Annals of International Medical and Dental Research
E-ISSN: 2395-2822 | P-ISSN: 2395-2814
Vol-8, Issue-4 | July-August 2022
DOI: 10.53339/aimdr.2022.8.4.5
Page no- 33-39 | Section- Research Article (Microbiology)

Prevalence of Sars-Cov-2 Detected Through Cartridge-Based Nucleic Acid Amplification Test (Cbnaat) Among Patients of Ili and Sari in Various Districts of Punjab

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Received: 04 March 2022
Revised: 28 April 2022
Accepted: 07 May 2022
Published: 23 June 2022

Abstract

Background: COVID-19 is an infectious disease caused by the SARS-CoV-2 virus. After a December 2019 outbreak in China, the World Health Organization identified SARS-CoV-2 as a new type of coronavirus. Currently, WHO recommends detection of unique sequences of virus RNA by rRT-PCR. ICMR also recommends use of CBNAAT using Cepheid Xpert Xpress SARS-CoV2. The aim of this study is to determine the prevalence of SARS-CoV-2 detected through CBNAAT. **Material & Methods:** This retrospective study was conducted from July 2020 to December 2021 at VRDL, GMC, Amritsar. The study group consisted of all the patients presenting with symptoms of Influenza Like Illness (ILI) and Severe Acute Respiratory Illness (SARI) who presented to hospital. The data was collected and subjected to statistical analysis. **Results:** During the present study, a total of 1,259 samples were analyzed for SARS-CoV-2 by CBNAAT from July 2020 to December 2021. Out of total 1,259 cases which were included in the study, 327 cases (25.97%) were found to be SARS-CoV-2 positive while 870 cases (69.10%) were SARS-CoV-2 negative and 62 cases were found to be inconclusive. 62 inconclusive samples were further tested by RT-PCR. Out of which, 15 were RT-PCR positive and 47 were RT-PCR negative. **Conclusions:** The COVID-19 pandemic has put forward unprecedented challenge to the public health system across countries to prepare themselves for this current crisis which included isolation, contact tracing, quarantine and enforcement of a nation wide lockdown starting 25th March, 2020.

Keywords:- CBNAAT, SARS-CoV-2, Coronavirus.

INTRODUCTION

Coronaviruses (CoV) are group of viruses that cause respiratory tract infections ranging from the common cold to more severe diseases such as Middle East respiratory syndrome (MERS)-CoV and severe acute respiratory syndrome (SARS)-CoV.[1] COVID-19 is an infectious disease caused by the SARS-CoV-2 virus.[2] It is a kind of common virus that causes an infection in your nose, sinuses or upper throat. It has been the major pandemic which the world has seen in recent times.

On December 31, 2019, China informed the World Health Organization (WHO) about

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cases of pneumonia of unknown etiology detected in Wuhan city, Hubei province of China. 44 patients with pneumonia of unknown etiology were reported from December 31, 2019 to January 3, 2020 to WHO by the national authorities in China. During this period, the causal agent was not identified.[3] After a December 2019 outbreak in China, the World Health Organization identified SARS-CoV-2 as a new type of coronavirus.[4] The outbreak quickly spread around the world. On January 30, 2020, WHO declared COVID-19 outbreak as Public Health Emergency of International Concern (PHEIC).[5] The World Health Organization (WHO) on March 11, 2020, has declared the novel coronavirus (COVID-19) outbreak a global pandemic.[6] In India the first coronavirus case was reported on 30 January 2020 in the state of Kerala.[3]

SARS-CoV-2 is a large positive-sense single-stranded ribonucleic acid (RNA) virus belonging to the family Coronaviridae. The SARS-CoV-2 genome is approximately 30,000 nucleotides in length and encodes several proteins including an RNA-dependent RNA polymerase (RdRP) and four structural proteins viz., nucleocapsid protein (N), spike protein (S), envelope protein (E), and membrane protein (M).[7] Transmission can be from an infected person's mouth or nose when they cough, sneeze, speak or breathe.[8] Wide range of symptoms which may appear 2-14 days after exposure to the virus ranging from mild symptoms to severe illness are seen in COVID-19 infected patients.[9]

Currently, WHO recommends detection of unique sequences of virus RNA by Nucleic Acid Amplification Test (NAAT) such as real-time reverse-transcriptase polymerase chain reaction (rRT-PCR). ICMR also recommends use of FDA approved Cartridge-Based Nucleic Acid Amplification Test (CBNAAT) using Cepheid Xpert Xpress SARS-CoV2 for use under an emergency use authorization (EUA) which only detects E and N2 gene.[7] CBNAAT is widely available as it is already being used for the diagnosis of tuberculosis and other infectious diseases.

Aims & Objectives

This retrospective study aims to determine the prevalence of SARS-CoV-2 detected through CBNAAT from nasopharyngeal/ oropharyngeal swab specimen collected from patients of various districts of Punjab and referred to Viral Research Diagnostic Laboratory [VRDL], Government Medical College [GMC], Amritsar.

