The detection of SARS-CoV-2 RNA in indoor air of dental clinics during the COVID-19 pandemic

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Abstract

In the indoor environment of dental clinics, dental personnel and patients are exposed to a risk of infection because of the transmission of SARS-CoV-2 via particles or droplets. This study investigated the presence of SARS-CoV-2 RNA in indoor air of dental clinics in Tehran, Iran. Air sampling was done ($n = 36$) collecting particulate samples on PTFE filters at flow rates of 30 to 58 L/min. The samples were analyzed with novel coronavirus nucleic acid diagnostic real-time PCR kits. Only 13 out of 36 samples were positive for SARS-CoV-2 RNA. Logistic regression showed that sampling site's volume, PM_{2.5} concentration, number of people, and number of active patient treatment units were significantly positively related with the presence of SARS-CoV-2 RNA. Thus, strategies to control the spread of COVID-19 should include reducing the number of infected people in dental clinics, adding filtration systems, and/or improving ventilation conditions.

Keywords COVID-19 · SARS-CoV-2 · Indoor air · Dental clinics · Airborne transmission · PM_{2.5}

Introduction

In December 2019, the severe acute respiratory syndrome coronavirus (SARS-CoV-2) emerged in Wuhan City, Hubei (China), and spread quickly throughout the world. On March 11, 2020, the World Health Organization (WHO) announced a pandemic

(FERNANDES et al. 2020; IZZETTI et al. 2020; MUNIYAPPA and GUBBI 2020; WANG et al. 2021). In Iran, the first officially known case of COVID-19 was identified on February 19, 2020, by the Iranian Ministry of Health and Medical Education (MOHME). After this date, the number of COVID-19 cases increased day by day according to MOHME data. The virus

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rapidly spread to other parts of Iran, and by March 5, 2020, all 31 provinces had significant numbers of infected individuals (RAOOFI et al. 2020).

The most common clinical manifestations of COVID-19 include sore throat, fever, cough, shortness of breath, and fatigue. According to previous studies, the incubation period of the virus varies between 1 and 14 days, and asymptomatic infections have been observed (HU et al. 2020; ISAIFAN 2020; KLOMPAS 2020; NOBEL et al. 2020). The primary transmission routes of COVID-19 virus are airborne and direct contact. Airborne infection occurs via droplets and particles emitted from infected individuals by speech, coughing, sneezing, and breathing. Direct contact infection occurs via contact with contaminated surfaces including people followed by touching of the eyes, mouth, or nose. Saliva can also play a role in transmission of infection via the airborne and direct contact routes (JARVIS 2020). During the COVID-19 pandemic, both dentists and dental assistants are at high risk of being infected in oral dental treatment practice due to their exposure to bacteria, viruses, and fungi resident in respiratory tract and the oral cavity. Furthermore, the high-speed gas and running water of dental handpieces generate high particle and droplet concentrations that incorporate the patient's saliva or blood. These fluids contaminate the clinic space and the surfaces of diverse dental devices, increasing the risk of infection for clinic personnel and subsequent dental patients. To stop cross-infection and the outbreak of the epidemic, most dental clinics around the world suspended dental and oral healthcare during a lockdown period (COULTHARD 2020). The American Dental Association (Corman et al.) stated that dental emergencies are life-threatening and need urgent therapy to stop tissue bleeding, reduce major infections, or intense pain. Thus, the entire dental team is at risk of acquiring COVID-19 through local transmission in the dental clinic. The closure of dental clinics at least for emergencies was not suggested. Some studies have reported negative results with respect to the presence of this virus in the indoor air of hospitals (FARIDI et al. 2020; MASOUMBEIGI et al. 2020). However, SARS-CoV-2 RNA was detected in the indoor air of different hospital wards (LIU et al. 2020; RAZZINI et al. 2020). Thus, concern about dentistry practice-driven coronavirus transmission has been broadly known worldwide. To our knowledge, no studies have been reported to date on the presence of SARS-CoV-2 in the indoor air of dental clinics. Therefore, the present research evaluated the contamination of the air by SARS-CoV-2 RNA in dentistry clinics during COVID-19 pandemic outbreak in Tehran, Iran.

