed that chlorhexidine (rinse or gel) can reduce risk of ventilator-associated pneumonia in patients undergoing mechanical ventilation [13].

Chlorine dioxide (ClO2) is an unstable gaseous compound. Therefore, salt of chlorine dioxide i.e. sodium chlorite is utilized which is termed as stabilized chlorine dioxide. Oral rinses containing ClO2 are now utilized in dental practices as an anti-microbial mouthrinse for the oral cavity or for dentures [14,15]. Previous studies have suggested that ClO2 and ClO2- are chemically reactive oxidants with powerful reducing capacity. Such oxidative mouthwashes are proven more effective

against coronaviruses.

In an in-vitro study, Noss et al found that chlorine dioxide reacted with viral proteins, namely cysteine, tyrosine, and tryptophan amino acid residues [16]. Ogata found that ClO2 caused denaturation of viral proteins of influenza virus by oxidative modification of the tryptophan and tyrosine residues in hemagglutinin spike protein, thus abolishing its receptor-binding ability [17]. The spike protein of the new coronavirus SARS CoV-2 also contains 54 tyrosine, 12 tryptophan, and 40 cysteine residues [18]. Another mechanism involving antiviral activity of ClO2 could be inducing the release of reactive oxygen species (ROS) which could be further enhanced with the use of antioxidants like Vitamin C and E [19].

COVID-19 positive tested patients can mainly spread the virus unknowingly during the subclinical period [20]. This can be tested by assessment of viral load in these patients. *Viral load* also known as viral burden or viral titer is a numerical expression of the quantity of virus in a given sputum or blood plasma [21]. Higher viral load of SARS-COV2 has been found in the saliva as compared to that of oropharynx. [3] The reason for higher transmission and viral load in saliva is related to the receptor binding tropism, SARS-CoV-2 binds to the angiotensin converting enzyme 2 (ACE2) receptor, which is present in abundance in the respiratory tract and the salivary gland duct epithelium [22,23].

This viral load can be reduced from the upper respiratory tract, which is quite accessible, by gargling of oral cavity with an antiseptic and use of nasal drops/ tampons for nasal cavity.

A randomized controlled trial by Satomura et al found that regular gargling with drinking water containing 0.5 mg/L of chlorine was effective in reducing the effect of upper respiratory tract infections including influenza like viruses [24].

A virus ready to infect a cell is typically in aqueous phase, e.g., in a fluid droplet, or in the epithelial lining fluid covering the mucous membranes, the size of which is much larger than that of the virus. Therefore, in such cases, the rate-limiting step probably would be the diffusion of a substance, in this instance, chlorine dioxide in the water [25].However, on rinsing with chlorine dioxide mouthwash, the gaseous compound is released in the mouth Taking this into consideration Chlorine dioxide in the form of mouthwash would be an effective modality for reduction of oropharyngeal viral load.

Use as preprocedural mouthwash

A study found out that pathogenic microorganisms are present in patients' mouths from day one and show an increasing trend during hospitalization. [26] Chlorhexidine, being a gold standard mouthwash, has already been used as a preprocedural mouthwash for COVID-19 with varying concentrations in dental practice, that acts by reducing the systemic complications of the disease and accelerating the recovery process [27]. Chlorhexidine mouthwash has been reported as effective in reducing the incidence of ventilator-associated pneumonia in critically ill patients [28].

Chlorine dioxide has also been proven to be an effective antiviral mouthwash against various RNA and DNA viruses. As a disinfectant, it is more effective than sodium hypochlorite and chlorine alone which releases trihalomethane. [29]. As discernable from our results, inclusion of Chlorine dioxide mouthwash before dental procedures could further reduce the risk of infection by reducing the oral viral load.

However, due to limited budgeting and risk of infection, the sample size of the present study was limited and further studies with greater sample size and quantitative viral load estimation methods could be undertaken to ascertain the effectiveness of mouthwashes in reduction of oral viral load. Yet another limitation of the study was that the patients continued receiving routine treatment regimen for COVID-19 alongside the mouthwash intervention. Thus, all the results could not be attributed entirely to the mouthwash although patients had similar baseline variables such as location and treatment provided.

Conclusion

We cannot be sure that such a treatment would be enough to prevent the development and spread of the illness, as viruses living in other parts of the body can survive. However, inactivating part of the viruses and reducing their load in the oropharyngeal region using Chlorine dioxide mouthwash would definitely aid the

reducing the transmission of the disease through oral droplets along with the social distancing protocol. Chlorine dioxide mouthwash could augment the improvement of symptoms in COVID-19 patients as well. Thus, it should be recommended as an adjunctive measure by implementation of policies by health authorities and government bodies.

Ethical Clearance:: The approval for trial was obtained from Medical Officer of Health, Department of Public Health, G/North Ward, Dadar, Mumbai before commencement of the study.

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