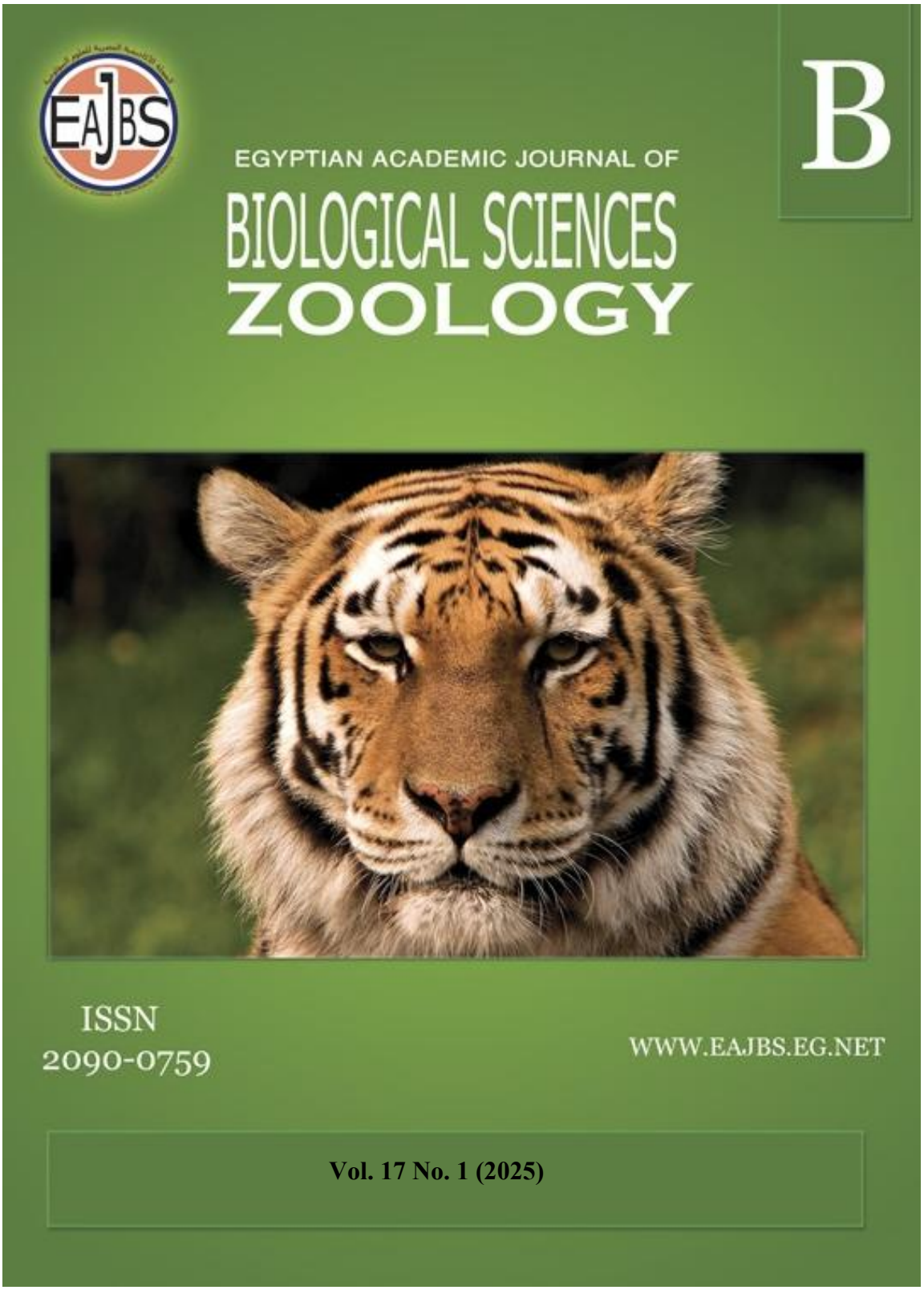

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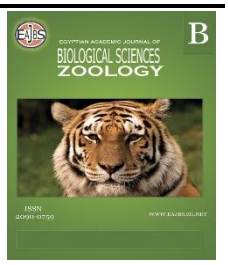

Detection and Persistence of SARS-CoV-2 in Air Samples from Admission Areas of Confirmed COVID-19 Patients: A Multicenter Study