Material and methods

Study area and air sampling

We conducted the present study between August and December 2020 in the dental clinics of Shahid Beheshti

University of Medical Sciences, Tehran, Iran. Tehran, with a population of around 9 million, is the most populous city in Iran and is the nation's capital. In total, 36 air samples were collected from seven clinics in the city. For all of the sampling sites, an AV1000 high-volume air sampler (China) was employed to collect fine particulate matter (PM_{2.5}) samples on polytetrafluoroethylene filters (PTFE, diameter = 9 cm, pore size = 0.2 µm). The sampling flow rates and sampling durations were 30–58 L/min and 1–2 h, respectively. The sampler height above the floor was between 1 and 2 m, and the approximate distance of 0.8 to 2 m was maintained with the individuals in the room. During the sampling process, several characteristics of sites including surface area, height, temperature, relative humidity, overall site volume, number of active treatment units, number of people, and ventilation conditions (type of ventilation, number of windows, number of ventilators, flow velocity of ventilator's outlet, and air change per hour) were measured and reported. Also, air changes per hour parameter for the mechanical ventilators were estimated by the following equation:

$$ACH = \frac{Q}{V} = \frac{\sum_{i=1}^n (A_i \times u_i)}{V}$$

where

ACH is air change per hour, Q is total outlet flow from mechanical ventilators (m³/h), V is site volume (m³), A_i is area of ventilation outlet channel (m²), u_i is outlet flow velocity of mechanical ventilator (m/s) (CHINN and SEHULSTER 2003).

Viral RNA concentrations

After the air samples were collected, they were transferred to the Virology Laboratory (RIGLD, Shahid Beheshti University of Medical Sciences) under cool conditions (temperature 4°C). Immediately in the laboratory, the filters were placed in 50-mL centrifuge tubes by adding 20 mL of Tris-glycine-beef extract buffer (TGBE, 100-mM Tris, 50-mM glycine, 3% (w/v) beef extract, pH = 9.5) followed by shaking at room temperature for 20 min. Then, the filters came out of the tubes, and 2.5 % (w/v) NaCl and 12.5 % (w/v) PEG-6000 at final concentration were added (approximately 25mL). The solution was mixed at 120 rpm by shaker for 2 h at 4°C and, after that, centrifuged for 30 min at 11,000 g (ZUO et al. 2013). The pellet was suspended in 200 µL of PBS (pH=7.2) and stored at –80 °C until subsequent analyses.

To estimate the performance of the virus extraction method, a virus should be selected that had a similar structure and similar target tissue. Also, the virus must have been from the same family as SARS-CoV-2. For this purpose, infectious bronchitis virus (IBV), a member of the family Coronavirus and belonging to Gammacoronavirus, was selected. The diluted IBV virus was inoculated on the surface of filter. Then,

filter was placed in a biosafety chamber for 2 h, and concentration process was conducted. After that, 20 μL of the vaccine was added to 120 μL of water and was extracted, and an RT-qPCR test was performed on the target RNA. The tests demonstrated a recovery percentage of about 25% in comparison with first value (HADEI et al. 2021).

Extraction of viral RNA

In the second concentration phase, 140 μL of final solution were obtained. QIAamp RNA Mini Kit (Qiagen, Germany) was used for extraction of viral nucleic acids from the final solution. Finally, final volume of 60 μL was produced, and the isolated nucleic acids were placed at $-20\text{ }^{\circ}\text{C}$ chamber until detection test.

Detection of SARS-CoV-2 RNA

The presence of SARS-CoV-2 RNA was investigated by the novel coronavirus nucleic acid diagnostic real-time PCR kit (Sansure Biotech, China) based on the manufacturer's protocol. Sequences of nucleocapsid protein N and ORF1ab genes were chosen as the target zone and detected using a Rotor-Gene Q instrument (Qiagen, Germany).

Quality assurance. Quality control (QA. QC)

In this study, to ensure the elimination of false-positive and false-negative results, quality control of the RT-PCR tests occurred using positive and negative control samples.

