Title: Efficacy of normal saline nasal spray and gargle on SARS-CoV-2 for prevention of COVID-19 pneumonia.

Abstract

Background: Role of microaspiration of mucus mixed with SARS-CoV-2 (severe acute respiratory syndrome corona virus 2) causing pneumonia is lacking in searched literature. Recently some authors have emphasized on microaspiration. SARS-CoV-2 primarily replicates in nasal mucosa and sheds in nasal mucus which travels down as microaspiration and causes pneumonia. We aimed to evaluate the efficacy of normal saline nasal spray and gargle (NSNSG) to wash off SARS-CoV-2 from nasal and pharyngeal mucosa to prevent microaspiration and pneumonia.

Methods: From RT-PCR (reverse transcriptase polymerase chain reaction) report, we selected 61 patients for study group and 64 patients for control, having higher virion load; cycle threshold (Ct) value 25 or less. Patients in study group were trained with NSNSG. We reviewed HRCT (high resolution computed tomogram) of lung in 56 patients of both groups for severity score in lung and were compared with initial HRCT.

Results: Twenty nine out of 61 (47%) of study group significantly (p=0·02) became negative following NSNSG compared to 17 out of 64 patients (26%) of control. Severity score (SS) in 31 out of 34 patients (91%) either decreased or became static in study group. In control group, 14 out of 22 patients (63%) also showed same findings. Nevertheless, study group significantly improved (p=0·028) in SS.

Conclusions: NSNSG significantly washes off SARS-CoV-2 from nasal cavity and pharynx and prevents microaspiration of SARS-CoV-2 in lung alveoli.

Trial Registration No: CTRI/2020/08/027465

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Main Text

Morbidity and fatality depends on the severity of pneumonia in COVID-19. Published literature has contributed a lot in management of COVID-19. However, role of microaspiration of SARS-CoV-2 from upper respiratory tract causing COVID-19 pneumonia is lacking. Recently Hou et al[1] have appreciated that SARS-CoV-2 migrates to lung alveoli by microaspiration from upper respiratory tract. Nasal mucosa is a fertile site for SARS-CoV-2 due highest expression of angiotensin converting enzyme2 (ACE2) receptor and transmembrane protease serine2 (TMPRSS2) in nasal epithelium.[1,2] Spike protein (S) of SARS-CoV-2 sticks to ACE2 receptor, and host cell TMPRSS2 cleaves and primes S to help transfer of RNA into epithelial cell (Fig.1). RNA replicates in epithelial cell to produce virions which shed in nasal mucus to alter it as VMS. Physiologically, nasal mucus is swept towards pharynx by mucociliary conveyer at a rate of ~0.5 ml/hour[1,3] and may progress to lung alveoli as microaspiration.[1] Similarly, microaspiration of VMS from nasal or pharyngeal mucosa may spread inwards to infect type II alveolar epithelial cells in lung alveoli to set pneumonia.

We hypothesize that normal saline nasal spray and gargle (NSNSG) would wash away VMS, and microaspiration would be prevented. So we tested such hypothesis and found positive outcome.

Patients: In this prospective study, a total of 251 (113 study group and 138 control group) RT-PCR (reverse transcription-polymerase chain reaction) positive, hospital admitted, symptomatic patients with co-morbidities were enrolled from 14th September 2020 to 4th January 2021 in phases. All patients were capable of performing NSNSG. Informed consents were obtained from all 251 patients. Research protocol was approved by institutional ethics committees.

Nasopharyngeal and oropharyngeal swabs were collected from all patients enrolled on their respective date of admission and sent to the referral laboratory for RT-PCR test to determine Ct (cycle threshold) value. On same day patients of study group were trained for NSNSG physically and audio-visually. We selected 61 patients for study group and 64 patients for control group, age ranged from 18 to 80 years, from both sex, having Ct value 25 or less in RT-PCR test.

Methods: All patients got same medical treatment as per hospital protocol. Enrolled patients in study group were taught to spray 25 ml of normal saline alternately in both nostrils from spray bottle (Fig. 2) and to gargle. They were trained to keep head slightly backwards and to opposite side to allow flow of normal saline through opposite nostril and were advised to collect effluent in a mug with soap water for safe disposal. During initial part of the study, 45 patients were on NSNSG every six hourly. In 19/45 patients, repeat RT-PCR test was done after 72 hours, and in 26/45 patients after 96 hours. Based on the results of the initial part of the study, another 16 patients were advised to increase frequency of NSNSG at three hourly intervals and RT-PCR was repeated after 120 hours. For repeat swab for RT-PCR test, NSNSG was kept on hold for six hours. RT-PCR for control group was also tested at same intervals in 21, 23 and 20 patients respectively (Table1). Serum sodium in study group and necessary investigations in both groups were done irrespective of Ct value in RT-PCR. Fifty six patients needed HRCT (high resolution computed tomogram) of lung on clinical suspicion of progression of disease and on some occasions to assure safety of NSNSG. Severity score (SS) was compared with initial HRCT of lung (Table 2).

