Atmospheric Pollution Research 15 (2024) 102245

Contents lists available at ScienceDirect

Atmospheric Pollution Research

journal homepage: www.elsevier.com/locate/apr

Identification of respiratory virus in indoor air of hospitals: A comparison of adult and children's hospital

Gholamreza Goudarzi [a,b,c], Yaser Tahmasebi Birgani [c], Niloofar Neisi [d,e], Ali Akbar Babaei [c], Mehdi Ahmadi [c], Zeynab Baboli [f,*]

[a] Air Pollution and Respiratory Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[b] Center for Climate Change and Health Research (CCCHR), Dezful University of Medical Sciences, Dezful, Iran
[c] Environmental Technologies Research Center (ETRC), Medical Basic Sciences Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[d] Department of Virology, the School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[e] Alimentary Tract Research Center, Imam khomeini Hospital Clinical Research Development Unit, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
[f] Department of Environmental Health Engineering, Behbahan faculty of Medical Sciences, Behbahan, Iran

ARTICLE INFO

Keywords:
SARS-CoV-2
Indoor air sampling
Adult and children's hospital

ABSTRACT

Air sampling was done inside adult and children's hospitals that were selected to treat severe cases of COVID-19. Influence of peripheral factors such as particle concentration, air velocity, sampling point dimensions, distance from the patient bed, sampling time, the flow rate of sampling pump, and factors related to COVID-19 patients (disease period, mask use, number, and age) were analyzed using multivariate analysis (RT-PCR). The results showed that 5.8% (N = 8) of indoor air samples were positive for the presence of the coronavirus. The presence of viruses in the indoor air of hospitals has a strong positive relationship with particles and the age of patients while it has a reverse relationship with the air cleaner, ventilation system, and distance from the patients. Therefore, the higher particle concentration, the age of hospitalized patients, and the remarkable number of patients increase the probability of the presence and identification of the coronavirus in the indoor air of hospital wards. Also, the presence of an air cleaner, a suitable ventilation system especially a mechanical one, and increasing the distance from the patients reduces the possibility of virus existence in the indoor air and its identification. In general, the results showed that the adult hospital has more polluted indoor air than the children's hospital in terms of the presence of SARS-COV-2. Sanitation and engineering measures like upgrading the ventilation system, particularly in vulnerable wards of hospitals are recommended.

1. Introduction

Individuals breathe an average of several million cubic feet of air during their lifetime, much of which contains dust-based bioaerosols and is a potential threat of upper respiratory tract. The most common route of transmission of respiratory infections is exposure to dust contaminated with bacteria, viruses, yeasts, and fungi. These bioaerosols can cause a variety of infectious and allergic diseases (O'Gorman and Fuller, 2008). Respiratory infections are of great importance due to their widespread and rapid spread and can play a very impressive role in the mortality of children and adults. Viruses are one of the most important causes of respiratory diseases that cause infection of the upper and lower respiratory tract. Viral airborne causing agents are very diverse and their distribution varies among patients based on age, season, and geographical areas (Rahmani et al., 2020; Lednicky and Loeb, 2013).

A new type of viral respiratory disease associated with 2019-nCoV disease is a new coronavirus called SARS-CoV-2 (WHO, 2020a; WHO, 2020b). With the increase in the incidence of this disease among people worldwide during the coronavirus pandemic due to quick transmission of the virus through inhalation of infected respiratory droplets, more attention should be paid to close contact (less than 2 m) with COVID-19 patients (Baboli et al., 2021; Seyyed Mahdi et al., 2020).

To investigate airborne transmission of virus spreading, it is necessary to take air samples using standard methods. There are several methods for sampling different air pollutants. Air sampling of viruses

Peer review under responsibility of Turkish National Committee for Air Pollution Research and Control.

* Corresponding author.
E-mail address: z.baboli@behums.ac.ir (Z. Baboli).

https://doi.org/10.1016/j.apr.2024.102245
Received 25 October 2023; Received in revised form 30 June 2024; Accepted 30 June 2024
Available online 4 July 2024

particularly SARS-COV-2 is a very tough task since they are affected by various factors such as temperature, humidity, concentration and type of virus, method of analysis, sampling duration, location, and target of sampling. Several active methods including impactors, impingers, filters, cyclone equipment, and passive methods have been used to take samples from different places (Booth et al., 2005; Verreault et al., 2008; Rahmani et al., 2020).

Due to the contradictions that are raised about the methods of transmission of respiratory diseases caused by the virus, as well as the existence of various instructions on how to protect and prevent the transmission of these diseases inside hospitals, and the pandemic nature of the disease, we decided to focus on the effective role of air and its quality in the transmission of the coronavirus. Accordingly, the present study was designed and carried out to investigate the factors affecting the spread, presence, and transmission of the virus through the air in adult and children's hospitals of Ahvaz city in Iran.

2. Materials and methods

2.1. Sampling location

This study was performed on the indoor air of two adult hospitals; Razi, and Sina, and one children's hospital which was Abuzar. Although these hospitals admitted a variety of patients before starting COVID-19, they were dedicated to the admission and hospitalization of COVID-19 patients during the pandemic. Air sampling was performed in two adult hospitals and the only children's hospital in July, August, and December 2021, simultaneously. Fig. 1 shows the Ahvaz city in Khouzestan province of Iran and Fig. 1c shows the location of the studied hospitals.

Air sampling was conducted in different parts of the COVID-19 ward. Sampling devices were installed in patients' rooms with distances of less than 1 m and more than 2 m from the patients' beds as the minimum and maximum distances, respectively. A total of 137 air samples were collected and analyzed in this study.