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ABSTRACT

Background: Implementing efficient infection control measures, and preventing nosocomial transmission is vital to have understand the transmission mechanism of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) within the hospital setting. **Aim of the Study:** to determine the sources of COVID-19 infection and the duration of the virus's presence in the working environment. This will allow the establishment of infection control measures and the timing of isolation, as well as the reduction of nosocomial infections among personnel working in healthcare and patients. **Methodology:** Our research is a multicentric observational study that was carried out in Egypt within the General Organization of Teaching Hospitals and Institutes. A total of 216 air samples were collected from intensive care units (ICUs) while 20 patients with confirmed SARS-CoV-2 infection were admitted for a period of four consecutive days between the months of May 2022 and April 2024. R***esults:*** Based on the findings, it was determined that 208 (96.5%) of the air samples were negative, while only 3.7% demonstrated positive results. The percentage of positive samples in the total samples increased from 3% on day 3 to 13% on day 4. **Conclusion*:*** Because the environment surrounding SARS-CoV-2 patients is a possible source of virus transmission for at least four days after admission, it is imperative that patients and healthcare personnel take the necessary precautions to prevent the spread of infection. These precautions include thorough surface disinfection, air filtering, and basic isolation practices.

INTRODUCTION

The pandemic transmission of coronavirus disease 2019 (COVID-19) caused by severe respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel β-coronavirus, has resulted in a public health concern that has never been seen before in the 21st century. Over 5.3 million cases of COVID-19 were reported worldwide, and there were 342,029 fatalities. It is possible that the rapid spread of disease can be attributable to the presence of asymptomatic patients who are actively shedding the virus. This can result in direct transmission through the droplet

route as well as indirect transmission through interaction with an environment that is contaminated. It is possible that the transmission dynamics of COVID-19 could be further complicated by super spreading occurrences that are accompanied with an enormous increase in the number of documented cases (Guo, Z. D., Wang *et al.,*2020)

It is possible for respiratory viruses to be transmitted through the inhalation of respiratory droplets, which are particles with a diameter greater than 5µm, and infectious aerosols, which have a diameter less than 5µm. Additionally, the transmission of respiratory viruses can occur through direct or indirect contact with respiratory droplets using contaminated surfaces. Numerous individuals have arrived to the conclusion that airborne transmission must be involved as a result of the rapid spread of COVID-19 (Andreoletti L *et al.,*2022).

Nevertheless, clinical and experimental tests have revealed that SARS-CoV-2 could be transmitted through the air from person to person. The SARS-CoV-2 virus was able to be detected in aerosols for a period of three hours in the experimental environment that involved the artificial creation of aerosol samples (Bennett, A. *et al.,* 2021)

The collection of air samples in the rooms of patients is the most straightforward method for determining the presence of airborne virus in a clinical setting; nonetheless, the results of this method have proven inconsistent. In a study conducted in Singapore, the presence of SARS-CoV-2 RNA was not found in the air samples that were taken near the heads of patients (Bennett, A *et al.,*2021).

In a second investigation, it was shown that SARS-CoV-2 RNA was present in 41 percent of the air samples taken from an intensive care unit (ICU) in Wuhan, China. This was despite the fact that the average viral load in the air samples was very low (Delagrèverie, H. *et al.,* 2022)

MATERIALS AND METHODS

Within the framework of the General Organization of Teaching Hospitals and Institutes, this is an observational study that uses many perspectives and was carried out in Egypt. A total of 216 air samples were collected from intensive care units (ICUs) while 20 patients with confirmed SARS-CoV-2 infection were admitted for a period of four consecutive days between the months of May 2022 and April 2024. The middle of the room, in front of the beds, and in front of the ventilators were all locations where air samples were taken with an air incubator tester. Air samples were allowed to contact PCR swabs for a period of one hour. PCR was used to determine whether any of the samples contained SARS-CoV-2 RNA.

We have included only patients with a confirmed SARS-CoV-2 infection by obtaining 2 consecutive nasal swabs and those admitted during the study period. We have excluded those with one positive nasal swab or indeterminate swabs.

Patients gave their consent to participate after receiving complete and accurate information. In this retrospective investigation, the form of informed permission that was applied was verbal consent of the participants. The confidentiality and security of the data were guaranteed for each and every participant. The participants were given the opportunity to withdraw from the research procedure at any moment, and they were told of the point at which this option would no longer be available any longer. Furthermore, users had the ability to withdraw their data if it was identifiable to them during the process. Any predicted benefits as well as any potential risks were communicated to the individuals who participated in the research.

