

Sniffer Dogs as a Screening/Diagnostic Tool for COVID-19, A Proof of Concept Study

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Abstract

Background: Sniffer dogs have been shown to detect certain chemical particles and to help diagnose many diseases and complications, such as colorectal cancer, melanoma, bladder cancer, and even dangerous states such as hypoglycemia in DM-1 patients. With the spread of COVID-19 throughout the world and the need to have a real-time screening of the population, especially in crowded places, this study aimed to investigate the applicability of these sniffer dogs.

Methods: This study was done in two phases. In the first step, three dogs (including one German shepherd, one German black, one Labrador) were intensively trained by the classical conditioning method for seven weeks. Human specimens were obtained from the throat culture and pharyngeal secretions of both definitely positive and negative COVID-19 participants. During the first seven weeks, each dog underwent the conditioning process for averagely 1000 times. In the verification process, 80 pharyngeal secretion samples consisting of 26 positive samples of hospitalized patients and 54 negative samples of hospitalized patients for other medical reasons were provided to the training team in a single-blind manner. The verification test was done using three dogs (Lexi, Sami, and Kozhi). Another similar conditioning process was done using COVID-19 patients' clothes and masks and the verification test was done using 50 positive and 70 negative samples by three other trained dogs (one Labrador, one Border gypsy, and one Golden retriever).

Results: In the pharyngeal secretions verification test, the sniffer dogs showed 65% sensitivity and 89% specificity. In this process, they could identify 17 out of the 26 true positive samples and 48 out of the 54 true negative samples. According to the results, the positive predictive value and negative predictive value for this experiment were 74% and 84%, respectively. In the next verification test for the patients' face masks and clothes, 43 out of the 50 positive samples were correctly identified by the dogs. Moreover, out of the 70 negative samples, 65 samples were correctly found to be negative by the canines. The sensitivity of this method was as high as 86% and its specificity was 92.9%. In addition, the positive and negative predictive values were 89.6% and 90.3%, respectively.

Conclusion: Dogs are capable of being trained as the identifier of people with COVID-19 by detecting their odor and can be used as a reliable tool in limited screening.

Background

Since late 2019, the SARS-COV-2 virus has emerged globally, leading to a pandemic. To the date of this writing, this has resulted in 14,189,223 confirmed cases and around 600,000 mortalities over the globe (1). This disease affected many sectors, even the provision of medical services and health education (2–4). In the first phase of the disease control, many countries adopted the quarantine policy to fight with the disease (5). However, the lockdown caused a massive breakdown in economy, especially in poor settings (6, 7). The central governments released the limitations gradually or abruptly based on their economic statuses, which has already resulted in the second wave of the disease in certain areas (8).

The primary modality in the diagnosis of COVID-19 seems to be Computed Tomography (CT-scan) and Polymerase Chain Reaction (PCR), both of which have absolute reliability (9). Nonetheless, the PCR test for COVID-19 is diverse in nature, which has resulted in different sensitivity and specificity values across the literature (10–12). Moreover, in addition to high expenses, CT-scan requires radiation and raises the risk of multiple morbidities, including malignancies (13, 14). Furthermore, these are not tools for public screening, nor are they available in all settings.

The meticulous canine olfactory system is well-known for human beings. The olfaction system has an extensive scent epithelium (170 cm² and 17 times greater than humans) as well as a large number of olfactory receptors (beyond 200 million receptors in comparison to 5 million in humans) (15, 16). This power is currently being used in exploring explosive materials, drugs, and even lost bodies. The use of sniffer dogs in medical literature dates back to a case report in 1989, in which a dog persistently smelled the mole of its owner (17). This habit was exacerbated over time and even when the trousers covered the mole. When she wore a skirt, the dog wanted to bite that mole. The dog was neutral concerning the other moles of the body. The owner got suspicious and consulted a dermatologist, and the diagnosis was a kind of melanoma (a type of skin malignancy). Following that event, many studies explored the utility of dog olfaction to have such screening or diagnosis. The utility of dogs in the diagnosis of multiple diseases, such as malignancies, diabetes, Parkinson's, seizures, and certain hormonal and enzymatic defects, has been shown by multiple studies (18–29). It is commonly believed that dogs can be trained to detect the "odor signature" of particular molecules and compounds. These are mainly believed to be Volatile Organic Compounds (VOCs) originating from altered biochemical interactions within the body in the presence of malignancies, inflammations, infections, and other related events. Metabolic changes in the body result in a set of certain odors that may be detectable for animals, such as dogs, as they have an extreme olfactory sensation and a very complex and unique analytical mechanism for detecting odors at the molecular level (25). In such a scenario, COVID-19 may not be an exception. At present, the diagnosis of this disease is based on sophisticated biochemical and genetic tests and CT-scan imaging. These modalities are relatively expensive and require accurate medical equipment and interpretation, which are usually positive during the initiation of symptoms. Thus, the golden time may be lost to cut the chain of transmission earlier (30). The use of trained dogs leading to earlier detection of infected persons may be possible to make the diagnosis much sooner. Use of canines for early detection of these viruses is less expensive compared to the abovementioned methods and helps separate asymptomatic carriers and patients quickly (each dog has a screening capacity of 250 samples per hour, which is effective in cutting the transmission chain of the disease and controlling its prevalence).

