

Incidence of SARS-Cov-2 in Patients Attending a Tertiary Care Hospital during Second Wave in Mahabubnagar, India- A Retrospective Study

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ABSTRACT

Introduction: In the year 2020, infectious outbreak in Wuhan city of China with SARS-CoV-2 and its rapid spread to most countries in the world lead to the pandemic. Ramping up the testing infrastructure played an important role in containing the spread of SARS-CoV-2 among people in a district. In this view, virology laboratory was set-up at Government Medical College, Mahabubnagar, Telangana in October 2020. In the beginning of COVID -19 in India, samples collected in Mahabubnagar for detection of SARS-CoV-2 were sent to a Virology lab which was 100 km away from the district.

Aim: The aim of this study was to observe the demographic details and the different variants of SARS-CoV-2.

Materials and Methods: A retrospective study was performed on the nasopharyngeal swab samples received for a period of one year (November 2020 to October 2021) at Government Medical College, Mahabubnagar, Telangana using rtRT PCR as a method of diagnosis. The total samples

received at the laboratory were 28221 out of which 28146 samples were included in the study. In addition, a total of 15 positive samples were sent to Centre for DNA Fingerprinting and Diagnostics (CDFD) for RNA sequencing to identify the variants of SARS-CoV-2. Demographic details were noted from the Indian council of Medical Research (ICMR) sample referral form. The data was analysed and presented as numbers and percentages.

Results: 28146 samples were accepted and tested. Among these, 5749 samples were tested positive (20.42%). Males were predominant with 60.54% and 27.50% of the patients were in the age group of 21-30 years. Out of 5749, 43.75% of the patients were tested for the ILI symptoms (category 9). Fever was the most common symptom and most of the positive cases during the second wave in India were due to delta variant.

Conclusion: In this study, delta was the only variant observed during the peak of second wave in Mahabubnagar district, Telangana.

Keywords: COVID-19, Delta variant, Molecular analysis, Sequencing

INTRODUCTION

On December 31st 2019, the World Health Organisation (WHO) was informed about an unknown etiology of pneumonia in Wuhan city of China and within 4 days, 44 such cases were reported [1]. WHO on 11th February 2020 announced that these cases were due to COVID-19 and cause respiratory tract infection in humans. Taxonomically these viruses belonging to Corona viridae and Betacoronavirus [2]. First case of COVID-19 in India was reported in 27th January 2020 in Kerala [3]. First case in the state of Telangana was identified on 2nd March 2020 after confirmation from National institute of Virology, Pune [4]. First wave of COVID-19 in India ended by December 2020; subsequently second wave started in February 2021 and peaked in May 2021.

Among the different methods available for diagnosis, Real time reverse transcriptase polymerase chain reaction (rtRT PCR) was considered as the gold standard test [5,6]. Molecular testing for COVID was not available in many centers in the country. Indian council of medical research (ICMR) laid down testing strategies on 17th of March 2020 [7]. Samples were collected from all over the state and were sent to the nodal testing centers as assigned by the State Governments and ICMR. As the numbers of cases were increasing all over the country, many testing labs were established in all the states following the guidelines laid down by ICMR [7]. According to National Medical Commission, Bio safety level (BSL)-II laboratory establishment was required for all the medical colleges [8]. At Government Medical College (GMC), Mahabubnagar(MBNR)

BSL-II lab was established after all the permissions from the State Government and ICMR in the month of November 2020. At that time this was the only Government run ICMR approved centre for diagnosis of SARS-CoV-2 in Mahabubnagar. This study was aimed to analyse and compare the patient demographics and Microbiological laboratory findings of COVID-19 positive patients.

MATERIALS AND METHODS

Study design: A Retrospective study was conducted at tertiary care center in Mahabubnagar. Data was collected for a period of one Year (Nov 2020-Oct 2021) from the hospital archives. Analysis was done over a period of 3 months (Jan 2022- March 2022). Samples were collected in the districts of Mahabubnagar, Wanaparthy and Narayanpet and transported for testing to Virology Lab, Government Medical College, Mahabubnagar, Telangana.

Inclusion criteria

1. Patients of all age groups with signs and symptoms of COVID-19.
2. Samples with ICMR specimen referral form for COVID-19 [9].
3. Properly transported samples.

Exclusion criteria

1. Samples leaking from Viral Transport Medium (VTM) tubes
2. Samples without ICMR specimen referral form for COVID-19
3. Samples sent without essential patient information labeled on the VTM tubes.

Sample collection

Nasopharyngeal or Oropharyngeal swabs were collected from the Patient according to the guidelines of ICMR [9] and transported in viral transport medium under proper conditions.

Sample Processing

After the samples were received, the condition of the sample was checked and if it doesn't pass through inclusion and exclusion criteria, it was rejected. Accepted samples were subjected for RNA extraction by ICMR approved kits-Qline manual extraction (POCT services private limited, New Delhi, India), Genes2Me automation extraction Kits, Genes2Me manual extraction kits (Member technologies-Ambala Committee, India, Genes2Me Private Limited, Gurugram, Haryana) in BSL-2 cabinet(Thermo Fischer Scientific A2 1300 series). Automation extraction was performed in Higenone Himedia Extraction machine.

