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DEMOGRAPHIC PROFILE OF SUBJECTS UNDERGOING REAL-TIME RT-PCR TEST FOR SARS-COV-2 AT A SINGLE CENTER IN NORTH INDIA DURING 3 WEEKS LOCKDOWN PERIOD: AN ANALYTICAL STUDY AND OPTIMAL STRATEGY AHEAD.

Medical Science

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ABSTRACT

The outbreak of Covid-19 due to SARS-CoV-2 has affected 210 countries around the world. Demographic profile of 1139 subjects, from eleven adjoining districts of south-west Uttar Pradesh, undergoing real-time RT-PCR test for SARS-CoV-2 at a single center during the lockdown period from 26th March to 16th April was analyzed. Results: Out of 1139 subjects screened 918 (80.6%) were male (male: female ratio 4.15:1). 53 (4.65%) were confirmed to be positive for SARS-CoV-2, out of which 43 (81.13%) were male (male: female ratio 4.3:1). Comparative analysis of the age distribution of the positive cases revealed bimodal pattern with peaks at 21-30 years and 51-60 years age group. Conclusions: Our cluster sampling approach, with an overall positive rate of 4.78% has identified 'hotspots' which might just be the tip of the iceberg of total case load. Comprehensive stratified sampling should be performed among all age groups and sex for positive case identification, whether symptomatic or not, for 'case isolation and contact tracing' strategy to be successful.

KEYWORDS

Covid-19, SARS-CoV-2, real-time RT-PCR assay, lockdown in India

Introduction:

The outbreak of Corona virus disease 2019 (Covid-19) due to Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has devastated Europe and America and is now threatening to engulf the Indian Subcontinent. 210 countries have been affected by Covid-19 around the world. As of 3rd July, 2020, the total number of Covid-19 diagnosed patients worldwide was 11,023,455 with 524,881 deaths. In India, the total number of diagnosed patients is 628,205 with 18,241 deaths. To combat the virus by containing its spread and 'flattening the curve', so that health care resources are not exhausted, India had gone into a 3 weeks nationwide lockdown on 25th March which had been further extended thrice.

The impetus of our government has been on isolating diagnosed cases, implementing social distancing and conducting extensive screening tests to identify as many cases as possible with the rather limited resources considering our vast population of 135 crores. As of 19th April, the state of Uttar Pradesh, with a population of 23.71 crores (more than the combined population of United Kingdom, Italy and Spain), has 24,825 confirmed Covid-19 cases with 735 deaths.

Viral Research and Diagnostic Laboratory (VRDL) at Department of Microbiology, Uttar Pradesh University of Medical Sciences (UPUMS), Saifai, Etawah District, has been listed by Indian Council of Medical Research (ICMR) as a center for conducting real time RT-PCR tests for SARS-CoV-2. It presently caters to samples from eleven adjoining districts of south-west Uttar Pradesh. Demographic profile of 1139 subjects undergoing real-time RT-PCR test for SARS-CoV-2 at our center during the 3 weeks lockdown period from 26th March to 16th April was analyzed and in-depth analysis of positive cases were performed to understand the distribution pattern and progress of Covid-19 in our geographical region, the efficacy of lockdown and the optimal strategy ahead.

Material and Methods:

We prospectively analyzed the demographic profiles and results of 1139 subjects undergoing real time RT-PCR tests at UPUMS, Saifai. Throat or deep nasal swab samples of patients from eleven districts of south-west Uttar Pradesh were taken in viral transport medium (VTM) and transported in cold chain to our centre by healthcare professionals with appropriate PPE with gloves, maintaining infection control when collecting the samples and proper disposal of all waste generated. All the appropriate operations from specimen collection, storage and transportation, and laboratory tests were carried out strictly in line with relevant regulations of biosafety and molecular laboratory management.

RNA extraction followed by real-time fluorescent RT-PCR assay was done for detecting SARS-CoV-2. From March 26th to April 9th, for a total of 327 samples, AgPath-ID screening kits (Thermo Fischer Scientific) targeting 'E' gene were used; the positive samples were further confirmed with AgPath-ID confirmatory kits targeting 'ORF' gene. From April 10th onwards BGI real-time fluorescent RT-PCR detection kits (BGI Europe A/S, Denmark) targeting 'ORF1ab' gene were used for all the subsequent samples tested. Both internal controls and negative controls were routinely performed with each batch of tests. All data (test dates, patient profile and results of RT-PCR assay) were collected on 'circa diem' basis.

