**Clinical Microbiology** 



## CORONAVIRUS DISEASE 2019- PREVALANCE AND RT PCR POSITIVITY- A TERTIARY CARE HOSPITAL EXPERIENCE

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**ABSTRACT** SARS – CoV – 2 virus has been reported since December 2019 in china and has been spreading rapidly all throughout the globe and we are in the middle of the pandemic. Globally there are around 171,782,908 positive cases, in India 28,574,350 positive cases and in Tamil Nadu 8,39,866 patients have been tested positive till now. RT-PCR were performed to detect the SARS-CoV-2-specific S gene (Spike protein), N gene (Nucleo capsid gene), ORF ab (Open reading frame) and RdRp (RNA-dependent-RNA-polymerase) gene fragment. Data collected for a period of five months at our centre shows that 31527 (18,57%) patients were Positive and 138206 (81.43%) were Negative, out of 169733 patients. Month of June had highest positive percentage with 41.22%. A total of 102371 (60.31%) Male with 84104 (82.16%) Negatives, and 18267 (17.84%) Positives and 67361 (39.69%) Female with 11917 (17.69%) Positives and 55444 (82.30%) Negatives were reported. Our study also reported more positivity percentage in adults (26.03%) followed by Elderly patients (22.57%). In conclusion, studying the evolving mutations and increasing the number of testing in symptomatic patients is urgent need of the hour for better understanding of SARS – CoV – 2 virus.

KEYWORDS: SARS - CoV - 2 virus, S gene, N gene, Rdrp gene

### INTRODUCTION

There have been 171,782,908 confirmed cases of COVID-19, including 3,709,397 deaths, reported to WHO, Globally, as of 4th June 2021. In India a total of 28,574,350 cases have been reported so far till May 31 2021 [1]. The largest single-day spike (30,987 cases) was reported on 13 May 2021 in Tamil Nadu and its fourth in India after Maharashtra, Kerala, and Karnataka [2] as far as total positivity is concerned. COVID 19 is characterized by high fever, cough, shortness of breath, pneumonia, loss of smell & taste and other respiratory tract symptoms, and became a great global public health concern. Respiratory specimens such as tracheal aspirate, nasopharyngeal swab, oropharyngeal swab and sputum are being collected for testing for COVID 19 virus by RT PCR. Some physicians argued that even the RT-PCR results were negative, Computed Tomography (CT) imaging may help the identification of SARS-CoV-2 infection, [3]. Since RT-PCR was one of the most quickly established laboratory diagnosis method in novel COVID-19 pandemic, it served efficiently to confirm a viral infection within 4 hrs.

Hence identifying and isolating the patients affected is critical in preventing transmission of virus from humans to humans and also in avoiding secondary infections to health care workers and immunocompromised patients [4].In our present study we retrospectively analyzed COVID-19 samples received at our VRDL lab, Madras medical College, from the month of May to September 2020 when the positivity of the infection was at its peak in the state. A total of 1,69,733 samples were tested for COVID 19 in this period by RT PCR method and 31527 samples tested positive.

## MATERIALAND METHODS:

## Sample Collection:

Nasopharyngeal and Oropharyngeal swabs were collected in viral transport medium according to ICMR guidelines from clinically suspected patients (i.e) Influenza like illness, severe acute respiratory illness patients, patients with travel history, close contact with positive patients and patients from containment zones.

#### **RNA extraction:**

The viral transport medium was subjected to vortex and 200µL of sample was transferred to blocks containing lysis buffer and proteinase K mixture and placed onto KingFisher<sup>™</sup> Flex automatic DNA/RNA extraction system. The eluted RNA is then subjected to RT PCR.

#### **RT PCR Testing:**

RT PCR was carried out in Roche light cycler 96 (Roche Diagnostic Systems, Basel, Switzerland) and Bio-Rad CFX 96 (Bio- Rad laboratories, Inc. USA), using LabGun Exo Fast kit(Lab Genomics, Republic of Korea) supplied by Tamil Nadu Medical Services Corporation Limited (TNMSC) which detects N gene and RDRP gene from the samples.