Statistical analyses

Descriptive statistics of characteristics of sampler and sampling sites were investigated. Descriptive statistics of the quantitative variables (sampling site's volume (m^3), number of people, number of units, temperature, relative humidity, $\text{PM}_{2.5}$ concentration, number of windows, and number of mechanical ventilators) and qualitative variables (type of ventilation and presence of SARS-CoV-2) were calculated. Also, the characteristics of sampling sites with presence/absence of SARS-CoV-2 were compared. Normality of data and equity of variances were tested using Shapiro-Wilk and Levene's test, respectively. If datasets passed both tests, a parametric test (independent *t*-test) was conducted; otherwise, non-parametric analysis (Mann-Whitney U test) was performed.

Logistic regression was used to explore the effects of the parameters on the presence of SARS-CoV-2. These parameters were used as continuous independent variables, and the presence/absence of SARS-CoV-2 was entered to the model as a binary variable of zero and one. The associations between the variables were expressed as odds ratios (ORs) with 95%

confidence intervals (CIs). The modeling was performed using R programming software v. 3.6.3.

Results and discussion

The details of sampling sites such as the sampling site's volume, surface area, number of people, number of units, temperature, relative humidity, $\text{PM}_{2.5}$ concentration, number of windows, and ventilation conditions (type of ventilation, number of windows, number of ventilators, flow velocity of ventilator's outlet, and air change per hour) are presented in Table 1. The sampled air volume at the various sites varied from 2900 to 5972 L. Table 2 summarizes the results of descriptive statistics for sampling sites and environmental conditions. Overall, the average (± 1 standard deviation) relative humidity and temperature during sampling periods were 45.6 (7.4%) and $28.5 (\pm 2.6)\text{ }^{\circ}\text{C}$, respectively. Thirty-six air samples were collected from the dental clinics. About 36% of the air samples were positive for SARS-CoV-2 RNA occurrence. These results are similar to previous studies that reported that SARS-CoV-2 was present in indoor air (JIN et al. 2021; LIU et al. 2020; RAZZINI et al. 2020). These results support the hypothesis of airborne transmission of SARS-CoV-2 in the indoor environment. A study in Iran examined the airborne potential of SARS-CoV-2 and stated there was no airborne transmission potential (MASOUMBEIGI et al. 2020). The effects of the sampling site's volume, distance between the individuals present, number of people present during sampling, number of active treatment units, number of ventilators, air temperature, relative humidity, $\text{PM}_{2.5}$, windows, speed of exhaust air, and air change per hour on the presence of SARS-CoV-2 RNA in the air are provided in Table 3. The number of people, number of dental treatment units, $\text{PM}_{2.5}$ concentration, and sampling site volume were positively related with the presence of SARS-CoV-2 RNA. All of these relationships were statistically significant. Air temperature, speed of exhaust air, and air changes per hour were negatively associated to SARS-CoV-2 RNA presence (Table 4). These results indicate that the increase in speed of exhaust air and number of air changes per hour reduced the presence of SARS-CoV-2 in indoor locations below the detection limits. Our results are consistent with other studies (HADEI et al. 2021; RAZZINI et al. 2020). They reported that the presence of SARS-CoV-2 in air of hospitals and public places could be associated to the number of people. More people lead to higher concentrations of SARS-CoV-2 RNA. Thus, reducing the number of people allowed to be present at any given time and number of active treatment units can likely reduce the transmission of the virus in dental clinics. Previous studies have reported that durability of SARS-CoV-2 decreases with temperature reduction in different environments and may elucidate the negative relation with temperature (CHENG et al. 2020; YAO et al. 2020). In