Earlier samples collected were mostly from home quarantined individuals with a history of travel to endemic areas or contact with positive patients (n=220). Later on samples were mostly from institutional quarantine or hospitalized individuals with contact history or symptoms (n=919). The samples with inconclusive results due to improper sampling were not considered for this study. Such samples were repeated and the results were included subsequently as per the date of reporting. The data collected were validated and analyzed descriptively for demographic distribution and result pattern.

Results:

Between March 26th 2020 and April 16th 2020, 1139 subjects were screened for SARS-CoV-2 by real-time RT-PCR assay. 918 (80.6%) subjects were male (male: female ratio 4.15:1). Age range was 02 months to 93 years with maximum 377 (33.09%) subjects screened in the 21-30 years age group, followed by 247 (21.68%) in 31-40 years, 153 (13.43%) in 11-20 years, 98 (8.60%) in 51-60 years, 62 (5.44%) in ≤10 years and 54 (4.74%) in 61-70 years age groups. The sampling in ≥71 years was the lowest at 12 (1.05%).

Out of the 918 males screened, 304 (33.11%) and 208 (22.65%) were in the 21-30 years and 31-40 years age groups respectively. Out of the 221 females screened, 73 (33.03%) and 39 (17.64%) were in the 21-30yrs and 31-40 years age groups respectively. (Chart-1) Comparative age & sex distribution of samples screened against total is depicted. (Chart-2)

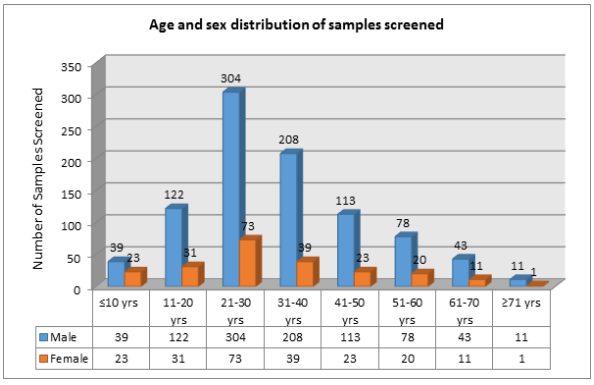


Chart-1: Age and sex distribution of samples screened

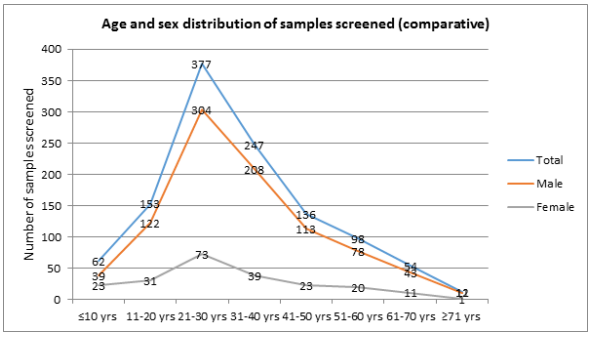


Chart-2: Age and sex distribution of samples screened (comparative)

Among the samples screened 73 (6.40%) samples were of individuals who had attended a religious congregation from 13th March to 15th March, at Delhi, capital of India.

Out of the 1139 total samples screened 53 (4.65%) were confirmed to be positive for SARS-CoV-2. The incidence of positive cases among the samples from the attendees of the religious congregation was 10 (13.69%) out of the samples tested (n=73), more than three times the incidence among non-congregation group samples (n=1066) which were positive in 43 (4.03%). Out of the 53 positive cases 10 (18.83%) were attendees of the religious congregation, 06 (11.32%) had history of contact present and 04 (7.54%) had travel history to endemic areas other than from Delhi itself.

Out of the 53 positive cases 43 (81.13%) were male (male: female ratio 4.3:1). Out of 918 samples screened in males 43 (4.68%) were confirmed to be positive which was higher than 10 (4.52%) confirmed positive out of 221 samples screened in females.

Age range was 02 months to 73 years with maximum 21 (39.62%) cases in the age group 21-30 years, 08 (15.09%) in age group 31-40 years, 07 (13.20%) in age group 51-60 years, 06 (11.32%) in age group 61-70 years and 05 (9.43%) in age group 11-20 yrs. The age group ≤10 years and ≥71 years showed 03 (5.66%) and 01 (1.88%) positive samples respectively.