When it came to conducting the statistical analysis, IBM Inc.'s SPSS version 26 (located in Armonk, New York, United States) was utilized. Histograms and the Shapiro-Wilks test were utilized in order to determine whether or not the data distribution in question was normal. To examine qualitative data, the Chi-square test or Fisher's exact test was utilized

where it was deemed acceptable. The results of this analysis were provided in the form of frequency and percentage. As long as the p-value was lower than 0.050, it was regarded as statistically significant; otherwise, it was regarded as not statistically significant.

RESULTS

A quick demographic screen is presented in Table (1), which contains the samples that were collected during the admission of twenty patients to intensive care units and isolation rooms.

Table 1: A summary of the genders, length of stay, and the quantity of samples collected each day.

Parameters		Total no. = 20
Gender	**Female**	7 (35.0%)
	Male	13 (65.0%)
N. of days	**3 days**	8 (40.0%)
	4 days	12 (60.0%)
No. of samples	**6 samples/day**	14 (100.0%)
Severity	**Non severe**	8 (40.0%)
	Severe	12 (60.0%)

There was a total of 216 air samples that were acquired from the intensive care unit (ICU) and isolation rooms. These samples were collected from several sampling points, which were evenly distributed between the middle of the room, in front of the ventilator, and above the patient. Of these samples, 1.4% (n=1), 8.3% (n=4), and 1.4% (n=1) were positive for the CoV-2 PCR and were presented in Table 2.

Table 2: Air samples collected from the intensive care unit room.

Parameters		Total No. = 72
Middle of room	Negative	71 (98.6%)
	Positive	1 (1.4%)
Front of vent	Negative	66 (91.7%)
	Positive	6 (8.3%)
Above of patient	Negative	71 (98.6%)
	Positive	1 (1.4%)

The data from 20 participants reveal various clinical parameters, including white blood cell count (WBCs) with a mean of 11.60 ± 7.86, indicating a moderate level of immune response variability in Table 3. Lymphocyte percentage shows a mean of 27.40 ± 13.66, reflecting a range between 10% and 60%, which suggests possible immune system activation or suppression. Hemoglobin (Hb%) is on the lower end, with a mean of 8.58 ± 1.87, indicating mild anemia in some participants. Platelet count (PLT) is within a normal range (221.50 ± 75.98). Liver function tests show an elevated mean ALT of 82.40 ± 69.07, potentially indicating liver stress or damage. Renal function markers such as urea (50.90 ± 37.05) and creatinine (1.52 ± 1.50) are slightly elevated, pointing to possible renal involvement. C-reactive protein (CRP) is significantly high with a mean of 184.41 ± 88.57, suggesting active inflammation or infection. Albumin levels are on the lower end with a mean of 3.06 ± 0.74, possibly indicating nutritional or hepatic issues.

Table 3: The laboratory parameters of the patients who were under study.

Parameters		No. = 20
WBCs	Mean±SD	11.60 ± 7.86
	Range	2.60 - 29.90
Lymphocytes	Mean±SD	27.40 ± 13.66
	Range	10.00 - 60.00
Hb%	Mean±SD	8.58 ± 1.87
	Range	5.60 - 12.90
PLT	Mean±SD	221.50 ± 75.98
	Range	120.00 - 403.00
ALT	Mean±SD	82.40 ± 69.07
	Range	29.00 - 342.00
Urea	Mean±SD	50.90 ± 37.05
	Range	38.00 - 206.00
Creat	Mean±SD	1.52 ± 1.50
	Range	.90 - 7.80
CRP	Mean±SD	184.41 ± 88.57
	Range	1.20 - 388.00
Albumin	Mean±SD	3.06 ± 0.74
	Range	1.60 - 4.00

The comparison between females and males for the presence of SARS-CoV-2 in air samples from different locations (middle of room, front of vent, and above the patient) shows no significant differences. In the middle of the room, 100% of females and 92.3% of males had negative results (P = 0.452), and in the area above the patient, 100% of females and 92.3% of males were negative (P = 0.452). For the front of the vent, both groups showed no positive results (Table 4).

