USE OF GENEXPERT (CBNAAT) IN DIAGNOSIS OF SARS-CoV-2.

DR. RENUKA MURHAR[1], DR. SUNITA GAJBHIYE[2], DR. ASHWINI PATIL[3], DR. SUNANDA SHRIKHANDE[4]

[1]Junior Resident-2, GMC, Nagpur.
[2]Associate Professor, GMC, Nagpur.
[3]Assistant Professor, GMC, Nagpur.
[4]Professor and Head, GMC, Nagpur.

DEPARTMENT OF MICROBIOLOGY,
GOVERNMENT MEDICAL COLLEGE, Nagpur.

Name and Address of the corresponding author: Dr. Renuka Aniruddha Murhar
Plot no. 172, 'Martand', Indraprastha Nagar, Pannase layout, Nagpur-440022.

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ABSTRACT

BACKGROUND & OBJECTIVE: The current SARS-CoV-2/COVID-19 pandemic poses significant diagnostic challenges on each level of pre- to post-analytical steps. CBNAAT/ GENEXPERT detects the pan-sarbecovirus E gene and also N2 region of the N gene as its SARS-CoV-2-specific target which helps in the diagnosis of COVID-19 infection. Rapid and simple assays for SARS-CoV-2 detection aid in early diagnosis in emergency situations thus provide important diagnostic information, improve patient management and provide infection control counter measurements. With this background, we estimated the usefulness of CBNAAT in diagnosis of COVID-19 infection.

METHODOLOGY: According to the advisory issued by ICMR, nasopharyngeal swabs of 1390 cases were tested by GeneXpert (CBNAAT).

RESULTS: Out of the 1390 cases tested, 469 cases (33.6%) were tested positive with a male to female ratio of 3:1. Out of 1390 cases, 1209 (83%) were dead patients and 181(9.2%) were ANC females.

CONCLUSION: GeneXpert Xpress SARS-CoV-2 (CBNAAT) is a closed cartridge-based system requires BSL-2 facility with minimal sample handling. GeneXpert is rapid and simple assay for SARS-CoV-2 detection with turn-around time: 50 mins. It is a valuable addition for affording laboratories in situations where rapid and accurate diagnosis are of essence.

Keywords: SARS-CoV-2, CBNAAT, cartridge- based, GeneXpert, pandemic

INTRODUCTION:

The current SARS-CoV-2/COVID-19 pandemic poses significant diagnostic challenges on each level of pre- to post-analytical steps [1]. Rapid and simple assays for SARS-CoV-2 detection with high sensitivity and specificity are very important for infection control counter measurements.

Cartridge-based diagnostic often allows the: diagnosis of critically ill cases in a short time period, assessment of suspected patients very rapidly, allowing for a specific epidemiological management, and transfer diagnostics to point-of-care scenarios including smaller laboratories. Rapid testing has been shown to provide important diagnostic information immediately improving patient management [2].

The Cepheid Xpert Xpress SARS-CoV-2 assay also detects the pan-sarbecovirus E gene but detects the N2 region of the N gene as its SARS-CoV-2-specific target [3]. The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (i.e., nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab or nasal wash/ aspirate). The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents. [4]

MATERIALS AND METHODS:

An observational study was done on a total of 1390 samples and were processed from May 2020 to December 2020. Nasopharyngeal swabs & throat swabs were collected from suspected patients of COVID-19.

Inclusion criteria: Samples collected from dead patients, ANC females.

Exclusion criteria: SARI patients, routine swabs collected for rt-pcr.

These samples were then processed in State Viral Research & Diagnostic Laboratory, Government Medical College, Nagpur. Samples were processed as per the instructions given by Cepheid GeneXpert Manual.

About 300 microlitre of sample is taken into the cartridge with the help of pipette. Cartridge is then loaded into CBNAAT machine and the programme is generated. Results are available within 50 mins.

A few samples were also tested by RT-PCR and results were compared with CBNAAT. Results were evaluated on the basis of different categories like age, gender, etc.
Permission from Institutional Ethics Committee was taken.