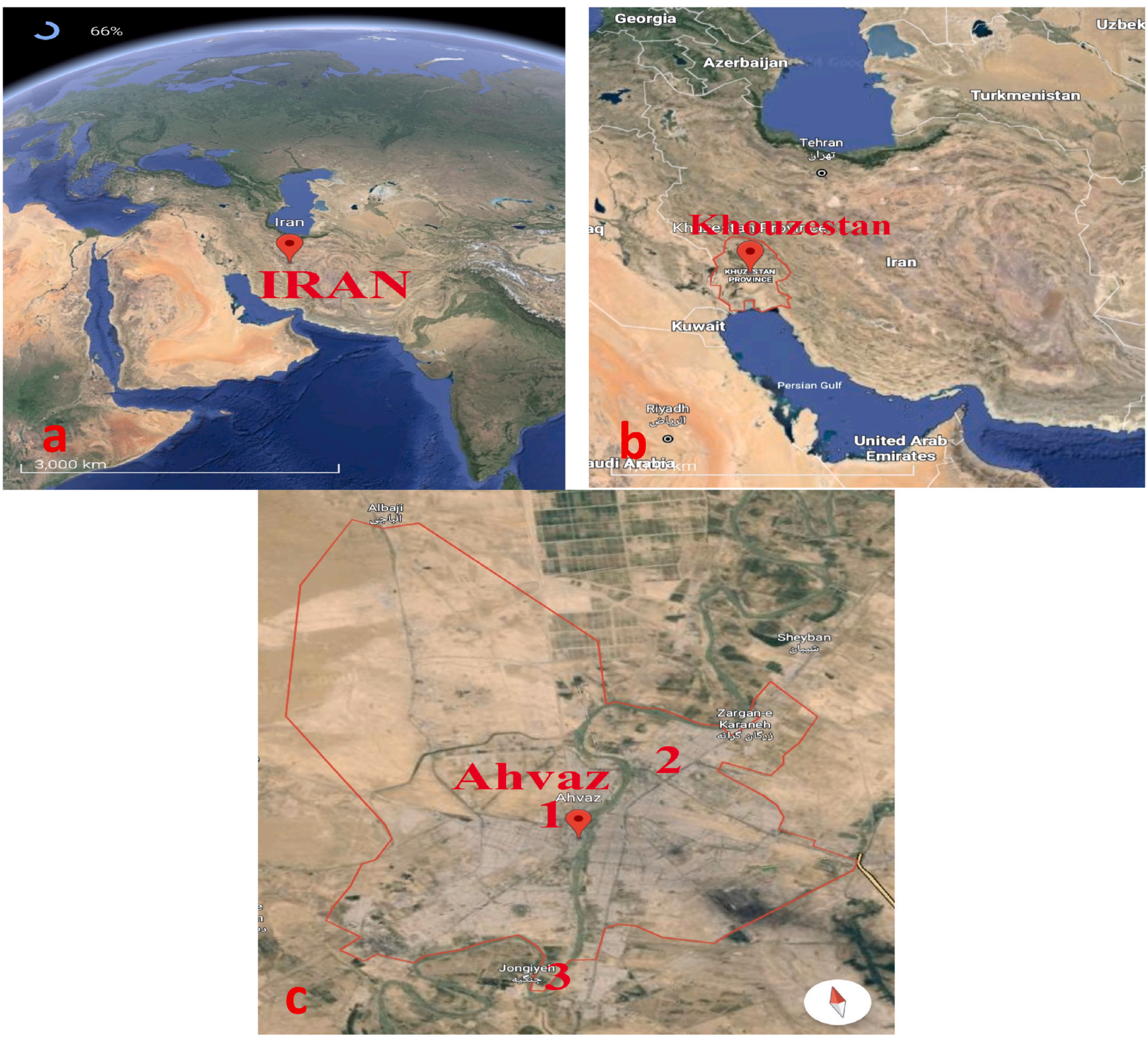

Fig. 1. The location of the studied hospitals in Ahvaz city; 1) Adult Hospital#1 (Razi); 2) Children's Hospital (Abuzar); 3) Adult Hospital#2 (Sina).

Out of 137 air samples in all three hospitals, 48 samples were taken at a distance of less than 1 m from the patient and 89 samples were taken at a distance of more than 2 m. In the first adult hospital (Razi), 51 air samples were taken from the COVID-19 ward, 16 samples from the corridor, 29 samples from the patients' rooms, and 6 samples from the nurses' station. In the second adult hospital (Sina), 21 air samples were taken from the COVID-19 ward, of which 9, 9, and 3 samples were taken from patients' rooms, corridors, and nurses' stations, respectively. In the children's hospital, 65 air samples were taken from the COVID-19 ward, of which 30, 19, and 10 samples were taken from patients' rooms, corridors, and nurses' stations. Also, six air samples were taken from the emergency department of the children's hospital due to the patient and outpatient referrals with COVID-19.

At the time of sampling, the age range of children admitted to the hospital was from 1 day to 12 years (<12 years), and the age range of the patients admitted to both adult hospital was from 20 through 80 years.

2.2. Air sampling

Active and passive methods were used for air sampling in the indoor air of hospitals. In the active sampling method, personal sampling pumps connected to the impinger containing 5 ml of the culture medium (VTM) or connected to the filter. The duration time of air sampling in the active method was from 3 to 480 min and the flow rate was 4–20 L/min (Pena et al., 2021; Rahmani et al., 2020). In the passive method, 5 ml of the culture medium was poured into an open Petri dish and the sampling time was from 15 to 240 min. After sampling, the culture medium was transferred from the sampler to a sterile container and transferred to the virology laboratory under a temperature of 4 °C (Baboli et al., 2021).

Out of 137 air samples in all three hospitals, 102 samples were taken by active sampling method and 35 samples were taken by passive sampling method. In the first adult hospital, 37 air samples were taken by the active method, and 14 air samples were taken by the passive method. In the second adult hospital, 16 air samples were taken by the active method, and 5 air samples were taken by the passive method. In the children's hospital, 49 air samples were taken by the active method, and 16 air samples were taken by the passive method. All samplers were mounted 1.5 m from the ground and at a horizontal distance of more than 1 m from the walls and windows (Rahmani et al., 2020; Borges et al., 2021).

2.3. Molecular tests

After sampling, the falcons containing the culture medium were kept in a freezer with a temperature of −70 °C until molecular tests. The air samples were analyzed by molecular methods to identify the coronavirus. First, the samples were concentrated. Second, the genetic material of the virus was extracted. Finally, molecular detection methods were performed using the RT-PCR technique. To concentrate air samples, 30 and 10 kDa MilliporeSigma ™ Amicon ™ Ultra Centrifugal Filter Units were used. Before performing the molecular tests, the frozen culture medium was thawed in the first step. Next, it was concentrated using 30 and 10 kDa Amicon filters for 10 min in a 4000 rpm centrifuge. It was sent to the extraction stage afterward (Ahmed et al., 2020; Walls et al., 2020; Lednicky and Loeb, 2013).

Sinaclon RNA Extraction Kit was used to extract RNA from existing samples. Next, the One Step Novel Coronavirus (2019-NCOV) Nocleic Acid Diagnostic Kit (PCR-Fluorencence Probing) by Sansure Biotech, Real-Time PCR testing for both RdRP and N genes containing specific FAM and TEXASRED and Internal Control gene (CY5) were employed. RT-PCR test results are determined based on the cycle threshold (Ct) value. Ct results below 40 and the sigmoid curve for both genes were considered as positive samples (Thuresson et al., 2022; Wang et al., 2022). Samples lacking the internal control gene Ct were replicated (Gorbalenya, 2020). In addition to air samples, mucus or saliva samples were taken from hospitalized COVID-19 patients at different sampling points and transported to the laboratory. All methods of maintenance, storage, freezing, and molecular tests were conducted for air samples and patient samples in the same way. Finally, viral load values related to molecular tests of positive air samples and positive patient samples were measured and presented.

2.4. Record the studied parameters

To find relationships between positive air samples and the most important factors all parameters including patient information, peripheral parameters, and conditions of sampling were recorded. The COVID-19 patients data including the number of patients admitted to each sampling point (No.p), the distance of sampling devices from patients (DIS), use of the mask by patients (mask), and age of patients (Age) were recorded in all sampling points. The indoor concentration of airborne particles was measured in three sizes of 10, 2.5, and 1 μm (PM_{10}, $PM_{2.5}$ & PM_1 ($\mu g/m^3$)) as environmental factors. Room dimensions such as area (A (m^2)) and volume (V (m^3)), presence/absence of air cleaner (AC), airflow velocity (AV (m/s)), and ventilation system (VS) were considered as sampling point conditions.

A flow meter device (KIMO brand, model LV110, made in Germany) was used to measure the speed of air indoors. Also, a portable dust monitor (Grimm Aerosol Technic, 11-B Model, Optical Spectrometer, Germany) was used to monitor particulate matter in the mentioned sizes.

2.5. Statistical analysis

A particular statistical method (multivariate) focusing on principal components analysis (PCA) and an informative map (self-organizing map, SOM) were applied to examine the statistically significant associations of positive air samples and the most important parameters. The PCA statistical test is one of the methods to reduce the dimension of multivariate data. A low-dimensional set of features is extracted from a high-dimensional set to help capture more information with fewer variables, and data visualization becomes more meaningful. To achieve dimensionality reduction, we select several components that explain an acceptable percentage of the total variance. On the other hand, to achieve the description of data dispersion structures, it is tried to relate the selected components to reasonable concepts. One of the tools to achieve this goal is the score chart and comparison charts. Another is to examine the role of variables in the components by examining the correlation coefficient of the variable and the desired component. After determining the influencing variables on each component and according to whether the correlations are positive or negative, interpretations are made about the concepts of the main components (Zuśka et al., 2019; Lovric, 2011). The SOM statistical test is a good tool for data clustering and can convert non-linear statistical relationships between input data into simple geometric relationships. The calculations of SOM are in the form of a non-parametric regression process that transforms the regression of a specific set of model vectors into the space of visible vectors in an algorithmic format. The clusters are regularized in a competitive learning process concerning the input variables. The two-dimensional map produced by SOM shows the similarity of the data while the identical output vectors are divided into groups separated by different colors. The method of implementing this type of network is in the Matlab collection, which is one of the strong and comprehensive software related to learning methods, including neural networks, which can be used for many applications(Guagliardi et al., 2022; De Oliveira et al., 2019).