Table 4: Relation between gender and the other parameters

Parameters		Gender		Test value	P-value	Sig.
		Female	Male			
Middle of room	Negative	7 (100.0%)	12 (92.3%)	0.567	0.452	NS
	Positive	0 (0.0%)	1 (7.7%)			
Front of vent	Negative	7 (100.0%)	13 (100.0%)	-	-	-
	Positive	0 (0.0%)	0 (0.0%)			
Above of patient	Negative	7 (100.0%)	12 (92.3%)	0.567	0.452	NS
	Positive	0 (0.0%)	1 (7.7%)			

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test

The comparison between females and males for various clinical parameters reveals no significant differences across most measures, as indicated by P-values greater than 0.05. White blood cell count (WBCs) (P = 0.285), lymphocytes (P = 0.319), hemoglobin percentage (Hb%) (P = 0.132), platelet count (PLT) (P = 0.469), alanine aminotransferase (ALT) (P = 0.905), urea (P = 0.629), creatinine (Creat) (P = 0.712), and C-reactive protein (CRP) (P = 0.579) all showed no statistically significant differences between females and males. Albumin levels (P = 0.233) also did not show a significant difference (Table 5).

Table 5: Relation between gender and laboratory parameters.

Parameters		Females	Males	Test value	P-value	Sig.
WBCs	Mean±SD	14.6 ± 9.37	9.98 ± 6.78	-1.070≠	0.285	NS
	Range	3.1 - 29.9	2.6 - 25			
Lymphocytes	Mean±SD	30 ± 12.75	26 ± 14.43	-0.997≠	0.319	NS
	Range	16 - 48	10 - 60			
Hb%	Mean±SD	9.37 ± 1.81	8.15 ± 1.83	-1.507•	0.132	NS
	Range	7.5 - 12.9	5.6 - 11.7			
PLT	Mean±SD	218.14 ± 83.92	223.31 ± 74.89	-0.723≠	0.469	NS
	Range	148 - 403	120 - 377			
ALT	Mean±SD	73.43 ± 36.21	87.23 ± 82.61	-0.120≠	0.905	NS
	Range	29 - 121	29 - 342			
Urea	Mean±SD	43 ± 5.13	55.15 ± 45.87	-0.483≠	0.629	NS
	Range	38 - 52	38 - 206			
Creat	Mean±SD	1.23 ± 0.24	1.67 ± 1.86	-0.370≠	0.712	NS
	Range	1 - 1.7	0.9 - 7.8			
CRP	Mean±SD	197.57 ± 73.94	177.32 ± 97.62	-0.556≠	0.579	NS
	Range	112 - 301	1.2 - 388			
Albumin	Mean±SD	2.8 ± 0.89	3.2 ± 0.64	-1.192•	0.233	NS
	Range	1.6 - 3.8	1.8 - 4			

P>0.05: Non-significant (NS); P <0.05: Significant (S); P <0.01: Highly significant (HS)
•: Independent t-test; ≠: Mann-Whitney test

At day three and day four, respectively, there is no statistical significance regarding the middle of the room, the front of the vent, or above the patient (Table 6).

Table (6): Relation between number of days and the sample sites

Parameters		No. of days		Test value	P-value	Sig.
		3 days	4 days			
Middle of room	Negative	23 (95.8%)	48 (100.0%)	2.028	0.154	NS
	Positive	1 (4.2%)	0 (0.0%)			
Front of vent	Negative	22 (91.7%)	44 (91.7%)	0.000	1.000	NS
	Positive	2 (8.3%)	4 (8.3%)			
Above of patient	Negative	24 (100.0%)	47 (97.9%)	0.507	0.476	NS
	Positive	0 (0.0%)	1 (2.1%)			

P-value > 0.05: Nonsignificant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test

The comparison between the 3-day and 4-day groups for various clinical parameters shows no significant differences across most measures, as indicated by P-values greater than 0.05. White blood cell count (WBCs), lymphocytes, hemoglobin percentage (Hb%), platelet count (PLT), alanine aminotransferase (ALT), urea, creatinine (Creat), C-reactive protein (CRP), and albumin all had P-values greater than 0.05, suggesting that the two groups had similar levels in these parameters. For instance, WBCs (P = 0.589), lymphocytes (P = 0.393), Hb% (P = 0.969), PLT (P = 0.784), ALT (P = 0.756), urea (P = 0.556), Creat (P = 0.603), and CRP (P = 0.817) did not show any significant differences between the 3-day and 4-day groups. Albumin levels (P = 0.262) also showed no statistically significant difference (Table 7).