Comparative analysis depicts a linear correlation between the total samples screened in different age groups and the positive results. (Chart-3)

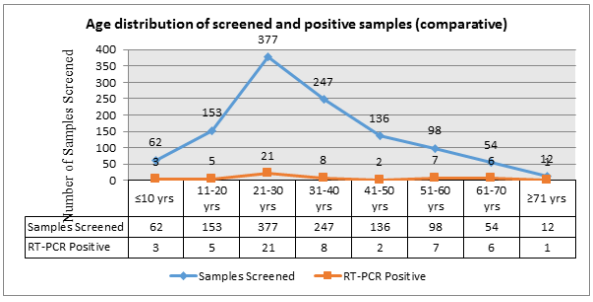


Chart-3: Age distribution of screened and positive samples (comparative)

The same correlation is evident in the sex specific age distribution of total samples screened compared to the positive results. Comparative analysis of the age distribution of the RT-PCR positive samples reveals bimodal pattern with peaks at 21-30 years and 51-60 years among total, male and female. (Chart-4)

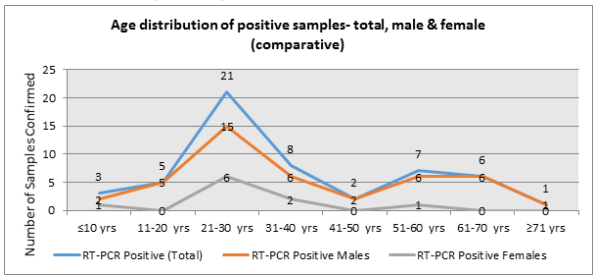
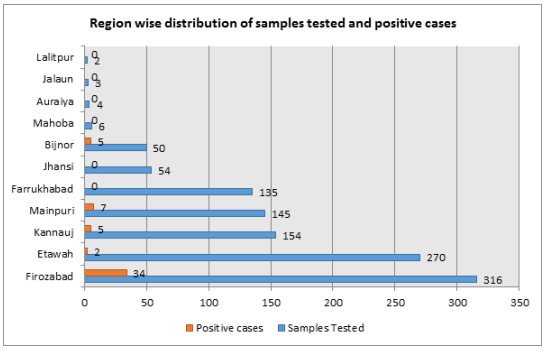


Chart-4: Age distribution of positive samples- total, male and female (comparative)

Among the 53 positive cases, maximum 34 (64.15%) were reported from Firozabad district. No positive cases were reported from Farrukhabad, Jhansi, Mahoba, Auraiya, Jalaun and Lalitpur districts (the latter four districts had negligible sampling). District wise 34 (10.76%) of 316

Chart-5: Region wise distribution of samples tested (n=1139) and positive cases (n=53)



samples tested from Firozabad were positive which was the highest. (Chart-05)

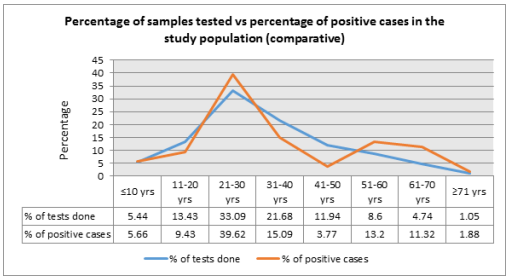
DISCUSSION:

In this study cluster sampling approach was followed based on the principle of case isolation and contact tracing. Earlier samples collected were mostly from home quarantined cases. Later on samples were mostly from institutional quarantined or hospitalized cases.

Among the samples screened, 73 (6.40%) samples were of individuals who had attended a religious congregation from 13th March to 15th March, at Delhi, capital of India. The attendees were from 19 states of India (156 from Uttar Pradesh, majority of which belonged to Firozabad district which is situated about 250km south-east of Delhi.) and 16 countries, including scholars from countries severely affected by Covid-19 outbreak. The incidence of positive cases in this group was more than 3-times that of non-congregation group. The hotspot areas in Firozabad and Mainpuri districts have been attributed to this congregation.

Out of the 1139 total samples screened 53 (4.65%) were confirmed to be positive for SARS-CoV-2. This is lower than the national screening positive rate of 4.78% as of March 16th, 2020 as per ICMR data.