The final PCR concentration was 20- $\mu$ l (15- $\mu$ l qPCR master mix containing 4- $\mu$ l primer/probe set, 4- $\mu$ l Buffer, 2- $\mu$ l RT Enzyme, 5- $\mu$ l nuclease free water and 5- $\mu$ l template). The nucleic acid amplification was performed with the following PCR steps: Reverse Transcription (500C, 5 min, 1 cycle), Initial activation (950C, 1 min, 1 cycle), Initial amplification (Denaturation: 950C, 1 sec, Annealing and extension: 600C, 1 sec, 10 cycles) and further amplification (Denaturation: 950C, 1 sec, 32 cycles). All samples were run together with a SARS-Cov-2 positive control, and negative control (H2O) and CT values were less than 32 was defined as a positive test.

#### Statistical Analysis:

The Statistical analysis t test was carried out using Graphpad prism version 9.0 software and p value is calculated. p value less than 0.05 is considered statistically significant with confidence interval at 95%.

#### **RESULTS & DISCUSSION:**

A total of 169733 samples were received and tested for RT PCR during the period of May to September and 31527 (18.57%) patients were found to be Positive and 138206 (81.43%) were found to be Negative (Table 1, Fig 1). Month of June had highest positive percentage with 41.22%. The statistical analysis showed that there was significant difference between positive and negative cases (P value 0.0005 (<0.05))

Table 1: Tota	l No	Of	SARS	_	CoV2	Testing	From	May	То
September.									

MONTH	TOTAL	POSITIVE	NEGATIVE
MAY	20434	3923	16511
JUNE	24940	10282	14658
JULY	42686	8669	34017
AUGUST	39246	4785	34461
SEPTEMBER	42427	3868	38559



Fig 1: Total No Of SARS - CoV2 Testing From May To September.

Different Age groups were taken into consideration and results were analyzed and summarized.

Table 2. Total	to Officer Doffi Cases	From May 100	eptember.
MONTH	TOTAL NO OF NEWBORN CASES	POSITIVE	NEGATIVE
MAY	255	20	235
JUNE	328	58	270
JULY	138	3	135
AUGUST	2	2	0
SEPTEMBER	1	0	1

#### Table 2: Total No Of New Born Cases From May To September.

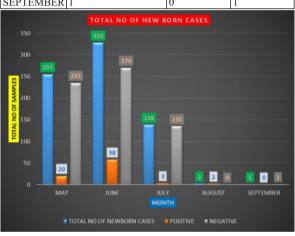


Fig 2: Total No Of New Born Cases From May To September.

Table 2, Fig 2 shows a total of 724 (0.42%) samples were received from May to September from New Born cases. 83 (11.46%) samples were tested positive and 641 (88.53%) patients were tested negative. The statistical analysis showed that there was no significant difference between positive and negative cases P value 0.0912 (>0.05).

# Table 3. Total No Of Children And Young Adolescent Tested From May To September.

MONTH	TOTAL NO OF CHILDREN AND YOUNG ADOLESCENT		NEGATIVE
MAY	1475	388	1087
JUNE	2023	931	1092
JULY	4410	1046	3364
AUGUST	4585	604	3981
SEPTEMBER	3545	338	3207



**Fig 3.** Total No Of Children And Young Adolescent Tested From May To September.

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Table 3, Fig 3 shows a total of 16038(9.44%) samples with 3307 (20.61%) positives and 12731 (79.38%) Negatives. The statistical analysis showed that there was significant difference between positive and negative cases P value 0.0042 (<0.05)

MONTH	TOTAL NO OF ADULT SAMPLES	POSITIVE	NEGATIVE
MAY	18063	14701	3362
JUNE	21194	8648	12546
JULY	35148	6942	28206
AUGUST	32797	3916	28881
SEPTEMBER	36603	3229	33374