3. Results

3.1. Distribution of samples based on sampling location

The information presented in Fig. 2 shows the number of samples

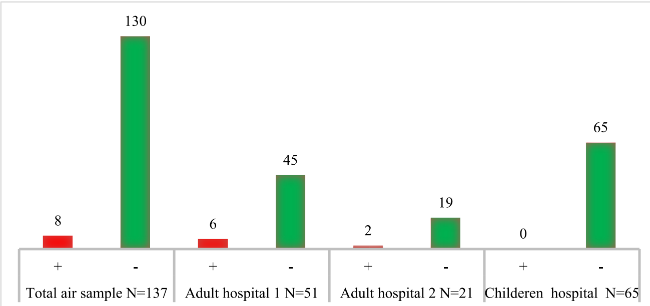

Fig. 2. Distribution of positive and negative air samples in adult and children hospitals.

prepared as well as the number of positive and negative samples in each hospital. Out of a total of 137 air samples, the presence of the SARS-CoV-2 virus was confirmed in 8 samples of adult hospitals while the children's hospital samples did not have any positive samples. In the first and second adult hospitals, 4 and 2 air samples were confirmed as positive at a distance of less than 1 m from the patient, respectively. In the first adult hospital, two positive air samples at a distance of more than 2 m from the beds were found in the hallway of the infectious ward.

In RT-PCR the Ct represents a specific detectable amplification signal which assesses recovery, transmission risk, and viral load. In the samples analyzed by PCR, the Ct value has an inverse relationship with the amount of target nucleic acid, so a lower Ct value indicates a higher amount of target nucleic. The infection status of the samples or the level of environmental contamination is evaluated based on the CT value. Those Cts <29 indicate a strong positive reaction and the presence of abundant target nucleic acid in the sample. The greater Cts in the range of 30–37 indicate a positive reaction and the presence of moderate target nucleic acid in the sample. The value of Cts from 38 through 40 indicates a weak reaction and the presence of very little target nucleic acid in the sample. The highest Cts, more than 40, indicate a negative reaction and the absence of very little target nucleic acid in the sample (Lu et al., 2020, Food and Administration, 2020). The Ct values obtained from the PCR tests of positive air samples at the sampling points and the positive samples of the hospitalized COVID-19 patients at the same points are presented in Table 1. The results show a lower viral load in positive air samples than in positive samples (in mucus or saliva) of COVID-19 patients. This indicates the low concentration of SARS-CoV-2 virus in the air compared to the respiratory system of infected patients. Thuresson et al. showed that there was a correlation between positive air samples for the virus and a lower CT value in patients (Thuresson et al., 2022).

3.2. Distribution of samples based on peripheral characteristics

The peripheral characteristics are needed to investigate the effect of air properties, environmental quality, and sampling point conditions on the distribution of the virus in indoor air. Environmental quality including room dimensions (surface area and volume), the concentration of airborne particles (PM_{10}, $PM_{2.5}$ & PM_1), and airflow velocity (AV) are presented in Table 2.

The results presented in Table 2 show that the average of A, V, and AV at sampling points in positive air samples is lower than in negative air samples while the average PM concentration of sampling points in positive samples is higher than in negative samples.

Air purifier existence in studied hospitals was recorded to evaluate the role of the air cleaner on the presence of viruses in the indoor air. Based on the results presented in Table 2 and it is clear that the first adult hospital had an air cleaner in some sampling points and it was turned on during sampling, and in some sampling points there was no air cleaner. There were no air purifiers in any of the sampling points in the second adult hospital and children hospital. The results show that 8 positive air samples were in places where there was no air cleaner. However, negative samples were recorded in some places where there was an air cleaner, but some places did not. Also, all the air samples were negative and the air cleaner was turned on during sampling.

The results of Table 2 show that all the positive air samples were recorded in both adult hospitals that had natural ventilation systems using doors and windows or split coolers. The negative results of air samples were obtained in pediatric hospital using negative pressure ventilation.

Table 1
Results of Ct value of RT-PCR tests of positive air samples and COVID-19 patients' samples at Adult Hospital.

No of adult hospital	Sampling points	Ct value of patient sample (mucus or saliva)	Ct value of positive air sample
1	hallway	–	38
1	hallway	–	38
1	patient room	19	38
1	patient room	20	34
1	patient room	25	37
1	patient room	21	32
2	patient room	25	37
2	patient room	25	37

Ct value: the cycle threshold value.

3.3. Multivariate statistical analysis

Principal component analysis (PCA) was used to investigate the statistically significant relationships between the presence/absence of SARS-CoV-2 in air samples and indoor air parameters. PCA analysis can be performed on raw data focusing on the correlation matrix or the variance-covariance matrix of the data. The results of the correlation matrix table express the correlation between the variables of the study with each other as shown in Table 3. In the correlation matrix table provided by this method, some coefficients should be more than 0.3, otherwise, the choice of variables should be reconsidered. On both sides of the matrix diameter, it should be noted that there are at least 4 to 5 numbers greater than 0.3 on each side. As can be seen in the table, there are a sufficient number of coefficients with this condition, which are marked with a thick line. For example, the results of this table show that the relationship between SARS-CoV-2 and VS quantities is negative ($r = -0.24$) while the relationship between SARS-CoV-2 and PM_{10} is positive ($r = +0.39$).

When performing PCA analysis, the first graph is the proportion of variance. This plot indicates the variance explained by each PC. In this diagram, the line means the variance expressed by each PC that this line is always downward. As the number of PCs increases, the variance expressed by each decreases. For this reason, we choose only two or three basic PCs. Based on the principal component analysis of the entire study data, the peripheral parameters were included in several principal components (PC). Based on PCA analysis, the studied parameters were divided into several principal components (PC). The number of components and the percentage of variance of each are shown in Fig. 3. The most important components are the first (PC1), and second (PC2) components can express 59.01% of the variance of the study variables, and these two components are used to examine the relationship between the parameters.

Based on PCA analysis, the 12 quantities or study variables are converted into 2 principal components. This is the main purpose of factor analysis. It means converting a large number of variables into a smaller number of PCs. Table 4 shows which PC each variable belongs to. The written numbers show the correlation values that are in the range of +1 to −1. Each number represents the relationship between the variable and the selected PC. For example, the number −0.852 indicates the existence of a strong and opposite relationship between variable and PC1. In the same way for other variables, each PC whose number was larger (in absolute terms and regardless of whether it is positive or

Table 2
The peripheral parameters in all sampling points and positive and negative air samples.

Air samples	Total data	adult's hospital 1	adult's hospital 2	children's hospital	Positive samples	Negative samples
	Mean ± SD					
A (m^2)	38.93 ± 23.72	34.22 ± 19.51	46.29 ± 12.17	40.26 ± 28.54	**30.00 ± 21.27**	39.49 ± 23.82
V (m^3)	116.80 ± 71.15	102.65 ± 58.52	138.86 ± 36.51	120.78 ± 85.62	**90.00 ± 63.82**	118.47 ± 71.47
AV(m/s)	0.18 ± 0.19	0.18 ± 0.13	0.04 ± 0.04	0.22 ± 0.23	**0.14 ± 0.12**	0.18 ± 0.19
presence AC	–	Yes/No	No	No	No	Yes/No
Type of VS	–	natural ventilation	natural ventilation	mechanical ventilation	natural ventilation	mechanical ventilation
Number of patients	–	2 or 3	6	1 or 2	2 to 6	2 to 6
PM_{10} (μg/m^3)	34.35 ± 20.75	52.94 ± 20.03	35.51 ± 1.99	19.38 ± 9.84	66.45 ± 20.45	**32.36 ± 19.14**
$PM_{2.5}$ (μg/m^3)	22.21 ± 21.75	44.98 ± 20.00	9.53 ± 0.71	8.45 ± 5.61	55.01 ± 28.59	**20.18 ± 19.62**
PM_1 (μg/m^3)	18.92 ± 20.99	41.33 ± 18.92	5.58 ± 0.60	5.64 ± 4.31	50.28 ± 28.29	**16.97 ± 18.95**

A: surface area (m^2); V: volume (m^3); AV: airflow velocity (m/s); AC: presence/absence of air cleaner in the sampling point (Yes: presence of AC; No; absence of AC, YES/No; AC was present in some sampling points and not in others); VS: type of ventilation system in the sampling point; PM_{10}, $PM_{2.5}$ & PM_1: the mass concentration of airborne particles in three sizes of 10, 2.5, and 1 μm (μg/m^3).