Comparative analysis of the age distribution of the RT-PCR positive samples reveals bimodal pattern with peaks at 21-30 years and 51-60 years among both male and female. There was a linear correlation between the samples tested and positive cases detected. As per the data, relatively higher positive cases were detected in the 51-60 years and 61-70 years age groups (24.52% combined) despite a lower testing rate (13.34% combined). Also relatively lower positive rates were detected in the 11-20 years and 41-50 years age groups (13.20% combined) despite a higher testing rate (25.37% combined). It is pertinent to sample more cases in the age groups 41-50 years and 51-60 years. (Chart-6)



	≤10 yrs	11-20 yrs	21-30 yrs	31-40 yrs	41-50 yrs	51-60 yrs	61-70 yrs	≥71 yrs
% of tests done	5.44	13.43	33.09	21.68	11.94	8.6	4.74	1.05
% of positive cases	5.66	9.43	39.62	15.09	3.77	13.2	11.32	1.88

Chart-6: Percentage of samples tested vs. percentage of positive cases in the study population among all age groups (comparative)

It is also evident that no particular age group or sex can be neglected or favored over others in sampling protocols. Comprehensive stratified sampling is needed among all age groups and sex for positive case identification, whether symptomatic or not, for case isolation and contact tracing strategy to be successful.

The region wise distribution of the positive cases revealed certain interesting data. 885 (77.69%) samples were tested from four districts [Firozabad (n=316), Etawah (n=270), Kannauj (n=154) and Mainpuri (n=145)] and these areas accounted for 48 (90.56%) of the 53 positive samples tested. (Chart-5) Though 135 samples were tested from Farrukhabad, no positive case was detected. The sampling at Mahoba, Auraiya, Jalaun and Lalitpur districts were negligible. Though certain districts have been favored for contact sampling, with larger positive case turnouts, other districts have been neglected in terms of cases sampled with evident nil positive case turnouts. This is a 'money begets money' phenomenon due to sampling bias based on geographical areas. Comprehensive stratified sampling is needed at all geographical locations in India, irrespective of contact or travel history, before declaring an area as 'Covid-19 free'. In the state of Uttar Pradesh, 31 districts have been labelled 'Covid-19 free'. This needs urgent attention lest these districts toe the line of areas with severe outbreaks at a later date due to initial complacency. Present study, representing south east Uttar Pradesh, is relevant pan country as the same testing protocol is being followed everywhere.

Based on the early experience in Wuhan, the number of Covid-19 cases could increase from 20 to 40 cases in 3 days (from Jan 06th-08th, 2020), and outbreak sizes doubled in every 7.4 days on average (in mid-February), highlighting the urgency of early detection and rapid response.[1] In our study in south-west Uttar Pradesh population, the number of Covid-19 cases increased from 21 (on March 12th) to 41 (on March 15th), a pattern consistent with other regions of India. Even the doubling rate in India is 7.4 days presently, an alarming similarity with the Wuhan statistics! (Charts-7 and 8)

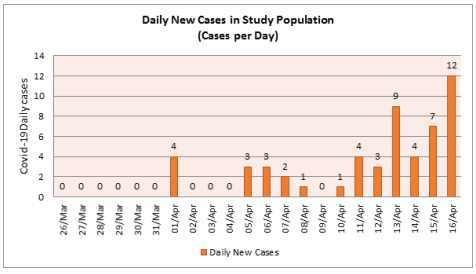


Chart-7: Daily new cases in study population (cases per day) during the study period

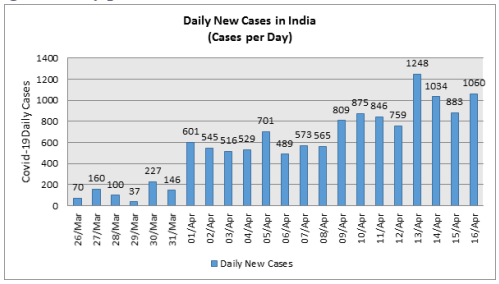


Chart-8: Daily new cases in India (cases per day) during the study period

In the initial few days, single digit tests were performed and no positive test result was identified. As the number of samples increased, the positive results increased correspondingly. The maximum new positive cases in a single day was 12 on April 16th. The pattern is consistent with that of the whole country. The number of new positive cases per day as well as total covid cases, in our study as well as the national data, are increasing linearly with the number of samples being tested per day. (Chart-7 to 10)