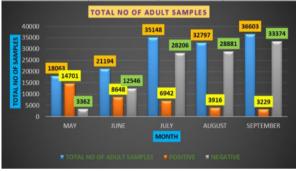


Fig 4: Total No Of Adult Samples From May To September

Table 4, Fig 4 shows a total of 143805 (84.72%) adult samples with 37436 (26.03%) Positives and 106369 (73.96%) Negatives. The statistical analysis showed that there was significant difference between positive and negative cases P value 0.0012 (<0.05)

Table 5: Total No Of Elderly Samples From May To September

MONTH	TOTAL NO OF ELDERLY SAMPLES	POSITIVE	NEGATIVE
MAY	641	153	488
JUNE	1395	645	750
JULY	2990	678	2023
AUGUST	1862	263	1599
SEPTEMBER	2149	301	1848



Fig 5: Total no of Elderly samples from May to September

Table 5, Fig 5 shows a total of 9037 (5.32%) samples with 2040 (22.57%) Positives and 6708 (74.22%) Negative samples in Elderly patients. The statistical analysis showed that there was significant difference between positive and negative cases - p value 0.0086 (<0.05)

Table 6: Male V	s Female Overa	all Cases From Ma	y To September.
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MONTH	Total MALE	Total MALE NEGA TIVE	Total MALE POSITI VE		Total FEMA LE POSITI VE	Total FEMALE NEGATI VE
MAY	12161	9857	2304	8272	1619	6653
JUNE	14314	8361	5953	10626	2987	7639
JULY	25456	20503	4953	17230	3715	13515
AUGUST	24190	21487	2703	15056	2082	12974
INDIAN	37					

SEPTEMB 26250 23896 2354 16177 1514 14663 ER Grand Total 102371 84104 67361 11917 18267 55444 m of MALE NEGATIV 14663 Sum of MALE POSITIVE 270 235 

Fig 6: Male vs Female overall cases from May to September.

Table 6 and Fig 6 shows a total of 102371 (60.31%) Male with 84104 (82.16%) Negatives, and 18267 (17.84%) Positives, p value 0.0004 (<0.05) statistically significant and 67361(39.69%) Female with 11917 (17.69%) Positives and 55444(82.30%) Negatives, p value 0.0002 (<0.05) which is statistically significant. One Transgender was excluded in the above results.

From all the above results we can see that male patients were predominant than female but the positivity rate more or less similar in both genders.

Positivity percentage is more in adults (26.03%) followed by Elderly patients (22.57%).

Although 2019-nCoV is similar to some betacoronaviruses detected in bats it is distinct from SARS-CoV and MERS-CoV. SARS-CoV strains from humans and genetically similar SARS-like coronaviruses from bats collected from southwestern China formed another clade within the subgenus sarbecovirus [5].

The 2019-nCoV infection caused clusters of severe respiratory illness similar to severe acute respiratory syndrome coronavirus and was associated with ICU admission and high mortality [6].

The positivity percentage of total samples received from May to September was 18.57% in our study which was seen in contrast to Liu et al [4] where they showed 38.42% of positivity percentage.

In our present study the total number of male patients were more when compared to female patient but the positivity was nearly equal male (17.84%), female (17.69%) which was in contrast to Pan et al where the positivity percent of female was 51.6% [7].

Wu and Mcgoogan [8] showed that positive percent was more in 30-79 (87%) our study which was similar to our study 48.6% which was also contrast to Karamese et al most positive cases were in the 21-40 (40.8%)[3].

#### **CONCLUSION:**

As a conclusion, SARS - CoV - 2 has had a severe impact globally in causing mortality and also affecting the economic status of many countries. There are continuous reports on mutations of the virus and this deadly pandemic is still not over. Hence continuous research and better understanding of the nature of virus and its mutations is the need of the hour.

#### **Conflict Of Interest:**

The authors have no conflict of interest in writing this paper.

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