According to the recommendations of the World Health Organization (WHO), the most critical issue in adopting strategies and policies to mitigate the spread of the disease is the ability to screen a large number of people, which is either impossible or difficult to do, especially in low-resource settings. In such a case, it was hypothesized that a defined population or environment could be easily screened in the most convenient time by using a few trained dogs. Therefore, this study aims to evaluate the feasibility of training and using the olfactory power of dogs to diagnose COVID-19.

Methods

Study design

This study was based on the hypothesis assuming that the sniffer dogs can detect the metabolism changes in human body as a result of being infected with SARS-Cov-2 virus. For this purpose, a training process was designed to condition five different breeds of German shepherd, German black, Labrador, Golden retriever, and Border gypsy dogs. The study was divided into two different training sections; the first study on the pharyngeal secretions detection and the second one on patients' face masks and clothes by both true positive and negative samples taken from infected hospitalized patients in SARS-Cov-2 ICU and other healthy individuals. The verification test was done in a single-blind process.

This study was approved by Iran's Ministry of Health, Treatment, and Medical Education (IR.AJAUMS.REC.139.055). Considering human resource management, all trainers were fully equipped with proper Personal Protection Equipment (PPE) while working with dogs and test specimens.

Sniffer dogs' introduction

Designing the study required making decisions about the dogs that could be involved in the study. The dogs belonged to the SK9 Dogs Training School. In this context, each dog's training background, age, and gender had to be taken into account. Since there was no evidence on the probability of success of each species in detecting the COVID-19 virus, the dogs were selected from different species at different ages. The dogs' characteristics have been presented in Table 1.

Table 1
Dogs' characteristics

Name	Age (year)	Gender	Species
Lexi	1	Female	Labrador
Sami	2	Male	Border gypsy
Saray (SY)	2	Female	German shepherd
Kuzhi (KZH)	1.5	Female	German black
Marco (MRC)	1.5	Male	Labrador
Zhico	3	Male	Golden retriever

Dog training

Three dogs (including one German shepherd, one Labrador, and one Border gypsy) were intensively trained by the classical conditioning method for seven weeks (31–33). For the first phase of the study, the training set included the pharyngeal secretions of both healthy individuals and COVID-19 positive patients. These specimens were transported daily from the hospital laboratory to the training site under safe and standard conditions. These samples were placed in groups of 10, consisting of one to three positive COVID-19 samples inside the dogs' training wheel. Positive samples were obtained from the patients admitted to the ICU. The samples were taken from both male and female patients at different ages before and after taking medicines. It should be noted that some patients were healthy before being infected with COVID-19, while others had diseases such as diabetes, diabetes mellitus, coronary diseases, heart failure, renal failure, respiratory disease (asthma and Chronic Obstructive Pulmonary Disease (COPD)), and Senecavirus A (SVA). In the second phase of the study, the training set included face masks and clothes. Each dog was trained by 1300 clothes and 1300 face masks of Covid-19 positive patients that were used for 24 hours. The control sample in this phase included the same hospital clothes and masks from the patients who were admitted in the hospital but were proven to be COVID-19 negative.

Safety and protection protocols

Testing

Before the initial stage of the project, all training team members as well as dogs were tested for COVID-19 (PCR method) to make sure they were not infected with the virus. In the course of the study also, the training team members were tested regularly on days 1, 21, 35, and 49. Two weeks after the final verifications, all team members were tested again not to be infected during the past week.

Personal protection

All team members were fully protected during the trainings. They all used the same PPE, face masks, and shields as those used in ICUs.

Quarantine

All training team members stayed in the training site during the study.

Samples delivery

Pharyngeal secretion samples, face masks, and clothes were safely delivered to the site.

Sanitizing

Training site, training equipment, and dormitory of the team were sanitized twice a day during the training period.

Dogs condition

The dogs were kept in standard cages under standard conditions and were fed with high-quality food. It should be noted that no forceful training equipment were utilized.