Table 7: Relation between no. of days and laboratory parameters.

Parameters		3 days	4 days	Test value	P-value	Sig.
WBCs	Mean±SD	12.91 ± 9.29	10.73 ± 7.06	-0.540≠	0.589	NS
	Range	2.8 - 29.9	2.6 - 25			
Lymphocytes	Mean±SD	24 ± 11.51	29.67 ± 14.97	-0.854≠	0.393	NS
	Range	10 - 38	13 - 60			
Hb%	Mean±SD	8.5 ± 1.37	8.63 ± 2.2	-0.039•	0.969	NS
	Range	6.9 - 10.9	5.6 - 12.9			
PLT	Mean±SD	230 ± 102.95	215.83 ± 56.02	-0.274≠	0.784	NS
	Range	120 - 403	148 - 372			
ALT	Mean±SD	73.75 ± 35.39	88.17 ± 85.74	-0.311≠	0.756	NS
	Range	39 - 123	29 - 342			
Urea	Mean±SD	64 ± 57.85	42.17 ± 5.8	-0.588≠	0.556	NS
	Range	38 - 206	38 - 58			
Creat	Mean±SD	2.03 ± 2.35	1.18 ± 0.27	-0.520≠	0.603	NS
	Range	0.9 - 7.8	0.9 - 1.9			
CRP	Mean±SD	187.62 ± 81.77	182.27 ± 96.34	-0.232≠	0.817	NS
	Range	96 - 301	1.2 - 388			
Albumin	Mean±SD	2.94 ± 0.53	3.14 ± 0.86	-1.122•	0.262	NS
	Range	1.8 - 3.6	1.6 - 4			

P>0.05: Non-significant (NS); P <0.05: Significant (S); P <0.01: Highly significant (HS)
•: Independent t-test; ≠: Mann-Whitney test

The comparison between non-severe and severe groups based on the presence of SARS-CoV-2 in air samples from different locations (middle of room, front of vent, and above the patient) reveals no significant differences. In the middle of the room, the majority of both non-severe (96%) and severe (100%) cases had negative results for SARS-CoV-2 (P = 0.167). Similarly, for the front of the vent, 96% of non-severe cases and 89.4% of severe cases were negative (P = 0.332), while for the area above the patient, 100% of non-severe cases and 97.9% of severe cases were negative (P = 0.463) (Table 8).

Table 8: Relation between severity and the other parameters

Parameters		Non severe		Severe		Test value	P-value	Sig.
		No.	%	No.	%			
Middle of room	Negative	24	96.0%	47	100.0%	1.906	0.167	NS
	Positive	1	4.0%	0	0.0%			
Front of vent	Negative	24	96.0%	42	89.4%	0.941	0.332	NS
	Positive	1	4.0%	5	10.6%			
Above of patient	Negative	25	100.0%	46	97.9%	0.539	0.463	NS
	Positive	0	0.0%	1	2.1%			

P-value > 0.05: Non-significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test

The comparison between non-severe and severe groups for various clinical parameters indicates no significant differences across most measures. White blood cell count (WBCs), lymphocytes, hemoglobin percentage (Hb%), platelet count (PLT), alanine aminotransferase (ALT), urea, creatinine (Creat), C-reactive protein (CRP), and albumin all showed P-values greater than 0.05, suggesting similar levels in both groups. For instance, WBCs (P = 0.316), lymphocytes (P = 0.485), and CRP (P = 0.231) did not exhibit statistically significant differences. Although albumin levels showed a trend toward significance (P = 0.082), this was still above the threshold for statistical significance (Table 9).

Table 9: Relation between severity and laboratory parameters.