Table 1 Environmental status of dental clinics

Samp.no.	Characteristics of sampling sites											
	Site volume (m ³)	T ^a (C)	RH ^b (%)	No. of people	No. of active units	PM _{2.5}	Ventilation condition		No. of mechanical ventilators	Outlet flow velocity of mechanical ventilators (m/s)	Total outlet flow from mechanical ventilators (m ³ .h)	No. of mechanical ventilation cycle (Fernandes et al.)
							VT ^c	No. of windows				
1	28	28.2	51.4	2	1	33	F	0	1	1.9	273.6	9.77
2	200	28.8	43.2	12	4	36	N & F	1	3	1.9	820.8	4.10
3	390	26.8	51.7	17	4	67	N & F	7	4	1.5	864	2.22
4	80	28.5	49.9	14	2	37.5	N	3	0	0.0	0	0.00
5	75	34.7	29.7	4	1	27	N	3	0	0.0	0	0.00
6	150	33.5	31.4	4	1	31.5	N	2	0	0.0	0	0.00
7	28	32.1	40.3	3	1	36	F	0	1	1.9	273.6	9.77
8	240	28.4	45	9	1	63	F	0	2	2.8	806.4	3.36
9	75	27.6	58.3	7	1	23.9	F	0	1	2.7	388.8	5.18
10	120	29.8	49.2	5	1	39	N	2	1	0.0	0	0.00
11	168	24.8	44	9	2	61.5	N & F	1	3	1.5	648	3.86
12	70	28	39.6	5	1	18	N	0	0	0.0	0	0.00
13	54	26.8	37.4	12	3	52.5	N & F	1	1	0.0	0	0.00
14	420	25.4	51.7	16	8	34.5	N & F	7	4	1.5	864	2.06
15	220	25.1	43.2	13	5	25.4	N & F	1	3	1.9	820.8	3.73
16	75	25.2	45	6	1	64.5	N & F	1	1	2.3	331.2	4.42
17	60	23.6	49.2	5	1	39	N	1	1	0.0	0	0.00
18	75	27.9	39.5	3	1	58.5	N	1	0	0.0	0	0.00
19	360	27.3	51.7	21	6	55.5	N & F	5	3	1.5	648	1.80
20	54	25.2	45.1	3	1	30	N & F	1	1	1.9	273.6	5.07
21	60	25.8	47.2	4	1	31.5	N & F	1	1	2.0	288	4.80
22	90	28.6	49.8	7	2	34.5	N	3	0	0.0	0	0.00
23	200	28.5	44.5	6	2	52.4	N & F	2	2	1.9	547.2	2.74
24	90	26.7	37.5	4	1	28.4	N & F	2	1	0.0	0	0.00
25	30	29.1	52.3	3	1	36	N & F	1	1	2.1	302.4	10.08
26	168	24.7	43.7	7	2	21	N & F	1	2	1.5	432	2.57
27	60	27.9	45.5	4	1	25.5	F	0	1	2.8	403.2	6.72
28	390	26.8	51.2	12	4	28.5	N & F	7	3	1.5	648	1.66
29	60	28.6	44.9	4	1	54	F	1	1	2.7	388.8	6.48

Table 1 (continued)

Characteristics of sampling sites												
Samp.no.	Site volume (m ³)	T ^a (C)	RH ^b (%)	No. of people	No. of active units	PM _{2.5}	Ventilation condition					
							VT ^c	No. of windows	No. of mechanical ventilators	Outlet flow velocity of mechanical ventilators (m/s)	Total outlet flow from mechanical ventilators (m ³ .h)	No. of mechanical ventilation cycle (Fernandes et al.)
30	45	27.6	58.4	3	1		N & F	25.65	N & 1	1.9	273.6	6.08
31	28	27.6	45.2	2	1		F					
32	60	25.1	45.2	3	1		N	25.3	N 0	2.0	288	10.29
33	30	32.1	41.3	4	1		N	54	N 1	0.0	0	0.00
34	85	29.7	54.2	4	1		F	37.5	F 0	1.9	273.6	9.12
							N & F	27	N & 2	1.8	259.2	3.05
35	70	30.1	48.7	3	1		F	31.5	N & 2	1.6	230.4	3.29
36	210	29.3	46.1	11	4		F	49.5	N & 1	1.9	820.8	3.91

^a Temperature; ^b relative humidity; ^c ventilation type: *N* natural, *F* forced, *N & F* combination of natural and forced ventilation

