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Research Article

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Usage of Cartridge Based Nucleic Acid Amplification Test (CB-NAAT/Xpert Xpress) in the diagnosis of SARS-CoV-2 among patients of tertiary care hospital in Amritsar, Punjab, India.

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Abstract

Cartridge based nucleic acid amplification test (CB-NAAT/Xpert Xpress) is an automated cartridge-based, in vitro molecular diagnostic test which is used for the qualitative detection of nucleic acid. It is a one-step confirmatory test for SARS-CoV-2 genes. Present study was conducted in VRDL, GMC, Amritsar. The objective of the study was to diagnose SARS-CoV-2 in patients, naso/oropharyngeal samples sent for CBNAAT from various districts of Punjab, referred to VRDL, GMC, Amritsar during the period from July to December 2020. Results were evaluated on the basis of different categories, gender and age. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were checked by comparing the results of 80 random samples tested by CB-NAAT/Xpert Xpress and RT-PCR. It was observed that the simplicity, speed and automation make CB-NAAT/Xpert Xpress a very promising diagnostic test for the detection of Covid-19 patients. The cost of the test however is a limiting factor.

Keywords: CBNAAT, SARS-CoV-2, RT-PCR

Introduction

An outbreak of respiratory illness of unknown etiology in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) on December 31, 2019 (Michaels et al., 2020). On January 30, 2020, WHO declared it a public health emergency of international concern. Subsequently, COVID-19 has spread over the world across 213 countries and territories. The most affected nations are the USA, Russia, Spain, Brazil, UK, Italy, France, Germany, Turkey, Iran, and India. On March 11, 2020, WHO declared COVID-19 as a global pandemic (Hsu et al., 2020). In India the first coronavirus case was reported on 30 January 2020 in the state of Kerala. The responsibility of formulating guidelines, coordination and monitoring of COVID-19 testing was given to the Indian Council of Medical Research (ICMR), the apex health research body of the country. ICMR has approved nucleic acid amplification tests RT-PCR and cartridge-based nucleic acid amplification test CBNAAT and Truenat™ and rapid antigen detection for diagnosis of COVID-19. The Xpert Xpress SARS-CoV-2 test is a molecular diagnostic test based nucleic acid amplification technology. These tests use customized cartridges and have quick turnaround time. The closed nature platforms and minimum sample handling, these tests pose a minimum bio-safety hazard and number of samples can be tested in a single run depending upon the model of the GeneXpert systems.

SARS-CoV-2 is an enveloped, positive-sense, single stranded ribonucleic acid (ssRNA) virus with a diameter of 50 to 200 nm which comprises four structural proteins, i.e., spike protein (SP), envelope protein (EP), membrane protein (MP), and nucleocapsid protein (NP). Infection is acquired either by inhalation of respiratory droplets or through contact with surfaces contaminated by them. After an incubation period of 2 to 5 days, the patient develops varying degrees of symptoms, ranging from fever, headache, fatigue and myalgia to sore throat, cough and shortness of breath. All ages and sex are susceptible. Severe infection and high-mortality are seen in old age patients with comorbid conditions like diabetes, hypertension, cancer, lung disease, cardiovascular disease, immunosuppressive drugs, old age and children (Singal, 2020) leading to complications like acute lung injury, acute respiratory distress syndrome (ARDS), shock, and acute renal injury (Sahin, 2019, Huang et al., 2020). Thus, the present study was aimed to diagnose SARS-CoV-2 in patients, naso/oropharyngeal samples sent for CBNAAT from various districts of Punjab, referred to VRDL, GMC, Amritsar, Punjab.