Table 3
The value of data correlation matrix by PCA analysis for the studied parameters.

	DIS'	'A'	'No.p'	'Age'	'Mask'	'AC'	'VS'	'AV'	' PM_{10}'	' $PM_{2.5}$'	' PM_1'	'SARS-CoV-2′
'DIS'	1.00	**0.38**	**−0.54**	**−0.48**	**−0.35**	0.16	0.25	−0.14	−0.24	−0.26	−0.28	−0.21
'A'	**0.38**	1.00	0.17	−0.05	**−0.55**	0.05	−0.12	**−0.56**	0.04	−0.02	−0.05	−0.09
'No.p'	**−0.54**	0.17	1.00	0.19	0.06	−0.16	−0.28	0.05	−0.03	−0.01	0.01	0.04
'Age '	**−0.48**	−0.05	0.19	1.00	−0.23	**−0.48**	**−0.81**	−0.23	**0.69**	**0.60**	**0.60**	0.24
'Mask'	**−0.35**	**−0.55**	0.06	−0.23	1.00	0.01	**0.42**	**0.45**	−0.13	0.04	0.08	−0.01
'AC'	0.16	0.05	−0.16	**−0.48**	0.01	1.00	**0.50**	0.03	**−0.35**	**−0.42**	**−0.44**	0.13
'VS'	0.25	−0.12	−0.28	**−0.81**	**0.42**	**0.50**	1.00	0.30	**−0.68**	**−0.59**	**−0.58**	−0.24
'AV'	−0.14	**−0.56**	0.05	−0.23	**0.45**	0.03	**0.30**	1.00	**−0.30**	−0.18	−0.15	−0.05
' PM_{10}'	−0.24	0.04	−0.03	**0.69**	−0.13	**−0.35**	**−0.68**	**−0.30**	1.00	**0.94**	**0.92**	**0.39**
' $PM_{2.5}$'	−0.26	−0.02	−0.01	**0.60**	0.04	**−0.42**	**−0.59**	−0.18	**0.94**	1.00	1.00	**0.38**
' PM_1'	−0.28	−0.05	0.01	**0.60**	0.08	**−0.44**	**−0.58**	−0.15	**0.92**	1.00	1.00	**0.37**
'SARS-CoV-2'	−0.21	−0.09	0.04	0.24	−0.01	0.13	−0.24	−0.05	**0.39**	**0.38**	**0.37**	1.00

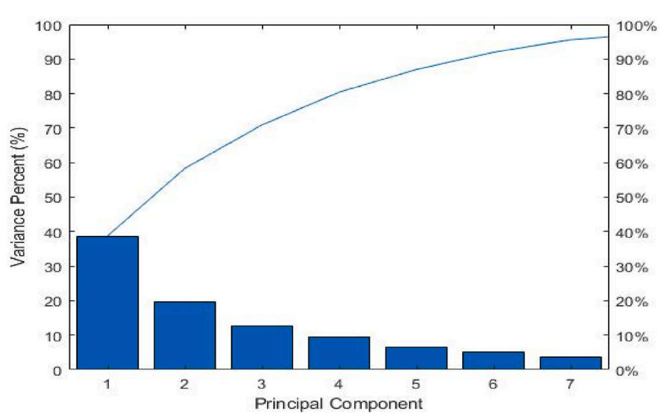

Fig. 3. Pareto plot of PCA for the proportion of variance.

Table 4
Principal component analysis coefficients (PCA) of samples.

% variance of each component		principal components	
		PC1	PC2
		38.98	20.03
parameters	**'DIS'**	−0.19	**0.40**
	'A'	0.01	**0.54**
	'No.p'	0.08	−0.12
	'Age'	**0.39**	−0.01
	'Mask'	−0.07	**−0.53**
	'AC'	**−0.25**	0.05
	'VS'	**−0.39**	−0.14
	'AV'	−0.14	**−0.46**
	' PM_{10}'	**0.43**	0.04
	' $PM_{2.5}$'	**0.42**	−0.06
	' PM_1'	**0.42**	−0.08
	'SARS-CoV-2′	**0.19**	−0.08

Abbreviations: DIS: distance sampling devices from patients; Age: age of patients; A: area (m^2); No.p: the number of patients admitted to each sampling points; mask: use the mask by patients; AC: presence/absence of air cleaner; VS: ventilation system; AV: airflow velocity(m/s); PM_{10}: up to 10 μm (μg/m^3); $PM_{2.5}$: particulate matter equal or less than 2.5 μm (μg/m^3); PM_1: mass concentration of airborne particles ≤1 μm (μg/m^3).

negative), is assigned to the same PC. The numbers written opposite each variable are the percentage of total variance explained by that PC. Therefore, the larger the number of the eigenvalue, the greater the effect of that variable on the PC.

Based on the explanation provided about the analysis, the coefficients related to PCA analysis are given in Table 4. In this table, you can see the variable weight in each component. The most important components are the first and second components and the most important variables are those that have more weight in these components. In the first component, the variables of Age, AC, VS, PMs, and SARS-CoV-2 are more important, respectively, so the effect of Age, PMs, and SARS-CoV-2 in this component is positive and the effect of AC and VS is negative. Also, in the second component, the coefficient or weight of each variable is specified in the same way. The positive sign of weights indicates the direct effect of that variable on the component and the negative sign of weights indicates the inverse effect of that variable on the component. The higher the coefficient of values, the greater the effect of the variable on the components.

The biplot image related to PCA analysis between the studied parameters is shown in Fig. 4. In the Biplot graph of Fig. 4, the correlation of each variable is reported as an array (x,y) where x is the quantity correlation with PC1 and y is the correlation with PC2. For example, the array of (0.19, −0.08) for SARS-CoV-2 shows that SARS-CoV-2 has a correlation of 0.19 with PC1 and a correlation of −0.08 unit with PC2. In the Biplot graph, you can see the correlation arrays for each variable.

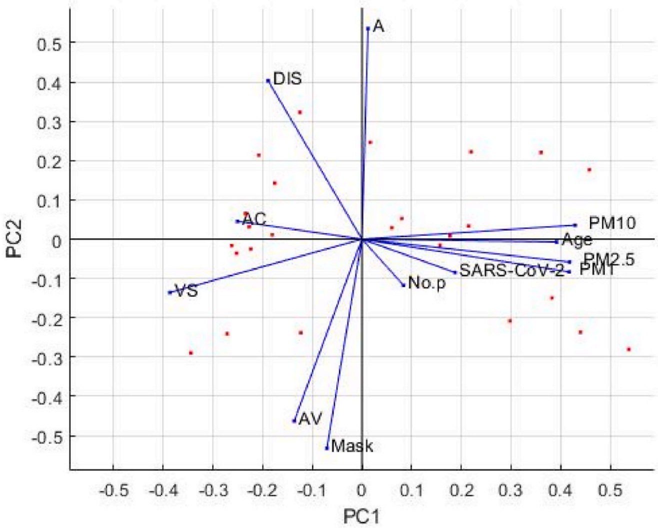

Fig. 4. Biplot image related to the relationship of the studied parameters (Abbreviations: DIS: distance of sampling devices from patients; Age: age of patients; A: area (m^2); No.p: the number of patients admitted to each sampling points; mask: use the mask by patients; AC: presence/absence of air cleaner; VS: ventilation system; AV: airflow velocity(m/s); PM_{10}: up to 10 μm (μg/m^3); $PM_{2.5}$: particulate matter equal or less than 2.5 μm (μg/m^3); PM_1: mass concentration of airborne particles ≤1 μm (μg/m^3).