After extraction, the RNA was subjected to RT-PCR using ICMR approved Kits (NIV RT-PCR Pune Kit, Qline RT-PCR Kit, Genes 2 Me RT- PCR Kit) Various RT-PCR tests targeting viral genes nucleocapsid (N), envelope (E), spike (S), RNA dependent RNA polymerase (RdRp) and open reading frames (ORF) were performed depending on the availability of test kits. Procedure was done according to the instruction given by the Manufacturer. RT-PCR was done in Quant studio 5 Thermo Fisher Scientific machine. Result of each sample was given based on the CT Values of the genes given in the Kit insert provided by the manufacturer.

Patient demographic details and other details (patient category [9], signs and symptoms) were collected from the ICMR specimen referral form for COVID-19. According to the Telangana State Government guidelines, 15 positive samples per month were also sent for whole genome sequencing (WGS) to state nodal center-Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad. WGS reports were received from CDFD through email.

STATISTICAL ANALYSIS

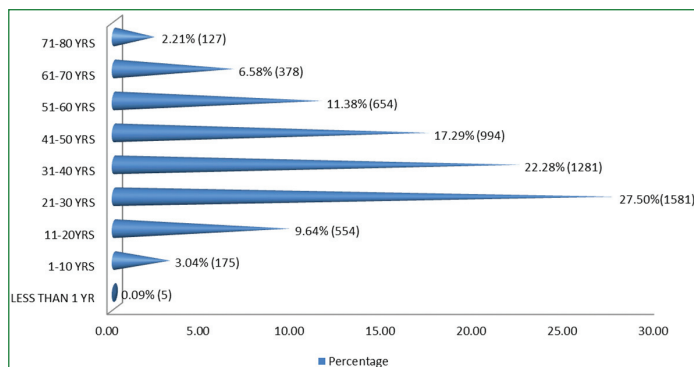
The data has been collected in Microsoft Excel sheet and results were presented as count and percentages.

RESULTS

A total of 28221 samples were received at the laboratory out of which 75 samples were rejected. 28146 samples were tested for SARS CoV-2, out of which 5749 samples were positive (20.42%) [Table/ Fig-1]. Among the 5749 positive samples, 2269 (60.54%) were male (predominance) and 3480 (39.46%) were females. Majority patients infected belong to age group 21 to 30 years [Table/ Fig-2]. Majority of positive samples were from patients belonging to category 9 with percentage of 43.75% [Table/ Fig-3]. Common symptoms were

Month	Percentage positivity
Nov-20	18.31
Dec-20	10.3
Jan-21	7.72
Feb-21	3.12
Mar-21	8.91
Apr-21	43.13
May-21	39.79
Jun-21	18.71
Jul-21	8.97
Aug-21	4.57
Sep-21	4.58
Oct-21	6.54
Not mentioned	6.2%

[Table/ Fig-1]: Percentage positivity every month (November 2020–October 2021).



[Table/ Fig-2]: Age wise distribution of positive patients.

fever, body aches, cough, sore throat, breathlessness, loss of smell, etc. [Table/ Fig-4]. Total 9.4% of positive patients had co-morbidities. 11.3% were inpatients and 88.7% were out patients.

Patient categories	n (%)
1: Symptomatic international traveler in last 14 days	180 (3.13%)
2: Symptomatic contact of lab confirmed case	72 (1.25%)
3: Symptomatic healthcare worker	72 (1.25%)
4: Hospitalised SARI (Severe acute respiratory illness) patient	395 (6.88%)
5a: Asymptomatic direct and high-risk contact of lab confirmed case	1221 (21.25%)
5b: Asymptomatic healthcare worker in contact with confirmed case without adequate protection	144 (2.50%)
6: Symptomatic influenza like illness (ILI) patient in hospital	72 (1.25%)
7: Pregnant women in/near labor	0
8: Symptomatic influenza like illness (ILI) among returnees and migrants (within 7 days of illness)	36 (0.63%)
9: Symptomatic influenza like illness (ILI) patient in Hotspot/ Containment zones	2515 (43.75%)
Others*	683 (11.88%)
Not mentioned	359 (6.25%)

[Table/ Fig-3]: Patient categories of SARS-CoV-2 positive cases (N=5749).

**Others* only if the patient doesn't belong to category 1-9

Symptom	Number of patients	Percentage
Fever	4070	70.8%
Cough	2465	42.87%
Sore throat	1450	25.23%
Body ache	2657	46.21%
Breathlessness	1334	23.2%
Vomiting	155	2.7%
Diarrhea	86	1.5%
Abdominal pain	109	1.9%
Nausea	92	1.6%
Nasal discharge	454	7.9%
Sputum	213	3.7%
Chest pain	80	1.4%
Headache	69	1.2%
Haemoptysis	11	0.2%
Loss of taste	592	10.3%
Blurring of vision and red eyes	0	0%
Asymptomatic	540	9.4%
Not mentioned	360	6.2%

[Table/ Fig-4]: Symptoms in positive patients (N=5749).