Parameters		Non severe	Severe	Test value	P-value	Sig.
WBCs	Mean±SD	13.6 ± 8.61	10.27 ± 7.4	-1.003≠	0.316	NS
	Range	5.7 - 29.9	2.6 - 25			
Lymphocytes	Mean±SD	24.25 ± 11.68	29.5 ± 14.95	-0.699≠	0.485	NS
	Range	10 - 38	13 - 60			
Hb%	Mean±SD	8.79 ± 1.22	8.43 ± 2.25	-0.695•	0.487	NS
	Range	7.3 - 10.9	5.6 - 12.9			
PLT	Mean±SD	240 ± 94.25	209.17 ± 62.47	-0.469≠	0.639	NS
	Range	152 - 403	120 - 372			
ALT	Mean±SD	73.88 ± 35.26	88.08 ± 85.79	-0.505≠	0.614	NS
	Range	40 - 123	29 - 342			
Urea	Mean±SD	43.13 ± 7.55	56.08 ± 47.56	-0.471≠	0.638	NS
	Range	38 - 58	38 - 206			
Creat	Mean±SD	1.2 ± 0.24	1.73 ± 1.93	-0.040≠	0.968	NS
	Range	0.9 - 1.7	0.9 - 7.8			
CRP	Mean±SD	203.13 ± 73.22	171.93 ± 98.55	-1.198≠	0.231	NS
	Range	100 - 301	1.2 - 388			
Albumin	Mean±SD	2.85 ± 0.45	3.2 ± 0.87	-1.741•	0.082	NS
	Range	1.8 - 3.2	1.6 - 4			

P>0.05: Non-significant (NS); P <0.05: Significant (S); P <0.01: Highly significant (HS)
•: Independent t-test; ≠: Mann-Whitney test

When it came to air samples, the front of ventilator samples had the highest rate of positive rate, which was also shown to be statistically significant (Table 10).

Table 10: Comparison between air sites.

	Middle of room	Front of vent	Above of patient	Test-value	P-value	Sig.
Negative	71 (98.6%)	66 (91.7%)	71 (98.6%)	6.490*	0.039	S
Positive	1 (1.4%)	6 (8.3%)	1 (1.4%)			

P-value > 0.05: Nonsignificant; P-value < 0.05: Significant; P-value < 0.01: Highly significant
*: Chi-square test

DISCUSSION

For the purpose of reducing the potentially harmful consequences of SARS-CoV-2, it is essential to do research on the transmission of the virus between patients and healthcare professionals. During the course of our research, we concentrated on analyzing samples that were collected from the peri-patient environment with the intention of developing new infection control techniques to inhibit the spread of infection between patients and healthcare staff.

When we were working with the first group of patients, we took air samples from different parts of the patient's environment in order to acquire a more definitive pattern of air contamination. The highest percentages of positive results were found in samples that were taken in front of the ventilator, which highlights the necessity of protective measures for healthcare professionals who are attending to the ventilator or during close clinical examination. In addition, we have collected samples from the area in front of the patients as well as the middle of the room, both of which have demonstrated positive findings that are not statistically significant. yet, because to the limited size of the sample, this cannot be deemed conclusive; yet, it does highlight the importance of having adequate ventilation and air filtering in intensive care units and COVID-19 entrance bays. Twenty-four observational studies with

a cross-sectional design were included in the systematic review that was carried out, 82 out of 471 air samples (17.4%) were found to be positive for SARS-CoV-2 RNA near the patient. In intensive care unit settings, the positivity rate was much greater, which suggests that the air near and far from patients with COVID-19 was frequently contaminated with SARS-CoV-2 RNA (Civra, A *et al.,* 2021).This was the case regardless of the distance between the patient which was keeping with the results in our study and supporting the fact that ICU air specifically close to the patient can be contaminated with COVID-19.

In a further observational study that was carried out in intensive care unit rooms with a single bed, it was discovered that 76% of the 100 surface samples and 30% of the forty air samples contained viral RNA environmental contamination. A high-flow nasal cannula system did not produce more viral aerosolization than a mechanical ventilation system in patients with COVID-19, according to the study; however, this information was not examined in our investigation (Civra, A *et al.,* 2021). The study also suggested that a mechanical breathing system also produced more viral aerosolization.

In comparing the results of the current study with those of previous studies, it is evident that the detection of SARS-CoV-2 in air samples across various settings, particularly healthcare environments, has been a focal point of research. (Guo, Z. D., Wang *et al.,*2020) in their study found SARS-CoV-2 and other respiratory pathogens present in continuous air samples from congregate settings, similar to the findings in the current study, where the highest detection rate of SARS-CoV-2 was observed in the air near the ventilator (8.3%). This aligns with the study by Moore *et al.,* 2021, which also emphasized the importance of sampling in healthcare environments, particularly in areas with direct airflow like in front of ventilators, where viral particles are more likely to accumulate and pose a transmission risk.