MATERIAL AND METHODS

This retrospective study was conducted for a period of one and a half year i.e. from July 2020 to December 2021 at VRDL, GMC, Amritsar. The study group consisted of all the patients presenting with symptoms of Influenza Like Illness (ILI) and Severe Acute Respiratory Illness (SARI) who presented to hospital. According to WHO, case of ILI is defined as an acute respiratory infection with measured fever of ≥38℃ and cough with onset within the last 10 days.[10] Case of SARI is defined as an acute respiratory infection with history of fever or measured fever of ≥38℃ and cough with onset within the last 10 days and requires hospitalization.[10] For initial diagnostic testing for current SARS-CoV-2 infections, CDC recommends collecting and testing an upper

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respiratory specimen i.e. nasopharyngeal and oropharyngeal specimen.[11] Specimens were obtained and transported in viral transport medium under cold chain. The rejection criteria for the samples includes the samples which were not transported in cold chain with proper packaging or were leaked or if the patient's details did not match with the label provided on the samples. SARS-CoV-2 was then identified by CBNAAT using Cepheid Xpert Xpress SARS-CoV2 according to manufacturer's protocol. The Cepheid Xpert Xpress SARS-CoV2 is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV2. The system consists of an instrument, computer and preloaded software for running tests and viewing the results. It uses single use disposable cartridges that hold RT-PCR reagents and host the RT-PCR process. The nasopharyngeal, oropharyngeal swab specimen is collected and placed into viral transport tube containing viral transport medium. The specimen is briefly mixed. Using the supplied transfer pipette, the sample is transferred to sample chamber of Xpert Xpress SARS CoV-2 cartridge. The cartridge is then loaded into GeneXpert Instrument System platform, which performs hands-off, automated sample processing and real time RT-PCR for detection of viral RNA. Testing is carried under BSL-2 conditions and with appropriate biosafety precautions.[12] No permission from institutional ethical committee was taken as data was collected from the old records of the department.

RESULTS

During the present study, a total of 1,259 samples were analyzed for SARS-CoV-2 by CBNAAT from July 2020 to December 2021. Out of total 1,259 cases which were included in the study, 327 cases (25.97%) were found to be SARS-CoV-2 positive while 870 cases (69.10%) were SARS-CoV-2 negative and 62 cases were found to be inconclusive. 62 inconclusive samples were further tested by RT-PCR. Out of which, 15 were RT-PCR positive and 47 were RT-PCR negative. Gender and age wise percent positivity of SARS-CoV-2 are shown in Table 1 and 2. Positivity rate SARI and ILI cases are shown in [Figure 1].

Table 1: Gender wise percent positivity of SARS-CoV-2

	Male	**Female**	**Total**
Positive	241(28.52%)	86(20.77%)	327
Negative	561(66.39%)	309(74.64%)	870
Inconclusive	43(5.09%)	19(4.59%)	62
Total	845(100%)	414(100%)	1259

Table 2: Age wise percent positivity of SARS-CoV-2

S.No.	**Age**	**Positive (327)**	**Total (1259)**	**Percent (%)**
1.	≤20	6	132	4.55
2.	21-40	108	589	18.34
3.	41-60	92	269	34.20
4.	≥60	121	269	44.98

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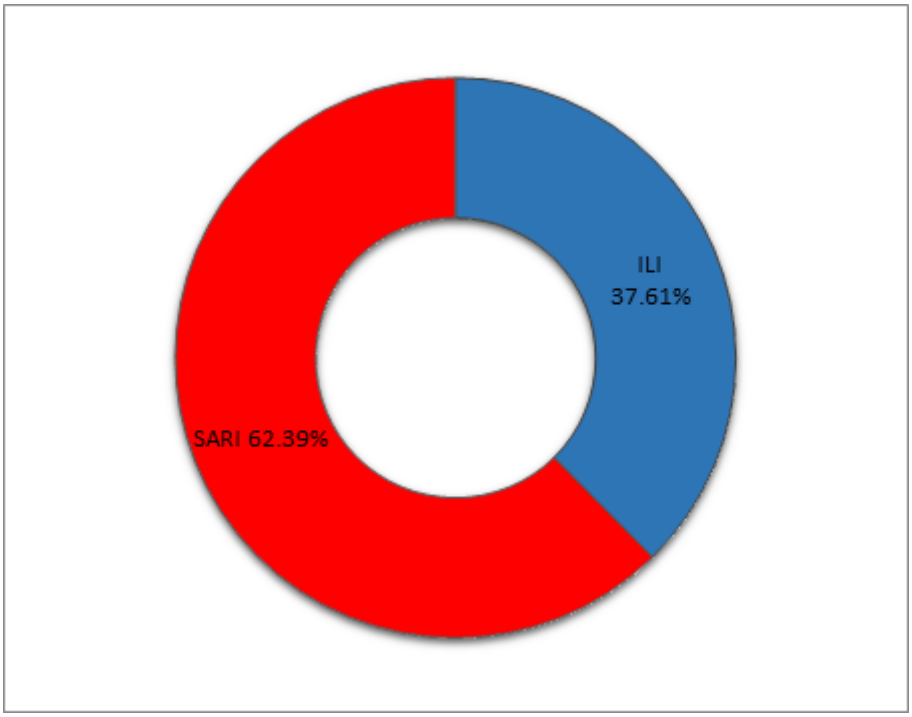