The lines are drawn from the origin of the coordinates and the point (0, 0), and the correlation of the quantity with any of the PCs is assigned to that PC. Another use of this graph is to check the relationship between variables. As shown in the graph, SARS-CoV-2, PM_{10}, $PM_{2.5}$, PM_1, Age, and number of patients are clustered close to each other, which shows that they have a positive correlation with each other. In comparison, AC and SARS-CoV-2 vectors or DIS and SARS-CoV-2 vectors form an almost right angle, indicating that they are not correlated with each other. Returning to the results of the correlation matrix tab, we can confirm that these assumptions are largely correct.

In order to interpret the Biplot results, three factors are used, including the angle between the vectors, the direction of the vector for each variable, and the length of the vector of each variable in each component (Baboli et al., 2021; Zuśka et al., 2019). Accordingly, the SARS-CoV-2 vector has the lowest angle with the parameters of particles (PMs), age of the patient (Age), and number of patients (No.P). On the other hand, all four parameters are in the same direction. Therefore, an angle of less than 90° between vectors and the alignment of vectors with each other indicate a direct relationship between these parameters and they are placed in a cluster. It can be explained that the presence of the virus in air samples has a direct and positive relationship with the increase in the concentration of particles, the increase in the age of patients, and the increase in the number of patients. So the results show that with the increasing age of patients, increasing concentration of particles, and increasing the number of patients, the concentration of the virus in the air will probably increase and positive air samples will increase. The SARS-CoV-2 vector also has an open angle (approximately 180°) with parameters such as distance from patients (DIS), air cleaner (AC), and ventilation system (VS). On the other hand, the vectors are in the opposite direction. Therefore, the wide-angle between the vectors and their inverse direction shows the inverse relationship of these parameters. Therefore, the results show that increasing the distance from patients, the presence of air cleaners, and the presence of an advanced ventilation system probably lead to a decrease in the concentration of the virus in the air, and positive air samples are reduced. Also, Among the parameters affecting the presence of the virus in the air, which was determined based on the angle between the vectors and the direction of the vectors; based on the length of the vector, it can be said that the parameters of the PMs, the age of the patient (Age), distance from patient (DIS), and the ventilation system (VS) have a longer length and therefore have the greatest impact on the presence of the virus in the air. Therefore, the parameters of the number of patients (No.p) and the air cleaner (AC) have a smaller effect on the presence of the virus in the air due to their shorter vectors. The relationship between other parameters cannot be expressed by principal component analysis, and in other words, the effective and meaningful relationship with other parameters is not shown.

The results of the analysis of self-organizing maps (SOM) between the different parameters studied at all sampling points are shown in Fig. 5. In these images, based on the pattern of the color spectrum and the location of the colors in the SOM maps, we will find out their relationship for each parameter; As long as the location of the colors in the SOM map corresponds to two parameters, it indicates a direct and strong relationship between these two parameters, and if the color pattern is shifted, it means the opposite relationship. In this study, the dependent variable is the presence/absence of the SARS-CoV-2 virus in air samples and the other independent variables are the studied parameters. Therefore, the color pattern of the SOM map of the SARS-CoV-2 parameter is compared with the color pattern of the parameters.

In Fig. 5, it can be seen that, like the results of PCA analysis, in SOM analysis, there is a direct and strong relationship between the SARS-CoV-2 parameter and PM_{10}, $PM_{2.5,}$ and PM_1 and the age of patients (Age). In other words, the number of positive samples increases with the increase in the concentration of particles and the age of the patients at the sampling points. Also, based on the results presented in Fig. 2, Table 2, and Fig. 4, similar results can be obtained. Thus, no positive samples were found in the air samples of hospitalized children with younger patients (<12 yeass), and all positive air samples were recorded in hospitals where older adult patients (>20 years) were hospitalized.

Also, in Fig. 5, there is a strong correlation between the SARS-CoV-2 parameter with VS, A, AV, AC, and Dis. Also, the number of positive air samples with the ventilation system (VS), the area of the sampling points (A), the amount of air velocity (AV), air cleaner (AC), and the distance of the sampling points from the patients (DIS) have an opposite and strong relationship, so that based on the results of Fig. 2, Table 2 and Fig. 4, this result can be approved. No positive samples were found in children's hospital with advanced mechanical negative pressure ventilation, larger dimensions of sampling points, and higher air flow speed. However, in adult hospitals where positive air samples were recorded, they had natural ventilation systems, smaller dimensions of patient rooms, and lower airflow velocity. Therefore, it can be seen that the results of SOM are in line with PCA analysis.

4. Discussion

Fig. 2 shows that SARS-CoV-2 virus was detected in 5.8% of air samples in the studied hospitals. The positive air samples were recorded in adult hospitals and no positive samples were recorded in children's hospitals. Similar to the results of the present study, many studies with different virus recovery rates have identified SARS-CoV-2 in indoor air samples from hospital COVID-19 wards (Chia et al., 2020; Lednicky et al., 2020; Razzini et al., 2020; Bazzazpour et al., 2021). Many researchers have also reported that SARS-CoV-2 was not detected in indoor air and air has no role in the transmission of SARS-CoV-2 (Ong et al., 2020; Faridi et al., 2020; Masoumbeigi et al., 2020). The results of Table 1 show that the genetic material of the virus in the positive air samples is less than in the throat samples of the hospitalized COVID-19 patients at the same sampling points, which indicates a lower concentration of the virus in the air than in the respiratory system of the patients. Therefore, methods of sampling and detecting viruses in the air require high accuracy and sensitivity. On the other hand, environmental conditions such as temperature, humidity and air pressure affect the survival and transmission of bioaerosols (Baboli et al., 2021; Ahlawat et al., 2020). Therefore, sampling and identifying viruses in air samples