180 positive samples collected during the one year study were sent to whole genome sequencing. B.1.1.306, B.1.36.29, B.1.1.25, B.1.1, B.1.36, B.1.456, B.1.36.8 variants were seen from November 2020 to March 2021. From April to August 2021, B.1.617.2 was the only variant identified in the samples tested

Variant	AY.20	AY.33	AY.39	AY.4	AY.41	B.1	B.1.1	B.1.1.25
Number of cases	8 (4.45%)	1 (0.55%)	1 (0.55%)	4 (2.23%)	1 (0.55%)	2 (1.2%)	4 (2.23%)	2 (1.11%)
Variant	B.1.1.306	B.1.1.326	B.1.36	B.1.36.29	B.1.36.8	B.1.456	B.1.617.2	Omitted
Number of cases	3 (1.66%)	4 (2.23%)	3 (1.66%)	27 (15)	4 (2.23%)	1 (0.55%)	106 (58.8%)	9 (5%)

[Table/Fig-5]: Covid-19 variants identified during the study (N=180).

at our Virology lab. After which AY.4, AY.20, AY.33, AY.39, AY.41 were identified. On the whole, B.1.617.2 (Delta) was the dominant variant during the second wave in the districts included in the study [Table/Fig-5].

DISCUSSION

As on 6th of March 2020, India had only one virology testing laboratory and ICMR has drafted the laboratory preparedness in India [11]. After which ICMR has activated 72 laboratories all over India by 17th March 2020 [12]. By the end of the first wave, laboratory was established to be in preparation for the 2nd wave. Since then over 28146 tests were done in one year.

In November 2020 percentage positivity was 18.38% which was during declining of 1st wave. Further the declining in percentage positivity was seen till February 2021 (percentage positivity 3.12%). After the 2nd week of March 2021, there was a sudden increase in the percentage positivity-8.9%, where peak was seen in April 2021 (43.13%). From May 2021 (39.79%) there was steady downfall of the positivity till Oct 2021 (6.54%). In the study of Ranjan R et al., [13] it was reported that test positivity rate started in Feb 2021 and peaked in April 2021.

In this study male predominance was 60.54%. This was in line with the studies of Reddy MM et al., [14] (67.80%), Kumar K et al., [15] (77.77%).

In this study, 27.50% patients belong to age group 21 to 30 years. This was similar with the studies done by Kumar K et al., [15] which showed mean age as 31±12 years, Wattal C et al., [16] showed common age group 31-40 years but not in line with study of Reddy MM et al., [14] which showed common age group as more than 75 years.

In present study, 43.75% of positive samples were from patients belonging to category 9. This was not in line with the study of Wattal C et al., [16] where category 6 was common with 31.04%. In the present study, only 1.25% cases were referred from category 6. Common symptoms were fever, body aches, cough, sore throat, breathlessness, loss of smell. This was similar with the study done by Wattal C et al., [16], whereas in the study of Ranjan R et al., [13] symptomatic cases were only 14.3% (This study didn't specify the different symptoms). In this study, 11.3% were inpatients and this is in the study of Wattal C et al., [16] 35.5% patients were admitted in hospital.

B.1.1.306, B.1.36.29, B.1.1.25, B.1.1, B.1.36, B.1.456, B.1.36.8 variants were seen till March 2021. All these variants were also reported in different parts of India [17-23]. After March 2021, B.1.617.2 was the only variant identified in the samples tested at our Virology lab till August 2021 after which AY.4, AY.20, AY.33, AY.39, AY.41 were also identified. On the whole, B.1.617.2 (Delta) was the dominant variant during the second wave in the districts included in the study. In India, Delta was the major variant during second wave, which further mutated into different sub lineages (Delta AY.1 and Delta AY.2) which were reported [24,25]. Next dominant variant of concern all round the world was B.1.1.529 (Omicron), the first case of which was reported from South Africa on 24th November 2021 [26-28].

The present study gives an overview of the COVID-19 infection rate during the second wave in Mahabubnagar, India which can be compared to other regions in further studies. The center in which the study was conducted was the only Government run ICMR approved COVID-19 testing centre in Mahabubnagar, India.

Limitation(s)

The limitations of this study were that the ICMR sample referral forms were incompletely filled with data and majority of the patients were on outpatient basis. Due to which other laboratory investigations couldn't be done and analysed.

CONCLUSION(S)

In this study we have observed the demographic details of the positive COVID-19 patients attending tertiary care government hospital. Also we have listed out and analysed different variants of SARS-CoV-2 identified in the samples collected. Delta was the only variant observed during the peak of second wave in Mahabubnagar district, Telangana. In anticipation of third wave in India, estimation of antibody titers in post vaccinated individuals might be useful in determining the effectiveness of vaccination.

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