Table 2 Summary statistics of sampling sites and environmental conditions

Parameter	Negative results					Positive results				
	Mean	Median	Std. deviation	Maximum	Minimum	Mean	Median	Std. deviation	Maximum	Minimum
Distance between sampler and individuals (m)	1.20	1.00	0.35	2.00	0.80	1.31	1.00	0.44	2.00	0.80
Site's volume (m ³)	85.57	70.00	78.81	390.00	28.00	203.85	200.00	122.67	420.00	54.00
Temperature (°C)	28.55	28.00	2.56	34.70	25.10	26.87	26.80	2.01	29.80	23.60
Relative humidity (%)	45.63	45.20	7.40	58.40	29.70	46.35	45.20	4.20	51.70	37.40
No. of people	5.13	4.00	3.39	14.00	2.00	10.23	9.00	5.33	21.00	3.00
No. of active dental units	1.39	1.00	1.03	5.00	1.00	3.00	2.00	2.16	8.00	1.00
PM _{2.5} (µg m ³)	33.49	31.50	11.30	64.50	18.00	48.07	52.40	13.29	67.00	21.00
No. of windows	1.39	1.00	1.59	7.00	0.00	2.31	1.00	2.39	7.00	0.00
No. of mechanical ventilator	0.91	1.00	0.79	3.00	0.00	2.23	2.00	1.24	4.00	0.00
Outlet flow velocity of mechanical ventilators (m s)	1.43	1.90	1.02	2.80	0.00	1.23	1.50	0.92	2.80	0.00
Air change per hour (Fernandes et al.)	4.33	4.42	3.74	10.29	0.00	2.05	2.22	1.59	4.10	0.00

our research, there was a positive relation between the presence of SARS-CoV-2 and PM_{2.5} concentration. These results have demonstrated that SARS-CoV-2 may have airborne transmission potential in dental clinics (KAYALAR et al. 2021). Also, airborne transmission of SARS-CoV-2 was previously examined by Setti et al. in air of industrial area of Bergamo (Italy) (SETTI et al. 2020). They stated that it could be concluded that the infection was transmitted via PM_{2.5} particles. Although the airborne transmission of SARS-CoV-2 has been evaluated, it was pointed out this virus had not airborne potential (FARIDI et al. 2020). Different analysis and sampling methods can cause these contradictions. The sampled air volume is a crucial agent for these studies, because collecting a low sampled air volume (low sampling duration or low sampling flow rate) could generate a false-negative result (HADEI et al. 2021). Van Doremalen et al. (2020) reported that SARS-CoV-2 survived in aerosols at least for 3 h under laboratory conditions (VAN DOREMALEN et al. 2020). These results indicated that the virus could be transmitted through aerosol processes in such environments (SANTARPIA et al. 2020). Clearly some aerosol-generating practices are related with a significant enhancement in the risk of disease transmission (LUO et al. 2021). However, it can be concluded that ventilation is an efficient technology for reducing exposures in dental clinics. WHO has recommended that a ventilation rate of at least 288 m³ h⁻¹ per person is an efficient method for control of airborne transmission virus such as influenza and SARS in healthcare settings. Some variables such as PM_{2.5} concentration, temperature, and relative humidity affect the stability and presence of SARS-CoV-2 in indoor air. Therefore, controlling these parameters in optimal values can play an important role in

preventing virus spreading (BABOLI et al. 2021). In addition, although research on COVID-19 transmission is not yet definitive, precautionary measures should be used including efficient ventilation, indoor air purifiers, and avoidance of air recirculation without adequate filtration in healthcare setting (ZHAO et al. 2020).

In the current study, there were several limitations. Because of the requirements of each site, the maximum distance maintained between the sampler and individuals was 2 m. Under these circumstances, it will be difficult to say with certainty that the virus is being transmitted over longer distances. However, the statistical relationship found between ventilation conditions, PM_{2.5}, and the presence of the viral RNA suggests its airborne nature. This study did not investigate the viability and infectivity of the virus. RT-PCR is a reliable tool for detecting the SARS-CoV-2 RNA in respiratory secretions (CORMAN et al. 2020), but it is important to note that PCR does not necessarily determine the presence of viable, infective virus (OTTER et al. 2016). Future study must focus on viability conditions of SARS-CoV-2 in dental practices.