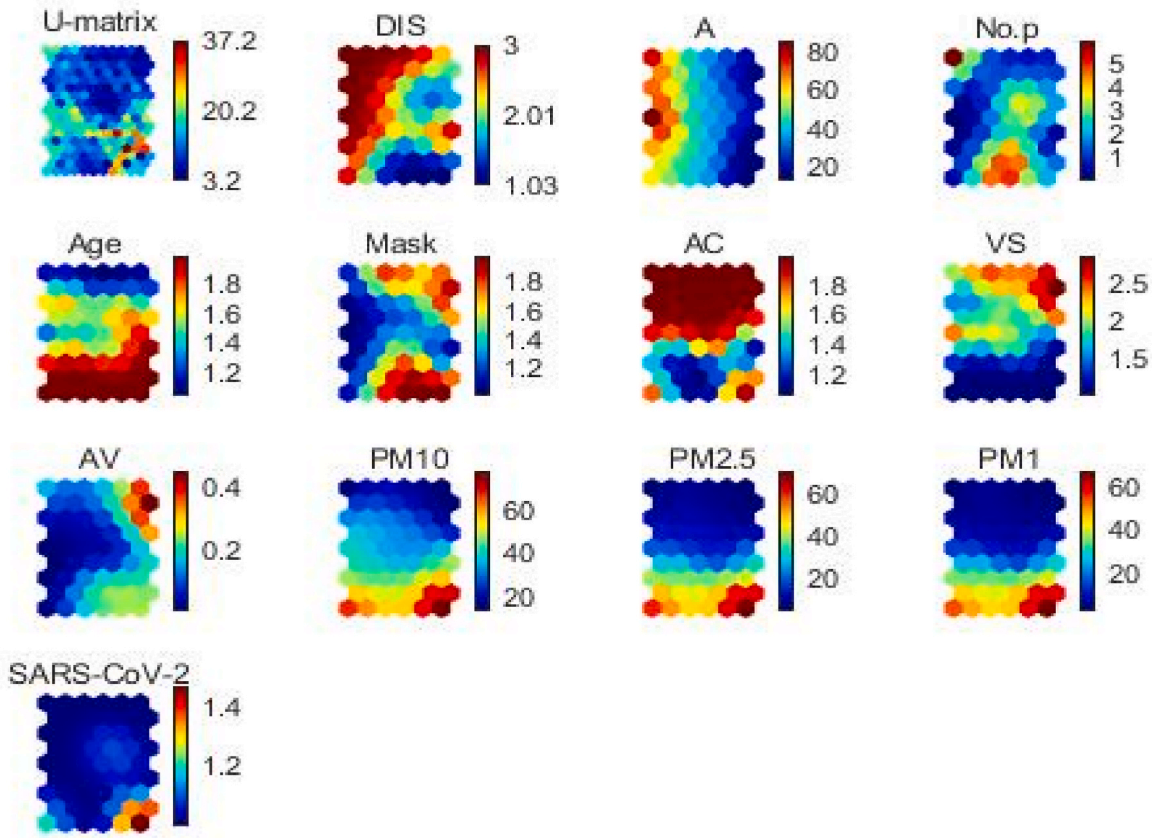

Fig. 5. The color scale of the component plane from SOM analysis.

is challenging. Sampling problems include the lack of proper ability to collect small bioaerosols, low concentration of virus in the air, damage to the genetic structure of the virus due to stress, and shear forces caused by sampling methods at the entrance or wall of the samplers. Compared to other microorganisms, airborne viruses are present in very low concentrations, meaning that relatively large volumes of air need to be sampled to obtain reliable analytical results (Borges et al., 2021). Based on this, various researchers have proposed the standard of air sucked during active air sampling with a volume of at least 1000 L (Pena et al., 2021). Of course, it should be noted that the extended duration of sampling have a negative impact on the structure of the virus and reduces its survival and contamination, and this problem is emphasized more in filter samples (Verreault et al., 2008).

PCA and SOM analysis results in Figs. 4 and 5 showed a direct and strong relationship between the presence of the virus and air particle concentration. Also, the results presented in Table 2 show that the average concentration of suspended particles in positive air samples is higher than in negative samples. Therefore, it can be concluded that as the concentration of particles in the air increases, the number of positive samples increases. Similar to the results of the present study, based on the direct relationship between the concentration of particles and the presence of the virus in the air, the possibility of SARS-CoV-2 transmission through airborne particles and droplets in restaurants (Lu et al., 2020) or in fitness classes (Cheng et al., 2020) has been expressed by different researchers. Transmission through airborne particles, especially in environments with high human density and poor ventilation, cannot be ignored if infected people are present for a long time. In another study, particulate matter with a diameter equal to or less than 2.5 μm was identified as carrying the microbiome, and a positive correlation was found between influenza-like illnesses and $PM_{2.5}$ concentrations, as smaller particles were able to remain suspended in the air while droplets large ones settled on the ground or surfaces due to their weight (Feng et al., 2016). Also, other studies have shown a direct relationship between the concentration of PM_{10} and $PM_{2.5}$ in the outdoor air with the increase in COVID-19 patients (Wangb et al., 2020) and between airborne particles with respiratory infections (Croft et al., 2019) and mortality caused by SARS (Kan et al., 2005). Therefore, the RNA virus of SARS-CoV-2 on airborne particles was expressed as a possible indicator of epidemic growth due to the concentration of particles (Setti et al., 2020). Therefore, based on the results of the present study in Table 2, Figs. 4 and 5, which showed a positive relationship between the PMs and the presence of the virus in air samples, It can be concluded that by reducing the concentration of indoor air particles, the dispersion and transmission of viruses in the air will probably decrease.

The Biplot images in Fig. 4 and SOM images in Fig. 5 show an opposite and strong correlation between the presence of the virus and the air cleaners (AC) in the sampling points. There is a reciprocal relationship between the presence of SARS-CoV-2 in the air and the existence of air cleaners in the hospitals. Similar to the results of the present study, Riediker and Tsai's study showed that the virus concentration is higher in areas without air purifiers indicating the positive effect of air cleaners in reducing the viral load of the indoor environment (Riediker and Tsai, 2020).

Based on the PCA Biplot in Fig. 4 and SOM images in Fig. 5, there is also a strong inverse relationship between the presence of the SARS-CoV-2 virus in the air samples and the ventilation system (VS) parameters at the sampling points. In adult hospitals, Sina and Razi, the natural ventilation method was using doors and windows or split coolers where positive air samples were detected. In children's hospital, Abuzar, no positive air samples were detected where the mechanical ventilation system was under positive or negative pressure. Therefore, positive samples were collected in places with natural ventilation. In sampling points with mechanical ventilation including negative and positive pressure all air samples were negative. Therefore, the type and performance of the ventilation system had an effective role in the concentration of viruses and air quality. Investigating the behavior of particles coming out of the respiratory system can show the role of the ventilation system in the presence of viruses in the air. During exhalation, the particles coming out of the respiratory system compete with two forces of gravity (downward movement) and air friction force (upward movement), and the balance of these two forces and the cross-currents determine the particle's buoyancy (Wells, 1934). Factors such as doors and windows, peo ple crowding, fans, and air conditioning affect the buoyancy and movement of particles in the indoor air. Viral load estimation by Tsai et al. showed that the concentration of the virus in the air of small rooms of patients with poor ventilation increases, particularly in the early days of the disease onset (Riediker and Tsai, 2020). Similar to the results of Tsai's study, we observed that the highest concentration of the virus was detected in areas with less ventilation indicating the important role of proper ventilation in controlling the viral load of indoor microenvironments (Riediker and Tsai, 2020). Thuresson et al. showed a correlation between positive air samples for the virus and room ventilation (Thuresson et al., 2022).

PCA Biplot in Fig. 4 and SOM images in Fig. 5 clearly show that there is a strong inverse correlation between the SARS-CoV-2 existence and distance of sampling devices from patients (DIS), as we observed positive air samples at closer distances of patients and farther distances leading to negative results. The results of Thuresson et al. study showed that there is a correlation between positive SARS-CoV-2 samples and nearer physical distance from patients (Thuresson et al., 2022). Other studies investigated the importance of physical distancing in the airborne transmission of the virus and suggested that an infected person may be responsible for the outbreak of COVID-19 among bus passengers in China (Shen et al., 2020). Also, air samples containing the virus in the rooms of patients with COVID-19 were detected in the absence of aerosol production methods (AGP) in the samples collected at a distance of 2 and 4.8 m from the patients' beds (Lednicky et al., 2020). The 2-m distance, which is widely used in aerosol-borne infections, based on Well's study in the 1930s, stated that large droplets (>100 μm) fall to the ground 2 m from the source and, depending on the speed (Wells, 1934). However new models show that at cough and sneeze flow rates, droplets can be transported up to 6 m, and patients with respiratory disease can transport super-drops up to 8 m (Borges et al., 2021). Santerpia et al. stated that viral bioaerosols are produced by COVID-19 patients and these viral bioaerosols can travel distances of more than 2 m and be transferred from inside the patient's room to outside the room (Santarpia et al., 2020). Another study showed that particles containing SARS-CoV-2 were widely dispersed in the indoor air of COVID-19 wards over distances of more than 4 m (Guo et al., 2020). Virus detection at a distance of more than 2 m from the virus source (patients) is classified as an airborne virus (CDC, 2020), so our results are due to the presence of SARS-CoV-2 in corridor samples and transmission of the virus at a distance of more than 2 m from patient beds shows evidence of airborne transmission. The results of the present study revealed that particles or droplets containing infectious SARS-CoV-2 are usually produced by infected patients, which emphasizes proper physical distance from the source of virus dissemination.