Furthermore, the current study's observation of a low detection rate of SARS-CoV-2 in other areas (middle of the room and above the patient) resonates with the results of Guo *et al.* (2020), who demonstrated that SARS-CoV-2 aerosols are less concentrated in non-ventilated or less active areas. The study by Robotto *et al.,*2021 highlighted the significance of methodological consistency in detecting SARS-CoV-2 in both indoor and outdoor air samples, which could explain the variation in detection rates between different sampling sites in the ICU and isolation rooms in the current study.

Additionally, van Doremalen et al.,2020 discussed the aerosol stability of SARS-CoV-2 and SARS-CoV-1, which further supports the notion that the virus can persist in the air for extended periods, particularly in environments with constant airflow like ventilation systems. This finding correlates with the higher detection rate near the ventilator in the current study as well persistence of the virus till day 4.

The findings of Solo-Gabriele et al., 2020 regarding the use of SARS-CoV-2 RNA in air, surface swabs, and wastewater samples provide an integrated approach to assessing the environmental presence of the virus, highlighting the need for comprehensive monitoring strategies in clinical and communal settings, which is consistent with the methodology used in this study.

This discovery is supported by the findings of a multi-center investigation that was carried out in England during the initial wave of the COVID-19 outbreak. The study indicated that SARS-CoV-2 RNA was found on 30 (8.9%) of 336 ambient surfaces that had a low bacterial count at the same time. According to the findings of the study, efficient cleaning has the potential to lessen the likelihood of fomite transmission through contact (Solo-Gabriele *et al.,* 2020). On the other hand, our experiment did not look into the surface samples nor number of bacteria, which may be a drawback that can be solved in subsequent research. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to detect SARS-CoV-2 RNA in surface, air, patient mask, and healthcare worker mask samples, respectively, in a study that investigated environmental SARS-CoV-2 contamination in hospital rooms of patients with acute COVID-19 in France (Nagle, S. *et al.,* 2022). As a result, this provides

evidence that airborne aerosols, as well as solid objects such as masks and the garments and masks worn by health care workers, can be sources of air contamination. In this context, regulations for infection control should take into consideration the changing of clothing worn by healthcare workers and the execution of appropriate decontamination procedures. Additionally, masks should be changed often between patients and at predetermined intervals of time, which should be further determined based on data.

After conducting research on the length of time that surfaces are polluted, it was discovered that surfaces can continue to be contaminated for up to four days. In this time period, it is necessary to disinfect the surface in the appropriate manner. Due to the fact that these samples were collected during the patient's hospitalization, we are unable to definitively determine whether the contamination occurred on the first day of the patient's stay or whether it was a repeated infection.Because of this, it is not possible to provide any exact advice concerning the amount of time that should pass between patients in terms of disinfection or room evacuation. On this subject, there is a paucity of published material, which suggests that it would be an intriguing subject for additional investigation.

CONCLUSION

The presence of CoV-2 in the air in intensive care units and isolation rooms is a fact that is supported by evidence. This fact necessitates the implementation of appropriate air filtering, adequate room ventilation, and surface disinfection in these environments in order to reduce the risk of infection between patients and between patients and healthcare workers. For the purpose of elucidating the length of time spent in isolation and decontamination, as well as the type of decontamination, additional research is required.

Declarations:

Ethical Approval: Patients gave their consent to participate after receiving complete and accurate information. In this retrospective investigation, the form of informed permission that was applied was verbal consent of the participants. The confidentiality and security of the data were guaranteed for each and every participant. The participants were given the opportunity to withdraw from the research procedure at any moment, and they were told of the point at which this option would no longer be available any longer. Furthermore, users had the ability to withdraw their data if it was identifiable to them during the process. Any predicted benefits as well as any potential risks were communicated to the individuals who participated in the research.

Competing interests: The authors declare that there is no conflict of interest.

Author's Contributions: Sahar Abdel Fattah carried out field execution to all experiment stages, collect blood samples and field data and contributed in wrote this article. Sahar Abdel Fattah wrote this article, helped in biochemical analysis, contributed in drafting the manuscript and revision as well as performing the statistical analysis of the results, contributed in drafting the manuscript and revision. Amira T. Mersal , Nadia F. Ibrahim and Mohamed M. Abdelghaffar wrote this article and contributed in drafting the manuscript and revision and performed the histo-morphological and immunohistochemical parameters. Responsible for paper idea; Sahar Abdel Fattah, Amira T. Mersal , Nadia F. Ibrahim and Mohamed M. Abdelghaffar.All authors approved the final manuscript.

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