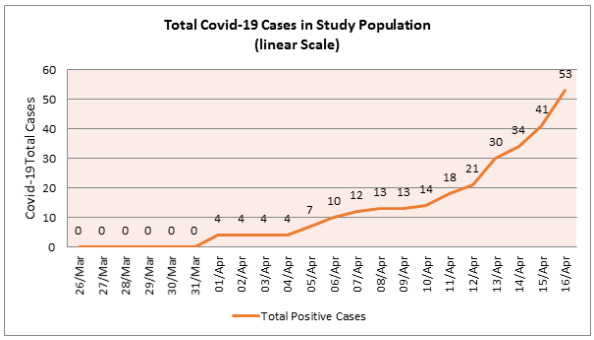


Chart-9: Total Covid-19 cases in the study population (linear scale) during the study period

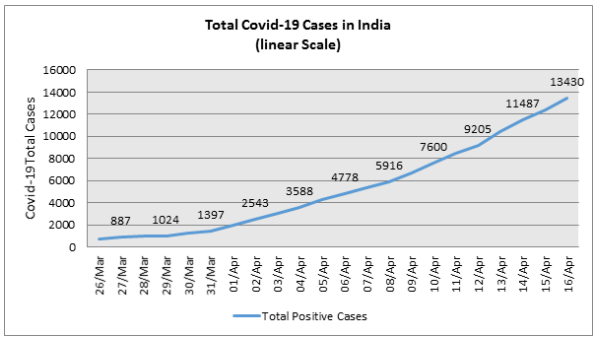


Chart-10: Total Covid-19 cases in India (linear scale) during the study periods

Case isolation and contact tracing are common interventions used to control the outbreak of infectious diseases. Historically, these have been successfully applied for controlling emerging infectious disease outbreaks, such as smallpox, but only partially effective in certain other diseases like influenza.[2] The countries successful in containing covid-19 up to now have utilized other containment strategies such as lockdown of the endemic zones, travel restrictions, mandatory facial masking, social distancing mandates, mass quarantine and school closures.[3,4] The timing of initiation of these measures as well as exit strategy is instrumental in the successful control of disease outbreaks and resurgence.[5]

Since the transmission of Covid-19 can occur before symptom onset, seroprevalence studies among the contacts are important. *Zou et al.* revealed that similar viral loads are seen in asymptomatic and symptomatic patients, indicating the transmission potential of asymptomatic patients.[6] Transmission by asymptomatic or mildly symptomatic cases can weaken the baseline strategy of case isolation and contact tracing because of reduced likelihood of isolating all cases and tracing all contacts. Extensive identification and testing of potential cases, among all age groups and geographical regions, including the asymptomatic cases, is prudent.

Another major hindrance to the case isolation and contact tracing strategy is the RT-PCR false-negative results (RT-PCR sensitivity ranges from 50 to 62% according to previous large-scale reports).[7,8] Cases are on record where symptomatic 'suspected' cases with typical clinical features and CT images have been set free, based on false negative RT-PCR tests, only to be confirmed positive by the same test subsequently.

A negative result cannot be used as the sole criterion for patient isolation or management protocols.[9,10] Until a highly sensitive, specific and rapid test is developed, a high margin of suspicion in clinically evident cases and subsequent isolation is advisable. False negative

results may also occur due to insufficient organisms in the sample due to inappropriate collection, transportation or handling.[11,12]

There were certain limitations to our study. This was a prospective study with a predominantly cluster sampling with inherent selection bias and potential under-coverage. As the sample size increased with time, participation bias crept in. Due to varied case load, samples from these eleven districts might have been sent to other ICMR designated centres, as per their testing capacities. The accuracy of RT-PCR assay might have varied due to the different kits used or due to improvement of the detection protocol with gain of experience in sampling and testing.

Conclusion:

Our cluster sampling approach, with an overall positive rate of 4.78% has identified 'hotspots' which might just be the tip of the iceberg of total case load. Comprehensive stratified sampling should be performed among all age groups and sex for positive case identification, whether symptomatic or not, for 'case isolation and contact tracing' strategy to be successful. Intensive random sampling is needed at all geographical locations in India, irrespective of contact or travel history, before declaring an area as 'Covid-19 free'.

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