Testing phase and verification phase

In late April 2020, following seven weeks of intensive training, the testing and verifying phases were initiated. Each dog underwent the training process for averagely about 1000 times, and nearly 120 tests were performed during the course. In the second phase of the study, the dogs were tested via 120 final samples (consisting of clothes and face masks), 50 positive samples and 70 negative ones. Notable to mention that the samples were taken from different medical centers and people with various levels of disease severity. For the verification stage, 80 samples of pharyngeal secretions (26 positive samples from patients with COVID-19 virus and 54 negative samples from healthy individuals) were obtained and provided in a single-blind manner.

At the end of the study, none of the dogs had COVID-19 and were completely healthy.

Statistical analysis

Epidemiological and statistical tests, including sensitivity or True Positive Rate (TPR), specificity or True Negative Rate (TNR), positive condition, negative condition, False Negative Rate (FNR), False Positive Rate (FPR), Positive Predictive Value (PPV), and Negative Predictive Value (NPV) were performed. The data were analyzed using the SPSS software, version 23.

Result

After completing the training phase, the testing phase was initiated. In this initial step, the dogs were able to identify the positive samples in all samples with an accuracy of over 80%. In the verification test, 80 samples were used in eight sets of ten (both positive and negative). Out of the 26 positive samples, the dogs identified 17 positive samples. Surprisingly, 48 out of the 54 negative samples in the test tubes were correctly identified by the dogs. Nevertheless, the performances of the three trained dogs were slightly different. The German shepherd dog named 'SY Saray' successfully identified six out of the ten positive samples. Kuzhy (KZH), the German black dog, was able to correctly identify six out of the ten positive samples in a set of 30 samples. The third dog in this experiment was Marco (MRC), a Labrador, that identified five positive samples out of 20 (6 positive and 14 negative) (Table 2).

Table 2
Description of the performances of different dogs in identifying the positive and negative samples of COVID-19 virus

	Positive condition		Negative condition		Predicted positive condition	Positive condition	Predicted negative condition	Negative condition
	True positive	False negative	False positive	True negative				
SY	6	4	3	17	9	10	21	20
KZH	6	4	2	18	8	10	22	20
MRC	5	1	1	13	6	6	14	14
Total	17	9	6	48	23	26	57	54

Summarizing the results obtained by each dog and detection of 17 out of the 26 positive samples in the experiment revealed a sensitivity of 65% as an average indicator of the total performance of the dogs. Moreover, the correct detection rate of the negative samples was 89%, as they correctly identified 48 out of the 54 negative samples. According to the results, the PPV and NPV for this experiment were 74% and 84%, respectively (Table 3) (Fig. 1).

Table 3
Statistical results of the dogs' performance in identifying positive and negative samples

	Sensitivity or true positive rate (TPR) %	False negative rate (FNR) %	False positive rate (FPR)	Specificity or true negative rate (TNR)	Positive predictive value	Negative predictive value
SY	60%	40%	15%	85%	67%	81%
KZH	60%	40%	10%	90%	75%	82%
MRC	83%	17%	7%	93%	83%	93%
Total	65%	35%	11%	89%	74%	84%

In the second phase of the study, the testing set included 50 positive samples (clothes and masks) and 70 control (negative) ones. Out of the 50 positive samples, 43 were correctly identified by the dogs. Moreover, out of the 70 negative samples, 65 were correctly found to be negative by the canines. The sensitivity of this method was as high as 86% and its specificity was 92.9%. Additionally, the positive and negative predictive values were found to be 89.6% and 90.3%, respectively (Table 4).

Table 4
The overall performance of the dogs in the second phase of the study

	Condition Positive		Condition Negative		Predicted positive condition	Positive condition	Predicted negative condition	Negative condition	Sensitivity or true positive rate (TPR) %	False negative rate (FNR) %	False positive rate (FPR)	Specificity or true negative rate (TNR)
	True positive	False negative	False positive	True negative								
Face Masks and clothes	43	7	5	65	48	50	72	70	86.0%	14.0%	7.1%	92.9%

Discussion

In this study, positive samples were provided for the dogs and trainers. However, neither the dogs nor the trainers were infected with COVID-19, which was a great achievement.

Since the historical case report by Williams et al. in 1989 (17), there have been few experiments in the utility of the canine olfactory detection in the medical field. These experiments were mostly related to the detection of ovarian, prostate, colorectal, and lung cancer (21, 23, 24, 27, 28, 34, 35). However, the use of canine olfactory detection in other areas, including infectious diseases, has been limited. The current study aimed to investigate the applicability of canine olfactory detection in mitigating the COVID-19 virus. In this field, a recent pre-print paper showed the efficacy of dogs in differentiating the COVID-19 positive and negative cases using armpit sweat (36).