Figure 2: Positivity rate of SARI and ILI cases

DISCUSSION

The ongoing outbreak of SARS-CoV-2 infection has emphasized the importance of quick and accurate laboratory diagnosis in order to limit the spread as well as help patients to prevent the illness progression. Currently, ICMR has approved RT-PCR, CBNAAT and rapid antigen detection for the detection of SARS-CoV-2 infection. Nucleic acid testing modalities are confirmatory but are expensive and require expertise. Considering the gold standard for COVID-19 test, the RT-PCR can take a day to declare results, whereas CBNAAT is much quicker and produces results within 45 to 60 minutes.[13] With RT-PCR, more than 90 samples can be processed at one time with long turn-around time, requires dedicated infrastructure and trained personnel. CBNAAT has limited sample testing capacity with less turn-around time, even single samples can be tested with results available within one hour and doesn't even require high infrastructure.[14]

In the present study, a total of 1,259 samples were analyzed for SARS-CoV-2 detected through CBNAAT from July 2020 to December 2021. Out of which, 327 cases (25.97%) were found to be SARS-CoV-2 positive, 870 cases (69.10%) were SARS-CoV-2 negative and 62 cases were found to be inconclusive. These 62 samples were further tested by RT-PCR, out of which, 15 samples came out to be positive on RT-PCR and 47 were negative. Thus, proving that Real Time RT-PCR is the gold standard test for detecting cases of COVID-19.[15] The foremost reason lies in its accuracy in detecting the virus along with the ability to run upto 90 samples in a single run. However, this test

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cannot be performed at every district level laboratory due to its explicit requirements. Nevertheless, wherever accessible, real time RT-PCR should be used as the frontline test for diagnosis of SARS-CoV-2.[15]

In our study, 28.52% SARS-CoV-2 positivity was seen in males whereas 20.77% was seen in females. This finding is well supported by a study conducted by Chen et al in 2020 in Wuhan, China as well as by a study conducted by Tuli AK et al in 2021.[16,17] [Table 1] was statistically analyzed and found to be significant (p value <0.05). The rationale for this gender disparity can be attributed to number of factors such as social factors, genetic, immunological factors as well as hormonal difference. According to a recent study, the SARS-CoV-2 positivity variation among the gender could be because of difference in the levels of angiotensin-converting enzyme-2 (ACE2) expression which is the first point of contact for SARS-CoV-2 and the human body in both men and women. ACE2 gene locus on X-chromosome, which makes women heterozygous and differently assorted as compared to men who are homozygous. In conjunction with this, testosterone also play role in the expression of *ACE2*. Testosterone generally inhibits immune functions which is a possible explanation for men higher susceptibility to infections.[18] The male preponderance to infection has been explained by some studies that mast cells in females can trigger a more active immune response, which may help them fight infectious diseases better than the males.[17]

Age wise percent positivity in our study was 4.55% in age group of ≤20, 18.34% in 21-40 yrs, 34.20% in 41-60 yrs and 44.98% in ≥60 yrs. This data clearly showed that rate of infection increases with age. This is in concordance with the data obtained from CDC in 2021,[19] as well as with the study conducted by Davies NG et al in 2020.[15] The age gradient in recent reported cases, result from children having decreased susceptibility to infection which could result from immune cross-protection from other coronaviruses or from non-specific protection resulting from recent infection by other respiratory viruses, which children experience more frequently than adults.[20] Elderly people are at a higher risk of COVID-19 infection due to their decreased immunity and body reserves as well as multiple associated co-morbidities.

In the present study, majority of the samples which were found COVID-19 positive were due to patients suffering from severe acute respiratory illness which were 204 (62.39%) followed by influenza like illness 123 (37.61%). The results were in concordance with study conducted by Pannu et al in 2021 as well as with other study conducted by Gupta et al in 2020.[21,22] [Figure 1] was statistically analyzed and found to be significant (p value <0.05). There is increased chance of morbidity and mortality in SARI associated COVID-19.[23] It is found that symptomatic patients tend to have increased susceptibility to COVID-19 infection as compared to asymptomatic ones.

CONCLUSIONS

The COVID-19 pandemic has put forward unprecedented challenge to the public health system across countries to prepare themselves for this current crisis. As SARS-CoV-2 set foot in India, government took a number of steps to

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limit its spread which included isolation, contact tracing, quarantine and enforcement of a nation wide lockdown starting 25th March, 2020. Early recognition and prompt management is the need of the hour to prevent any mortality and morbidity.

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Source of Support: Nil, Conflict of Interest: None declared