Materials and Methods

During the study period from July 2020 to Dec 2020, 657 nasopharyngeal/oropharyngeal samples for CBNAAT/Xpert Xpress analysis, from various districts of Punjab were received at VRDL, GMC, Amritsar, under cold chain conditions. Samples were processed in BSL-2 cabinet in lab and inoculated to GeneXpert cartridges as per manufacturer's instructions. The inoculated cartridge was inserted into CB-NAAT/Xpert Xpress SARS-CoV-2 test (Cepheid) system which performs automated specimen processing, RNA extraction, RT-PCR of SARS-CoV-2 RNA, and amplicon detection in a single run. The test detects nucleocapsid gene (N) and envelope gene (E) genes. Results were analyzed in approximately 50 minutes using E gene, N gene and sample processing control (SPC) and were interpreted as the positive, negative and inconclusive. Out of 657 CBNAAT tested samples, 80 samples were further tested with RT-PCR to compare by taking RT-PCR as a gold standard.

Results

During the present study, a total of 657 samples were analyzed for SARS-CoV-2 by CBNAAT/Xpert Xpress from July to December 2020. The percent positivity for SARS-CoV-2, gender and age wise distribution of SARS-CoV-2 positivity are shown in Table 1, 2 and 3.

Out of 657 CBNAAT tested samples, 80 samples were randomly selected and were further tested with RT-PCR to compare by taking RT-PCR as a gold standard. Table 4 showed comparative results of CBNAAT/Xpert Xpress and RT-PCR.

Table 1: Percent positivity of SARS-CoV-2

S No.	Results	Numbers	Total	Percent (%)
1	Positive	218	657	33.18
2	Negative	439	657	66.81

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

	Male	Female	Total
Positive	143(33.4%)	75(32.7%)	218
Negative	285(66.6%)	154(67.3%)	439
Total	428	229	657

Table 3: Age wise distribution of SARS-CoV-2 cases

No.	Age	Positive	Total	Percent (%)
1	≤20	7	190	3.6
2	21-40	77	210	36.6
3	41-60	88	187	47.0
4	≥60	46	70	65.7

Table 4: Comparative evaluation of samples in CBNAAT/Xpert Xpress and RT-PCR.

CBNAAT/Xpert Xpress Sars-Cov-2		RT-PCR Sars CoV-2		
		Positive	Negative	Total
	Positive	38	2	40
	Negative	3	37	40
		41	39	80
Sensitivity				95.0%
Specificity				92.5%
Positive Percent Agreement (PPA)				92.7%
Negative Percent Agreement (NPA)				94.9%

Discussion

The COVID -19 outbreaks had a major impact on clinical microbiology laboratories during the year 2020. Timely and accurate COVID-19 testing is an essential part of the management of COVID-19 for slowing down the pandemic. The gold standard method is molecular testing by RT-PCR but this test requires well-equipped laboratory facilities, highly skilled technologists and multiple reagents. Present study highlights the use of CBNAAT/Xpert Xpress in molecular diagnostic of COVID-19 which is a closed nature platform, requires minimum sample handling, pose minimum bio-safety hazard and has less turnaround time.

In the present study, a total of 657 samples were analyzed for SARS-CoV-2 by CBNAAT/Xpert Xpress from July to December 2020 and 218 samples were tested positive for SARS-CoV-2 and 439 were SARS-CoV-2 negative. Laxminarayan et al., (2020) reported that among 575,071 individuals, 84,965 were confirmed cases, and infection probabilities ranged from 4 to 10%, which was based on comprehensive surveillance data from the two Indian states: Tamil Nadu and Andhra Pradesh.

In our study, gender wise distribution of SARS-CoV-2 shows 32% samples were positive in females and 33% were positive in males. No significant difference was observed among both the genders. On the contrary, epidemiological findings of impact of COVID-19 reported across different parts of the world indicated higher infection in males than females (Bwire, 2020). This may be due to the higher proportion of males in their study population. Our results do not support a previous report that SARS-CoV-2 generally affects more males rather than females in this epidemic (Chen et al., 2020).