Statistical analysis: Updated excel format exported to Epi-info software version 4.4 for statistical analysis for both data of Table1 and Table2. *P*-value of significance (<0.05) for chi-square test and 95% Confidence interval for Odds ratio (OR) checked. For Table1, OR of RT-PCR negative report of study group is 2.5 times higher than control. So 95% CI for OR is

1.2-5.3. For Table 2, OR improvement of SS on HRCT in study group is 6.1 times higher than control. So 95%, CI for OR is 1.4-26.4. As both the 95% CI don't include value 1, so the OR is significant. However, statistical analysis in SS of HRCT lung has got limitation due to its heterogeneity in comparison.

Results: We chose 61 patients in study and 64 patients in control group; having Ct value ranged 10-25 (mean 20.3) in RT-PCR. Eight out of 19 patients (42%) and 10 out of 26 patients (38%) of study group who did six hourly NSNSG became negative on RT-PCR test done after 72 hours and 96 hours respectively. In comparison, RT-PCR became negative in 28% and 21% of patients in control group after 72 hours and 96 hours respectively (Table-1). Subsequently 16 patients was on three hourly NSNSG and 11 of them became negative (68%) in repeat RT-PCR done after 120 hours, compared to six out of 20 patients (30%) of control group. Ultimately 29 out of 61 patients (47%) of study group and 17 out of 64 patients (26%) of control group became negative (Table1). Serum sodium was within normal range in study group. Efficacy of NSNSG found to be significant (p = 0.02, OR 2.5, 95% CI, 1.2-5.3) in this study.

We evaluated HRCT chest of 34 patients from study group and 22 patients from control done on different days and compared with primary HRCT. Thirty one out of 34 patients (91%) of study group either had improvement or no progression of severity score (SS) in lung HRCT. Similarly, 14 out of 22 patients (63%) of control group showed favorable findings in lung SS (Table 2). Nevertheless, in study group, SS was restricted significantly (p = 0.028, OR 6.1, 95% CI, 1.4-26.4).

Discussions:

In earlier part of this study, we advised six hourly NSNSG to assess tolerability as well as to assess outcome in repeat RT-PCR test done after 72 hours and 96 hours. Tolerability with NSNSG was found acceptable but relative outcome in RT-PCR was not

satisfactory. Six hourly NSNSG appeared insufficient to combat continuous shedding of SARS-CoV-2 from nasal epithelial cells. So we increased frequency to three hourly and found substantial clearance of virions in repeat RT-PCR done after 120 hours. It has been found that progeny virions, following replication, are continuously released from cells over a relatively longer period instead of burst-like disposal; in an *in vitro* study of influenza virus.[4] These progeny virions following replication exploit the secretory pathway to be released out of the cells.[5] This might be the basis of predilection of SARS-CoV-2 to infect type II alveolar epithelial cell which has got adequate secretory apparatus, like goblet cell in nasal epithelium,[6] unlike type I alveolar epithelial cell. Hence continuous shedding of progeny might be expected from SARS-CoV-2. So, frequent NSNSG is necessary to wash off continuous shedding of virions to protect lung from microaspiration of VMS and pneumonia. Hematogenous spread of SARS-CoV-2 to lung is unlikely; as demonstrated by absence of infection of micro vascular endothelial cells.[1] Microaspiration from pharyngeal mucus is a well known "oral-lung aspiration axis" for many lower airways diseases.[1,7,8] Macroscopically, in lung autopsy, COVID-19 infection appears patchy, segmental, and peripheral. These features are consistent with microaspirations.[1]

Anatomical barriers, immune mechanisms, mucociliary conveyor and cough might protect lung from microaspiration. Possibly those factors inhibited severity score from escalating in 63% patients in control group. Similarly, three patients (13%) in control group showed no sign of microaspiration in initial HRCT as well as in HRCT done on sixth day following admission (Table 2). It appears that some patients are physiologically protected from microaspiration. Nevertheless microaspiration from nasal cavity might gain access into lungs within 10 hours particularly during sleep, as shown in earlier study with technetium 99m-labeled macro-aggregated albumin.[9] On the other hand, microaspiration is frequently observed in older, diabetic, and obese subjects who as well are at risk for severe COVID-19.[1]

Appreciating the behavior of infection, replication, shedding and microaspiration of SARS-CoV-2, it might be considered as surface virion to comprehend the events of SARS-CoV-2 and to restrain its activities. With similar consideration, Hou et al[1] speculate that nasal lavage, topical antiviral, or immune modulation, might be beneficial to reduce viral titer in the nose. Soluble protease TMPRSS2 cleaves and primes S protein to facilitate fusion and helps to deliver RNA from virion to epithelial cell (Fig 1). In this study we have not examined the effect of NSNSG on soluble TMPRSS2. Nevertheless, it appears that TMPRSS2 might either be washed off or efficacy of TMPRSS2 might be decreased by NSNSG.

