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Practice Points

Gargling with povidone iodine has a short-term inhibitory effect on SARS-CoV-2 in patients with COVID-19

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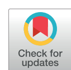

It is known that povidone iodine (PVP-I) solutions have virucidal action against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) *in vitro* [1–3]. In this study, the saliva of patients with coronavirus disease 2019 (COVID-19) was collected up to 2 h after gargling with PVP-I, and the dynamics of SARS-CoV-2 infectivity in saliva were assessed by real-time reverse transcription-polymerase chain reaction (rRT-PCR) and determination of the infectious viral load.

Patients (aged ≥20 years) who had symptoms indicative of SARS-CoV-2 infection within the last 7 days or asymptomatic patients with a cycle threshold value <40 for SARS-CoV-2 ribonucleic acid (RNA), as determined by rRT-PCR of saliva, were included in this study (N=35). Patients who had an iodine allergy or thyroid disease were excluded. This study was approved by the Institutional Review Board (Hokkaido University Hospital Division of Clinical Research Administration Number: 020-0111), and written informed consent was obtained from all participants.

Baseline saliva samples were collected prior to intervention with iodine. Patients rinsed their mouths for 20 s with 20 mL of PVP-I gargle solution (Meiji Co., Ltd, Tokyo, Japan), which was diluted 15 times with water. Patients repeated gargling with PVP-I three times, then rinsed their mouths with water. After gargling, saliva was collected at four time points: immediately after gargling, and 30, 60 and 120 min later. Patients collected saliva samples themselves by spitting into a sterile cup (PP Screw Cup 50; ASIAKIZAI Co., Tokyo, Japan). Viral RNA was quantified in the samples by RT-PCR and the virus was titrated in cultured cells.

For RT-PCR, 200 μL of saliva was added to 600 μL of phosphate buffered saline, mixed vigorously, then centrifuged at 20,000 × g for 5 min at 4 °C; 140 μL of the supernatant was used as the sample. rRT-PCR was conducted in accordance with the manual for the Detection of Pathogen 2019-nCoV Version 2.9.1. (https://www.niid.go.jp/niid/images/lab-manual/2019-nCoV20200319.pdf). Total RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany), and rRT-PCR was performed using

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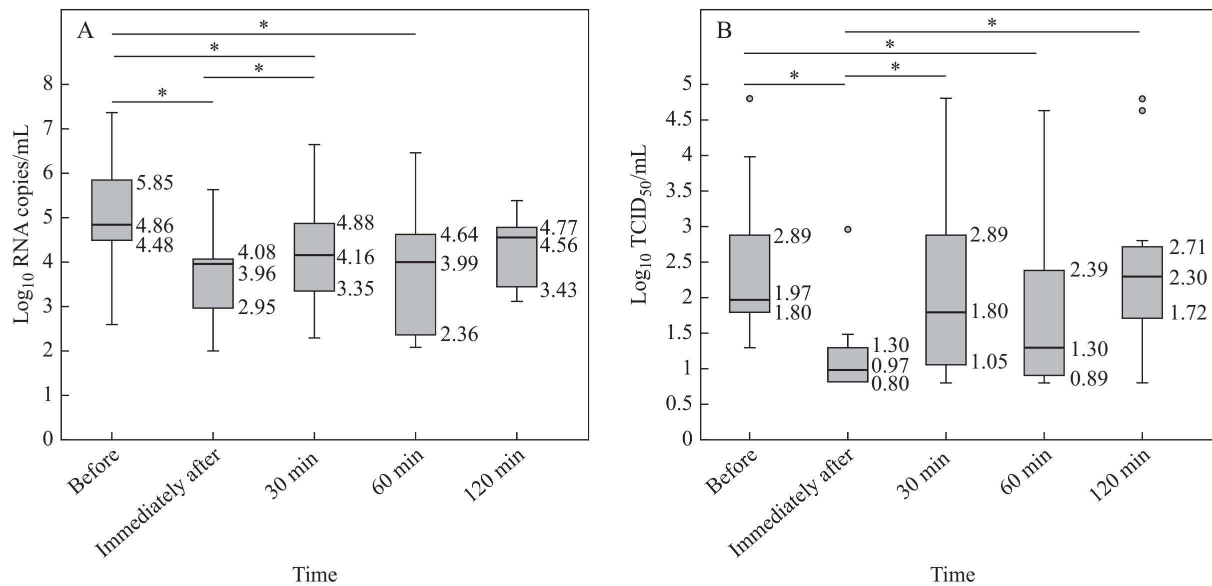

Figure 1. Changes in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) RNA level (A) and viral titre (B) in saliva samples before and after gargling with povidone iodine. Box plots [median, interquartile range (5th and 95th percentiles)]. $TCID_{50}$, tissue culture infectious dose; RNA, ribonucleic acid; before, before gargling; immediately after, immediately after gargling; min, minutes after gargling. *$P<0.05$.

the QuantiTect Probe RT–PCR Kit (QIAGEN) in the QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). The sequences of the primers and TaqMan probe used for detection of the SARS-CoV-2 genome were as follows: forward primer (NIID_2019 -nCOV_N_F2, 5′ AAATTTTGGGGACCAGGAAC 3′), reverse primer (NIID_2019-nCOV_N_R2, 5′ TGGCAGCTGTGTAGGTCAAC 3′), and TaqMan probe (NIID_2019-nCOV_N_P2, 5′ FAM-ATGTCGCGCATTGGCATGGA-BHQ 3′).

Viral titres were determined as the 50% tissue culture infective dose ($TCID_{50}$) of the virus. Vero E6 cells expressing the type II transmembrane serine protease (Vero-TMPRSS2) [4] were seeded into 96-well plates and incubated with a serial dilution of patient saliva. Three days later, cytopathic effects were examined. The samples in which infectious SARS-CoV-2 was detected before PVP-I gargling (i.e. $>10 \times TCID_{50}$ of the virus) were targeted in this study.

Of a total of 35 patients with COVID-19, 24 were excluded from the study because they had undetectable SARS-CoV-2 RNA or a viral titre $<10 \times TCID_{50}$ in their baseline saliva sample. Thus, 11 patients were analysed in this study. The average viral RNA copies and viral titres were compared at each time point using the Wilcoxon rank sum test. $P<0.05$ was considered to indicate significance.

Figure 1A shows the change in viral RNA copies (log_{10} copies/mL) after PVP-I gargling. A significant decrease in viral RNA was observed in the samples taken immediately after gargling and 30 and 60 min after gargling, compared with before gargling.

Figure 1B shows the change in viral titre (log_{10} $TCID_{50}$/mL) after PVP-I gargling. A significant decrease was observed in viral titre immediately after gargling and 60 min after gargling, compared with before gargling. The viral titres in the samples 30 min after gargling showed no significant difference (P=0.055), but the median value was lower compared with the samples taken before gargling.

In conclusion, viral copies and titres were significantly decreased 60 min after gargling. The reason for the temporary increase in viral titre 30 min after gargling may become clear as the number of examined cases increases. However, importantly, these data indicated that PVP-I gargling effectively suppressed SARS-CoV-2 infectivity in saliva for 60 min. The application of PVP-I may be an effective measure to reduce the infection risk in situations such as during dental treatment and oral examination by physicians.

A limitation of this study is that it was performed under simple conditions to minimize the risk of infection, and was carried out without a control group gargling with water. Despite this limitation, the findings support the use of PVP-I gargling for the prevention of infections via saliva over a short period.

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Conflict of interest statement
None declared.

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