Conclusions

This research provides the first report of the presence of SARS-CoV-2 RNA in the indoor air of dental clinics. In this study, 36 samples were analyzed for the presence of SARS-CoV-2 RNA in the indoor air of dental clinic (Tehran, Iran). The results of the research have identified the presence of SARS-CoV-2 viral RNA in about 36% of these samples. Thus, these results provide an alert with respect to the airborne transmission of SARS-CoV-2 in dental practices. Positive

Table 3 Presence of SARS-CoV-2 in indoor air samples of dental clinics

Samp. no.	Sampling duration (h)	Characteristics of sampler				
		Sampling flow rate (L min ⁻¹)	Sampling volume (m ³)	Height of sampler (m)	Distance from human individuals (m)	SARS-CoV-2
1	1.00	50	2.972	1.4	1.0	Negative
2	1.00	50	2.973	2.0	1.0	Positive
3	1.00	50	2.971	1.4	1.0	Positive
4	1.00	50	2.973	1.6	0.8	Negative
5	1.00	58	3.483	1.0	1.2	Negative
6	1.00	58	3.448	1.0	1.2	Negative
7	1.50	35	3.146	1.2	0.8	Negative
8	1.00	49	2.912	1.6	2.0	Positive
9	1.00	50	2.972	1.6	1.5	Negative
10	1.00	50	2.974	1.4	1.5	Positive
11	2.00	35	4.101	1.5	1	Positive
12	1.00	55	3.287	1.4	1	Negative
13	1.50	40	3.577	1.6	2	Positive
14	2.00	50	5.897	1.4	1	Positive
15	2.00	50	5.971	2	1	Negative
16	2.00	40	4.768	1.6	1	Negative
17	2.00	50	5.972	1.4	1	Positive
18	1.00	50	2.981	1.2	1.5	Negative
19	2.00	45	5.391	1.0	1.5	Positive
20	1.00	49	2.901	1.6	2	Negative
21	1.50	50	4.462	1.4	1	Negative
22	1.00	50	2.987	1.0	1	Negative
23	2.00	30	3.551	1.0	2	Positive
24	1.00	55	3.279	1.0	1.5	Negative
25	1.50	40	3.582	1.2	0.8	Negative
26	1.50	45	4.036	1.6	0.8	Positive
27	1.00	50	2.978	1.4	1.0	Negative
28	2.00	30	3.588	1.4	1.0	Negative
29	1.50	55	4.943	1.2	1.5	Negative
30	1.50	35	3.145	1.2	1.0	Negative
31	1.00	50	2.987	2.0	2.0	Negative
32	1.00	50	2.984	1.0	1.0	Positive
33	2.00	30	3.542	1.4	1.0	Negative
34	1.00	50	2.981	1.0	1.4	Negative
35	1.00	50	2.921	1.0	1.5	Negative
36	2.00	45	5.372	1.6	1.2	Positive

Table 4 Results of the logistic regression models for the effects of different parameters on the presence of SARS-CoV-2 in air

Independent variables	Odds ratio (95% CI)	<i>p</i> value
Sampling site's volume	1.02 (1.01, 1.03)	0.01
Distance	2.05 (0.33, 13.08)	0.43
Temperature	0.71 (0.46, 0.98)	0.06
Relative humidity	1.02 (0.91, 1.14)	0.74
PM _{2.5}	1.09 (1.03, 1.17)	0.01
Number of people	1.31 (1.09, 1.64)	0.01
Number of units	2.02 (1.21, 4.09)	0.02
Windows	1.28 (0.89, 1.93)	0.19
Number of ventilators	1.22 (0.89, 1.71)	0.22
Speed of exhaust air	0.81 (0.39, 1.64)	0.55
Air change per hour	0.77 (0.56, 0.98)	0.06

relationships between SARS-CoV-2 RNA and site volume, PM_{2.5}, number of people, and number of dental treatment units were obtained. These findings suggest the need to control the occupancy of these spaces (number of individuals in certain environments) especially in hospitals and dental clinics. Rigorous strategies need to be implemented to diminish the risk of infection for patients and healthcare professionals working in dental clinics through improved ventilation and/or effective air cleaning systems. Also, there is a need to reassess the commonly used recommendations for ventilation rates in closed environments relative to that provided by the WHO to reduce the potential for infections in locations where active generation processes are ongoing.

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Data availability The datasets used and/or analyzed during this study are included in this published article.

Declarations

Ethics approval The approval number of ethics of the present study was “IR.SBMU.PHNS.REC.1399.075.”

Consent for publication Not applicable

Competing interests The authors declare no competing interests.

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