Age wise percent positivity in our study was 3.6% in age group of ≤20, 36.67% in 21-20 yrs, 47.0% in 41-60 yrs and 70.0% in ≥60. The rate of infection increased with age. In children or teenagers, (3.6%) infection was observed which is much lower than old people (70.0%). This observation matched with the study by Li et al., (2020) in which he reported the same trend. Similarly, Stoltenberg 2020, also observed lower infection in children than for the middle and elder age group in China and US. Li et al., also reported the maximum infection rate in people aged 30-69 years. Our study indicated that risk of infection is low in children and teenagers but rapidly increase for adults and old age people. Clark et al., (2020) reported that 66% of people aged 70 and above have increased risk of infection. This might be due to the fact that elderly patients are more susceptible to adverse clinical outcomes in SARS CoV-2 infection and assessment/treatment is also a challenging. Therefore, it is prudent to strengthen the tertiary preventive and clinical care of old-aged patients to reduce COVID-19 infections. Present study recommends, until more progress in treatment is achieved, that the elderly population should be shielded during COVID-19 outbreaks.

Amplification of N and E genes were interpreted by Cycle threshold value (Ct). During this study, a Ct value more than 40 was considered insignificant. Rao in 2020 highlighted the importance of Ct values in predicting the clinical course and prognosis of patients with COVID-19.

Out of 657 CBNAAT tested samples, 80 samples were randomly selected and were further tested with RT-PCR keeping it as a gold standard. A sensitivity of 95% and specificity of 92.5% was observed. The Positive Percent Agreement (PPA) and the Negative Percent Agreement was 92.7% and 94.9%, respectively. This is comparable with the studies done by Priyadarshi et al., (2020).

Present study concluded that in developing countries like India with large population and insufficient availability of tests, CBNAAT/Xpert Xpress could be adopted as rapid and reliable test strategy for COVID 19 confirmation. CBNAAT/Xpert Xpress has greatly reduced the turnaround time, which is a key factor in emergency cases. The burden on testing centers can also be reduced by using high throughput CBNAAT/Xpert Xpress machines. However, cost of high-throughput machines and cartridges is a limiting factor. Large scale studies are required for further validation of the test to establish the hypothesis.

Conflict of Interest: None

References

1. Bwire GM. Coronavirus: Why Men are More Vulnerable to Covid-19 Than Women? PMC, 2020. Doi: 10.1007/s42399-020-00341-w.
2. Chen N, Zhou M, Dong X, et al., Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet, 2020. Doi: 395:15–21. 10.1016/S0140-6736(20)30211-7
3. Clark A, Jit M, Warren-Gash C, et al., Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. CMMID. https://cmmid.github.io/topics/covid19/Global_risk_factors.html
   COVID-19 in Hubei Province and Other Parts of China. Front Med, 2020, 7: 190.
4. Hsu LY, Chia PY, Lim JFY. The Novel Coronavirus (SARS-CoV-2). Pandemic. Annals, 2020, 49: 105-07.
5. Huang C, Wang Y, Li X. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 2020, 395: 497–506.
6. Laxminarayan R, Wahl B, Dudalahttps://science.sciencemag.org/content/370/6517/691 - aff-5 SR, et al., Epidemiology and transmission dynamics of COVID-19 in two Indian states. Sci, 2020, 360: 691-97.
7. Li H, Wang S, Zhong F, et al., Age-Dependent Risks of Incidence and Mortality of
8. Michaels MG, La Hoz RM, Danziger-Isakov L, et al., Coronavirus disease 2019: Implications of emerging infections for transplantation. Am J Transp, 2020, 20: 1768-72.
9. Priyadarshi K, Vijaya LN, Sarika PK, et al., Molecular Diagnosis of COVID-19: An Update and Review. Annals of the National Academy of Med Sci, 2020. DOI: 10.1055/s-0040-1713836.
10. Rao SN, Manissero D, Steele V. Clinical utility of cycle threshold values in the context of COVID-19. Infect Dis Thera, 2020, 2020.
11. Sahin A. 2019 novel Coronavirus (COVID-19) outbreak: a review of the current literature.

12. Singhal T. A review of Coronavirus disease-2019 (COVID-19). Indian J Pediatr, 2020, 87: 281–86.
13. Stoltenberg M, Jacobsen J, Wilson E, et al., Emergency Department-Based Palliative Care during COVID. J Pall Med, 2020, 23: 1151-53.

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