RESULTS:

In this study conducted from July 2020 to December 2020, a total of 1390 samples were tested for SARS-CoV-2. Out of which 469 were tested positive and 921 were tested negative. Of 469 positive cases, 334 (71.2%) were males & 135 (28.7%) were females. Positive cases peaked during the months of August to October and highest was during the month of September (57.03%). A few samples (90) samples were tested by RT-PCR as well by taking RT-PCR as gold standard. Comparative study between CBNAAT and RT-PCR is shown below

GROUPS TESTED BY CBNAAT	CASES TESTED	POSITIVE CASES
DEAD PATIENTS	1209	411 (33.99%)
ANC FEMALES	181	58 (32.04%)
TOTAL	1390	469 (33.66%)

Table 1: Total no. of cases tested by CBNAAT & positive cases with male female ratio (3:1)

	Male	Female	Total
Positive	334 (71.2%)	135 (28.7%)	469
Negative	612 (66.4%)	309(33.5%)	921
Total	946	444	1390

Table 2: MONTH-WISE DISTRIBUTION OF POSITIVE CASES (n=1390)

MONTHS	TESTED	POSITIVE	PERCENT POSITIVE
MAY	101	3	2.97%
JUNE	35	5	14.28%
JULY	177	39	22.03%
AUGUST	211	100	47.36%
SEPTEMBER	**263**	**150**	**57.03%**
OCTOBER	236	88	37.28%
NOVEMBER	248	64	25.80%
DECEMBER	118	18	15.25%
TOTAL	1390	466	33.54%

Table 3: DISTRIBUTION OF POSITIVE CASES IN EACH GROUP (n=1390)

Table No.4: Comparative study between CBNAAT and RT-PCR.

CBNAAT/ Xpert Xpress SARS-CoV-2		RT-PCR SARS-CoV-2			Sensitivity	Specificity	PPV	NPV
		Positive	**Negative**	**Total**	95.83%	92.85%	93.89%	95.12%
	Positive	46	3	49				
	Negative	2	39	41				
				90				

DISCUSSION

CBNAAT detects E & N2 genes for testing of SARS CoV-2. CBNAAT is a random-access system suitable for molecular point of care testing that is highly specific and sensitive for detection of SARS CoV-2 in emergency conditions. The present study highlights use of CBNAAT/ XPERT Xpress in diagnosis of COVID-19. CBNAAT is a closed cartridge-based system, requires minimal sample handling and has less turnaround time.

In this study, a total of 1390 samples were tested by CBNAAT/ Xpert Xpress from May to December 2020, out of which 921 samples were negative, 469 were found to be positive (33.66%). Number of positive cases were 469 out of which 334 (71.2%) were males and 135 (28.7%) were females with a ratio of 3:1 as shown in table no. 1.

In a correspondence published in the Lancet in December, a study was conducted in Madagascar during July 5- July 28 2020and a total of 2733 specimens from 15 different regions were tested, of which 877 (32.1%) were found positive for SARS CoV-2 by CBNAAT [5].

A study conducted by Kanwardeep Singh et al.[9], a total of 657 samples were analysed 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. 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)[6]. This may be due to the higher proportion of males in their study population.

Laxminarayan et al., (2020)[10] 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.

The highest number of positive patients were detected from August to October which peaked in the month of September (57.03%). Month wise distribution of positive cases is shown in table no. 2.

Distribution of total cases tested by GeneXpert/CBNAAT where total no. of cases was 1390 and 1209 were dead patients & 181 ANC females is shown in Table no. 3. The percentage of total positive cases was 33% while that of dead patients (34%), and ANC females (32%).

OUT 469 positive samples tested by CBNAAT, a few samples (90) were also tested for

RT-PCR. Sensitivity was 95.83% and Specificity was 92.85%. Positive Predictive value was 93.89% and Negative predictive value was 95.12%, as shown in table no. 4. Compared to Kanwardeep Singh et al.[9] which tested a total of 80 samples out of 657 positive samples by RT-PCR, 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.

CONCLUSION:

CBNAAT is a closed cartridge-based system that can be performed with minimal hands-on training and requires BSL-2 laboratory facility with minimal sample handling. There is less exposure to health care workers and technicians. It is simple highly performing test with a short turnover time (50 min).

Cartridge based system allows diagnosis of critically ill cases in a short period of time, assessment of suspected patients very rapidly, allowing for a specific epidemiological management and transfer diagnostics to point of care scenarios including smaller laboratories. [8]

CBNAAT/GeneXpert is therefore a valuable addition for laboratories where rapid and accurate diagnosis are of essence.

In developing countries where there is large population with insufficient availability of tests, CBNAAT is a useful tool for diagnosis of COVID-19. However limited number of samples can be tested. Thus, it may not be an ideal choice during the peak of pandemic in a core laboratory where high number of samples have to be tested each day. Also, the high cost of machines and cartridges is a limiting factor.

Conflict of Interest: None

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