Based on Figs. 4 and 5, there is a strong direct relationship between the SARS-CoV-2 parameter and the patient age parameter as we observed there was an increase in positive samples by the age of infected patients. The number of positive samples was zero in places where children and adolescents were hospitalized like Abuzar hospital while all positive samples were collected in places where adult patients were hospitalized (Razi and Sina hospitals). As the dynamic of the airways will be different with age, the mechanism of the generation of droplets in the indoor air is different depending on hospital type (pediatric or adult). The production of respiratory droplets and aerosols depends on the thickness of mucus, viscoelastic properties, surface tension at the mucus-air interface, and the achievement of critical air velocities (Wei and Li, 2016). Transmission of SARS-CoV-2 by children is influenced by the mechanisms of airborne transmission and differences in the lower and upper tract airways. Although children can have a higher viral load of SARS-CoV-2 than adults and transmit the infection, adults are more capable of transmitting the infection. The reasons include the simpler structure of the airway, the lower number of alveoli and terminal bronchioles, the lower flow rate and speed of exhalation, and the lower collapse of the airway in children than in adults (Riediker and Tsai, 2020; Castagnoli et al., 2020). Other studies have shown a positive correlation between respiratory aerosol concentration and age, which is associated with age-related airway collapse. Therefore, previous investigation revealed that adults can produce and disseminate more suspended particles and respiratory droplets in the air than children which is in line with the present research (Johnson and Morawska, 2009).

5. Limitation

The present study was designed and conducted at the beginning of the COVID-19 epidemic. Since the emerging disease of the SARS-CoV-2 virus, the lack of sufficient recognition, the continuous change of strains, the lack of well-organized equipment for air sampling, and the uncertainty of molecular identification methods, all conducted sampling and experimental activities in the present study were based on literature review, investigations, and sometimes innovative methods. Therefore, it is possible that the trapping of the viruses due to their low concentrations in the air or their molecular extraction and identification have been affected by the mentioned shortcomings.

6. Conclusion

The results of various studies show that SARS-CoV-2 is present in the air of COVID-19 wards and the research findings confirm that SARS-CoV-2 has been detected in air samples leading to the airborne transmission of SARS-CoV-2 as a major route in indoor microenvironments. According to the results of the present study, the most important conditions correlated with the presence of the virus in the air inside the COVID-19 ward include high levels of airborne particles (PMs), poor ventilation system (VS), absence of air cleaners (AC), older age of patients (Age), and distance lower than 1 m from patients (DIS). The risk of SARS-CoV-2 airborne transmission also depends on the number of COVID-19 patients present in the environment (No.p). Therefore, these parameters can be considered indoors to reduce virus transmission. Other basic control measures such as controlling the source of dissemination by quarantining patients in rooms with mechanical ventilation systems, especially negative pressure ventilation systems, using face masks, reducing the number of patients in the hospital and their duration in closed environments, maintaining physical distance between patients from each other or with the therapist staff and other responsible individuals from the patient. It is recommended to improve engineering measures related to adjusting and upgrading the performance of the ventilation system particularly in Razi and Sina hospitals. Air purifiers can also be recommended as an urgent requirement in the COVID-19 outbreak and similar biological threats. Preparedness of personnel in terms of Early Warning Systems (EWS) related to biological disasters, protocol preparation combating pandemics, and proper training can help hospital staff to enable them to act further and smarter during pandemics.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Gholamreza Goudarzi: Writing – review & editing, Writing –

original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Yaser Tahmasebi Birgani:** Writing – review & editing, Writing – original draft, Supervision, Software. **Niloofar Neisi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology. **Ali Akbar Babaei:** Writing – original draft, Visualization, Validation, Supervision, Methodology. **Mehdi Ahmadi:** Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology. **Zeynab Baboli:** Writing – review & editing, Writing – original draft, Software, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank the Iran National Science Foundation (INSF) and Ahvaz Jundishapur University of Medical Sciences (AJUMS) for their financial support with project numbers INSF-99017229 and APRD-9909, respectively. Also, the authors would like to thank the personnel of these three hospitals for helping us to take samples.