The first achievement of the present study was that during the ten weeks of continuous training, none of the trainers and dogs was infected with the SARS-COV-2 virus due to the observance of appropriate protection protocols. Another achievement of the research was a proof of concept in which the presence of a specific and traceable odor in people with COVID-19 disease was proved with a significant probability. In the first phase of the study, the dogs successfully distinguished over 65% of the positive specimens and 89% of the negative samples. In the second phase, using clothes and masks, they could differentiate 86% of the positive samples and 92.9% of the negative ones. It should be noted that laboratory samples containing the COVID-19 virus used in this study were taken from different people with different clinical conditions on different days to reduce the possible environmental effects. What can be certainly said is that the presence of this virus in human body led to a specific chain of chemical and metabolic reactions that caused the release of a specific odor compared to the odor of a negative sample.

In the first phase of the study, sensitivity and specificity values of 65% and 89% were noticed. In the second phase, the values were as high as 86% and 92.9%, respectively. This was easily comparable to those of laboratory kits (37, 38). In terms of diagnosis speed, dogs are real-time detectors that are highly required in the setting of pandemics. Furthermore, dogs are supposed to have better performance with more intense training. Thus, such detecting dogs can be used to identify suspicious COVID-19 cases in places with a high population density, such as airports, passenger terminals, and important national centers.

Conclusion

Dogs can be trained to identify people with COVID-19 disease and can be used as a reliable tool in limited screening. This study was a limited experience and could serve as a basis for directing future research in this field. This study also emphasized the need for detecting the 'signature odor' of COVID-19. As we currently know, there are at least 3481 VOCs in human breath. We hope that using the biological system can help detect the specific VOCs for this disease, which can possibly lead to designing biosensors and electronic noses. Currently, the VOCs associated with several malignancies have been identified. Accordingly, 4-methyl decane, dodecane, and undecane have been reported to be associated with malignant melanoma (39). Identification of the VOCs associated with COVID-19 and development of biosensors can screen the mass population via deep learning algorithms.

Abbreviations

Computed Tomography Scan: CT-scan

Polymerase Chain Reaction: PCR

Volatile Organic Compounds: VOCs

World Health Organization: WHO

Personal Protection Equipment: PPE

Chronic Obstructive Pulmonary Disease: COPD

Senecavirus A: SVA

True Positive Rate: TPR

True Negative Rate: TNR

False Negative Rate: FNR

False Positive Rate: FPR

Positive Predictive Value: PPV

Negative Predictive Value: NPV

Declarations

Ethics approval and consent to participate: This study was approved by Ethics committee of Iran's Ministry of Health, Treatment, and Medical Education (IR.AJAUMS.REC.139.055). Considering human resource management, all trainers were fully equipped with proper Personal Protection Equipment (PPE) while working with dogs and test specimens. Informed consent, written was obtained from all participants.

Consent for publication: All authors are fully satisfied with the publication of the article.

Availability of data and material: All data is available.

Competing interests: The authors have no conflict of interest to declare.

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Authors' contributions: MS, EE, MYZ, HA, RHF, AN, RL, HR, ADM, RZ, MAM, and HA were responsible for the study conception and design and Mehdi Shiri supervised the whole study. MS, EE, HA, RHF, AN, RL, HR, ADM, RZ, MAM, and HA prepared the first draft of the manuscript. MS, EE, HA, RHF, AN, RL, HR, ADM, RZ, MAM, and HA analyzed the results and supervised the study. All authors have read and approved the final manuscript.

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Figures

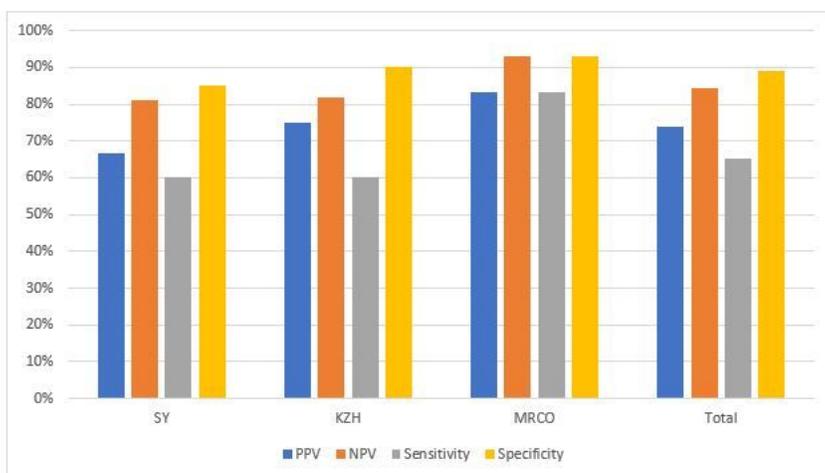


Figure 1

Statistical results of the dogs' performances