Co-infection with bacteria increases severity and fatality in pneumonia during influenza outbreaks.[10] In many situations, bacteria and virus mutually perform to increase pathogenicity.[11] Some bacteria enhance viral shedding.[12] It is found that 50% of COVID-19 patients who died had bacterial co-infections in pneumonia.[13] Understandably, NSNSG might wash off bacteria along with SARS-CoV-2 from nasal and pharyngeal mucosa and might decrease chance of co-infection.

Our study has got limitations. We could not study the efficacy of NSNSG on prevention of microaspiration in lung with HRCT lung in matched groups. So, we had to evaluate HRCT lung done at various intervals following admission. Secondly, patients were trained with audio-visual and physical demonstrations for NSNSG in a short period. This rapid learning appeared to be tricky in some patients. Three patients enrolled in study group failed to follow instructions correctly. They were excluded from study on 2nd day. Two patients in study group showed six and eight points increase in SS in HRCT done on sixth and seventh day (Table 2) respectively; possibly due to inadequate NSNSG following inadequate training. We increased frequency and duration of NSNSG in phases, as we could not anticipate patients' compliance beforehand. Afterwards, three hourly NSNSG for 120 hours was found

to be more effective to wash off SARS-CoV-2. We empirically used the volume of normal saline and frequency of NSNSG.

It appears that NSNSG would prevent pneumonia in COVID-19 if this procedure is applied once suspicion of contract or onset of symptoms is appreciated. Similarly, outcome would be better with proper training of patients by dedicated health workers.

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Author Contributions: U.S.C conceptualized the hypothesis of this study. A.K.C., B.S., S.K.N., U.S.C. designed the study protocol. S.K.N., U.S.C. recruited patients. A.K.C., S.K.N., U.S.C. performed the study and data acquisition. A.K.C. supervised or managed research. B.B., B.S., S.K.N. supervised the RT-PCR test. A.K.C., B.B., B.S., S.K.N., U.S.C. conducted formal data analysis. Department of Health & family welfare, Government of West Bengal, India was convinced by A.K.C. to allocate their infrastructure for this study. A.K.C., B.B., B.S., S.K.N., S.S., U.S.C. drafted the original manuscript. SS reviewed and performed statistical analysis.

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Legends:

Fig.1. TMPRSS2 from host cell to facilitate transfer of RNA in epithelial cell.

TMPRSS2 is coming out of epithelial cell (a, b), cleaving S protein (c, d) and making vestibule (priming) between virion and epithelial cell (e).

Fig.2. Spray bottle for NSNSG procedure. It delivers 0.5 ml with each full stroke

Table1. Total number of RT-PCR negative patients in both groups following 72, 96 and 120 hours.

Table2. SS in HRCT lung done on different days compared with the SS found on admission.

Table 1. Total number of RT-PCR negative patients

	Case	Control
Total enrolment	113	138
Inclusion (ct value 10–25)	61	64
RT-PCR Negative on 6 hourly NSNSG for 72 hours	8/19 (42%)	6/21 (28%)
RT-PCR Negative on 6 hourly NSNSG for 96 hours	10/26 (38%)	5/23 (21%)
RT-PCR Negative on 6 hourly NSNSG for 120 hours	11/16 (68%)	6/20 (30%)
Total Number of patients became RT-PCR Negative	29/61 (47 %)	17/64 (26%)

Table 2. Compared SS in HRCT lung

	Severity score (SS) either was static or came down or increased.	HRCT done on						Total- n=56	SS Improved (%).
		4th Day (n=7)	5th Day (n=6)	6th Day (n=17)	7th Day (n=14)	8th Day (n=6)	9th Day (n=6)		
Case	Static (n=13), came down (n=18) (range 1-6 points, mean 2.71)	4	4	7	9	5	2	n=31	91
	Increased: 4-8 points.	–	–	1	2	–	–	n=3	
Control	Static (n=5), came down (n=9) (range 1-5, mean 2.23)	–	1	9	2	–	2	n =14	63
	Increased (n=8) (range 1-12, mean 4.375)	3	2	–	1	1	1	n=8	

Involvement of each lobe is expressed in points score. Point 1 = < 5%, Point 2 = 5-25%, Point 3 = 25-50%, Point 4 = 50-75%, Point 5 = >75% involvement. Lung has got five lobes. SS is calculated on adding the points. So total of severity score may be up to 25 points