References

Ahlawat, A., Wiedensohler, A., Mishra, S.K., 2020. An Overview on the role of relative humidity in airborne transmission of SARS-CoV-2 in indoor environments. Aerosol Air Qual. Res. 20, 1856–1861.
Ahmed, W., Bertsch, P.M., Angel, N., Bibby, K., Bivins, A., Dierens, L., Edson, J., Ehret, J., Gyawali, P., Hamilton, K.A., 2020. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers. J. Trav. Med. 27, 116.
Baboli, Z., Neisi, N., Babaei, A.A., Ahmadi, M., Sorooshian, A., Birgani, Y.T., Goudarzi, G., 2021. On the airborne transmission of SARS-CoV-2 and relationship with indoor conditions at a hospital. Atmos. Environ. 261, 118563.
Bazzazpour, S., Rahmatinia, M., Mohebbi, S.R., Hadei, M., Shahsavani, A., Hopke, P.K., Houshmand, B., Raeisi, A., Jafari, A.J., Yarahmadi, M., 2021. The detection of SARS-CoV-2 RNA in indoor air of dental clinics during the COVID-19 pandemic. Environ. Sci. Pollut. Control Ser. 1–9.
Booth, T.F., Kournikakis, B., Bastien, N., Ho, J., Kobasa, D., Stadnyk, L., Li, Y., Spence, M., Paton, S., Henry, B., 2005. Detection of airborne severe acute respiratory syndrome (SARS) coronavirus and environmental contamination in SARS outbreak units. The Journal of infectious diseases 191, 1472–1477.
Borges, J.T., Nakada, L.Y.K., Maniero, M.G., GuimarãES, J.R., 2021. SARS-CoV-2: a systematic review of indoor air sampling for virus detection. Environ. Sci. Pollut. Control Ser. 1–14.
Castagnoli, R., Votto, M., Licari, A., Brambilla, I., Bruno, R., Perlini, S., Rovida, F., Baldanti, F., Marseglia, G.L., 2020. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children and adolescents: a systematic review. JAMA Pediatr. 174, 882–889.
CDC, 2020. Scientific Brief: SARS-CoV-2 and Potential Airborne Transmission. Centers for Disease Control.
Cheng, V.C.-C., Wong, S.-C., Chan, V.W.-M., So, S.Y.-C., Chen, J.H.-K., Yip, C.C.-Y., Chan, K.-H., Chu, H., Chung, T.W.-H., Sridhar, S., 2020. Air and environmental sampling for SARS-CoV-2 around hospitalized patients with coronavirus disease 2019 (COVID-19). Infect. Control Hosp. Epidemiol. 1–32.
Chia, P.Y., Coleman, K.K., Tan, Y.K., Ong, S.W.X., Gum, M., Lau, S.K., Sutjipto, S., Lee, P. H., Young, B.E., Milton, D.K., 2020. Detection of air and surface contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in hospital rooms of infected patients. medRxiv.
Croft, D., Rich, D., Hopke, P., Thevenet-Morrison, K., Thurston, S., Georas, S., Sime, P., Utell, M., Falsey, A., 2019. Risk of Influenza and Respiratory Syncytial Virus Infection Associated with Particulate Air Pollution: an Adult Case-Control Study. *D26. HEALTH EFFECTS ASSOCIATED with INDOOR and OUTDOOR AIR POLLUTION*. American Thoracic Society.
DE Oliveira, R., Carneiro, C.D.C., DE Almeida, F., DE Oliveira, B., Nunes, E., Dos Santos, A., 2019. Multivariate air pollution classification in urban areas using mobile sensors and self-organizing maps. Int. J. Environ. Sci. Technol. 16, 5475–5488.
Faridi, S., Niazi, S., Sadeghi, K., Naddafi, K., Yavarian, J., Shamsipour, M., Jandaghi, N.Z. S., Sadeghniiat, K., Nabizadeh, R., Yunesian, M., 2020. A field indoor air measurement of SARS-CoV-2 in the patient rooms of the largest hospital in Iran. Sci. Total Environ., 138401
Feng, C., Li, J., Sun, W., Zhang, Y., Wang, Q., 2016. Impact of ambient fine particulate matter (PM 2.5) exposure on the risk of influenza-like-illness: a time-series analysis in Beijing, China. Environmental Health 15, 17.
FOOD & ADMINISTRATION, D, 2020. Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) for Emergency Use Only Instructions for Use (24 Tests/kit and 48 Tests/kit) for in Vitro Diagnostic (IVD) Use for Prescription Use Only for Emergency Use Authorization Only [Internet] [cited 2020 Aug30].
Gorbalenya, A.E., 2020. Severe acute respiratory syndrome-related coronavirus–The species and its viruses, a statement of the Coronavirus Study Group. bioRxiv.
Guagliardi, I., Astel, A.M., Cicchella, D., 2022. Exploring soil pollution patterns using self-organizing maps. Toxics 10, 416.
Guo, Z.-D., Wang, Z.-Y., Zhang, S.-F., Li, X., Li, L., Li, C., Cui, Y., Fu, R.-B., Dong, Y.-Z., Chi, X.-Y., 2020. Aerosol and surface distribution of severe acute respiratory syndrome coronavirus 2 in hospital wards, Wuhan, China, 2020. Emerg. Infect. Dis. 26 (10), 3201.
Johnson, G.R., Morawska, L., 2009. The mechanism of breath aerosol formation. J. Aerosol Med. Pulm. Drug Deliv. 22, 229–237.
Kan, H.D., Chen, B.-H., Fu, C.W., Yu, S.-Z., Mu, L.N., 2005. Relationship between ambient air pollution and daily mortality of SARS in Beijing. Biomed. Environ. Sci. 18, 1–4.
Lednicky, J.A., Loeb, J.C., 2013. Detection and isolation of airborne influenza A H3N2 virus using a Sioutas personal Cascade impactor sampler. Influenza research and treatment, 2013.
Lednicky, J.A., Lauzard, M., Fan, Z.H., Jutla, A., Tilly, T.B., Gangwar, M., Usmani, M., Shankar, S.N., Mohamed, K., Eiguren-Fernandez, A., 2020. Viable SARS-CoV-2 in the air of a hospital room with COVID-19 patients. Int. J. Infect. Dis. 100, 476–482.
Lovric, M., 2011. International Encyclopedia of Statistical Science.
Lu, Y., Li, L., Ren, S., Liu, X., Zhang, L., Li, W., Yu, H., 2020. Comparison of the diagnostic efficacy between two PCR test kits for SARS-CoV-2 nucleic acid detection. J. Clin. Lab. Anal. 34, e23554.
Masoumbeigi, H., Ghanizadeh, G., Arfaei, R.Y., Heydari, S., Goodarzi, H., Sari, R.D., Tat, M., 2020. Investigation of hospital indoor air quality for the presence of SARS-Cov-2. Journal of Environmental Health Science and Engineering 1–5.
Ong, S.W.X., Tan, Y.K., Chia, P.Y., Lee, T.H., Ng, O.T., Wong, M.S.Y., Marimuthu, K., 2020. Air, surface environmental, and personal protective equipment contamination by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from a symptomatic patient. JAMA 323, 1610–1612.
O'Gorman, C.M., Fuller, H.T., 2008. Prevalence of culturable airborne spores of selected allergenic and pathogenic fungi in outdoor air. Atmos. Environ. 42, 4355–4368.
Pena, P., Morais, J., Gomes, A.Q., Viegas, C., 2021. Sampling methods and assays applied in SARS-CoV-2 exposure assessment. Sci. Total Environ., 145903
Rahmani, A.R., Leili, M., Azarian, G., Poormohammadi, A., 2020. Sampling and detection of corona viruses in air: a mini review. Sci. Total Environ. 740, 140207.
Razzini, K., Castrica, M., Menchetti, L., Maggi, L., Negroni, L., Orfeo, N.V., Pizzoccheri, A., Stocco, M., Muttini, S., Balzaretti, C.M., 2020. SARS-CoV-2 RNA detection in the air and on surfaces in the COVID-19 ward of a hospital in Milan, Italy. Sci. Total Environ. 742, 140540.
Riediker, M., Tsai, D.-H., 2020. Estimation of viral aerosol emissions from simulated individuals with asymptomatic to moderate coronavirus disease 2019. JAMA Netw. Open 3, e2013807.
Santarpia, J.L., Rivera, D.N., Herrera, V., Morwitzer, M.J., Creager, H., Santarpia, G.W., Crown, K.K., Brett-Major, D., Schnaubelt, E., Broadhurst, M.J., 2020. Transmission potential of SARS-CoV-2 in viral shedding observed at the University of Nebraska Medical Center. medRxiv.
Setti, L., Passarini, F., DE Gennaro, G., Barbieri, P., Perrone, M.G., Borelli, M., Palmisani, J., DI Gilio, A., Torboli, V., Fontana, F., 2020. SARS-Cov-2RNA found on particulate matter of Bergamo in Northern Italy: first evidence. Environ. Res., 109754
Seyyed Mahdi, S.M., Nadji, S.A., Mohammadi, H., Farhang Dehghan, S., Vaziri, M.H., Jamaati, H., Jabbari, M., Varahram, M., Zareei, S., 2020. Assessment of SARS-CoV-2 in air and surfaces of ICU ward in one of the designated hospitals in Tehran. Iran. Occup. Health 17, 1–10.
Shen, Y., Li, C., Dong, H., Wang, Z., Martinez, L., Sun, Z., Handel, A., Chen, Z., Chen, E., Ebell, M.H., 2020. Community outbreak investigation of SARS-CoV-2 transmission among bus riders in eastern China. JAMA Intern. Med. 180, 1665–1671.
Thuresson, S., Fraenkel, C.J., Sasinovich, S., Soldemyr, J., Widell, A., Medstrand, P., Alsved, M., LöNDAHL, J., 2022. Airborne severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in hospitals: effects of aerosol-generating procedures, HEPA-filtration units, patient viral load, and physical distance. Clin. Infect. Dis. 75, e89–e96.
Verreault, D., Moineau, S., Duchaine, C., 2008. Methods for sampling of airborne viruses. Microbiology and molecular biology reviews 72, 413–444.
Walls, A.C., Park, Y.-J., Tortorici, M.A., Wall, A., Mcguire, A.T., Veesler, D., 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181, 281–292. e6.
Wang, K., Ho, K.-F., Leung, L.Y.-T., Chow, K.-M., Cheung, Y.-Y., Tsang, D., Lai, R.W.-M., Xu, R.H., Yeoh, E.-K., Hung, C.-T., 2022. Risk of air and surface contamination of SARS-CoV-2 in isolation wards and its relationship with patient and environmental characteristics. Ecotoxicol. Environ. Saf. 241, 113740.
Wangb, B., Liu, J., Li, Y., Fu, S., Xu, X., Li, L., Zhou, J., Liu, X., He, X., Yan, J., 2020. Airborne particulate matter, population mobility and COVID-19: a multi-city study in China. BMC Publ. Health 20, 1–10.
Wei, J., Li, Y., 2016. Airborne spread of infectious agents in the indoor environment. American journal of infection control 44, S102–S108.
Wells, W.F., 1934. On air-borne infection. Study II. Droplets and droplet nuclei. Am. J. Hyg. 20, 611–618.

WHO, 2020a. Transmission of SARS-CoV-2: Implications for Infection Prevention Precautions: Scientific Brief, 09 July 2020. World Health Organization.
WHO, 2020b. WHO Coronavirus Disease (COVID-19): Situation Report, p. 182.
Zuśka, Z., Kopcińska, J., Dacewicz, E., Skowera, B., Wojkowski, J., Ziernicka–Wojtaszek, A., 2019. Application of the principal component analysis (PCA) method to assess the impact of Meteorological Elements on concentrations of particulate matter (PM10): a case study of the mountain valley (the Sącz basin, Poland). Sustainability 11, 6740.