

SARS COV-2 PANDEMIC: EXPERIENCE AT REFERENCE VIROLOGY LABORATORY

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ABSTRACT

Objective: To share our large scale SARS CoV-2 PCR test experience in Northern Pakistan.

Study Design: Prospective observational study.

Place and Duration of Study: Virology Department, Armed Forces Institute of Pathology, Rawalpindi, from Feb to Dec 2020.

Methodology: All the patients reporting to COVID-19 desk both indoor and outdoor were included in study. Nasopharyngeal swab specimen was taken from the patients arriving at reception. For hospitalized patient's samples were received at reception placed in viral transport medium maintaining cold chain.

Results: Among 193656 samples tested for SARS CoV-2 RNA by RT-PCR, 24338 (12.6%) were found positive and 169318 (87.4%) were negative. Mean age of patients was 38.25 ± 16.73 (1-110 years). 138781 (71.7%) were males and 54875 (28.3%) were females. 109765 (56.7%) samples were received from in patient department and 83891 (43.3%) samples were received from outpatient department. Highest number of cases (n=6224) seen during month of June followed by 5813 cases during May and 4786 cases during November (p -value <0.001). Most of the positive cases were in age group 21-40 years; 11122 (6%), followed by age group 41-60 years; 8133 (4.2%). More positive samples 14890 (7.7%) were received from in patient department and males 17928 (9.3%) were affected more than females.

Conclusion: The two peaks of COVID-19 pandemic in Pakistan were observed during the months of May to July and again during October to December. Most positive patients in our setup were males in age group 21-40 years as this age group is more exposed to external environment.

Keywords: COVID-19, Pandemic, Polymerase chain reaction, SARS cov-2.

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INTRODUCTION

In December 2019, a cluster of pneumonia cases of unknown etiology was reported from Wuhan, Hubei province of China¹⁻³. It was discovered that the causative virus was found to belong to Coronaviridae family and was initially named as 2019 Novel Coronavirus (nCoV-2019)³. Later, it was renamed as SARS CoV-2 and the respiratory ailment caused by it was termed as COVID-19 by Coronavirus Study Group of the International Committee on Taxonomy of Viruses (ICTV)⁴. Over the period of few months the disease spread globally affecting most of the countries and posing a serious threat to human health and economy^{4,5}. Due to rampant increase in cases World Health Organization (WHO) initially, declared Global Health Emergency of International Concern and then Pandemic in March 2019^{6,7}.

Coronaviruses are enveloped RNA viruses of zoonotic origin. Seven coronaviruses are known to cause human disease. Among them SARS CoV-1 and MERS (Middle East Respiratory Syndrome) have caused

epidemics in past. Due to high transmissibility SARS CoV-2 emerged as most widely spreading Coronavirus and the first among the family to cause a global pandemic⁶. The genome of SARS CoV-2 shares 80% similarity with SARS CoV-1 and 50% with MERS-CoV⁹. The full length genome of SARS CoV-2 was made available to public in January 2020 and shortly, after that many reverse transcriptase polymerase chain reaction (RT-PCR) assays were available for diagnosis across the world¹⁰. During initial phase of pandemic mass testing was initiated to contain the spread of virus as there was no treatment or vaccine available⁴. Armed Forces Institute of Pathology (AFIP), Rawalpindi being a reference center for diagnosis of viral illnesses in Northern Pakistan, started PCR testing for SARS CoV-2 on 18th February 2020 and first positive case was diagnosed on 16th March 2020. Since, then PCR is routinely being done AFIP and also experts in virology at this institute helped in establishment of PCR labs across the country.

METHODOLOGY

This prospective observational study was conducted at Virology Department, AFIP, Rawalpindi, from February to December 2020. The study was approved by the ethics review committee of the institute (VIR-3/READ-IRB/ 21/056). Informed written consent was

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taken from all the patients. Non probability consecutive sampling was done. All the patients reporting to COVID-19 desk both indoor/outdoor were included in the study. Nasopharyngeal swab (NPS) specimen was taken from the patients arriving at reception. For hospitalized patient's samples were received at reception placed in viral transport medium (VTM) maintaining cold chain. All samples were accompanied by a dully filled patient form. Samples were stored in refrigerator and processed on same day. Specimens that could not be dealt immediately were stored at 2-80C. All the specimens were processed in Biosafety Cabinet Class-II A2 as per standard laboratory guidelines. The nucleic acid extraction was done by an automated extraction system using magnetic beads technology (Taiwan Advanced Nanotech Inc. Taiwan). Amplification was performed by using two different kits i.e. SARS-CoV-2 ARGENE, BIOMERIEUX (Biomeriux SA, France) on CFX96 TM Thermal Cycler (BIO-RAD, USA) and SARS CoV-2 RT-PCR Scacce (Scacce biotechnologies, Italy) on Sa Cycler. The final results were interpreted on basis of cycle threshold value (Ct value). All samples with a Ct value 35 or below were considered positive as per kit manufacturer's instructions. Samples with Ct value >35 were considered negative. Results of samples positive for two genes of SARS CoV-2 were reported immediately while all samples positive for single target gene were repeated before declaring results.

Data was entered using SPSS-21. Mean \pm SD was calculated for quantitative variables while qualitative variables were expressed in frequencies along with percentages. Pearson's Chi square test was used to determine association between age groups, gender, month and SARS CoV-2 PCR positivity. A *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS

A total number of SARS CoV-2 RT-PCR tests carried out were 193656. Among the total tested, 24338 (12.6%) were positive and 169318 (87.4%) were negative. Mean age of patients was 38.25 ± 16.73 (1-110 years). 138781 (71.7%) were males and 54875 (28.3%) were females. About 109765 (56.7%) of samples were received from in patient department and 83891 (43.3%) of samples were received from outpatient department. Maximum number of patients belonged to age group 21-40 years i.e. 105363 (54.4%) (table-I).

Most of the positive cases were in age group 21-40 years 11122 (6%) followed by age group 41-60 years 8133 (4.2%). More positive samples 14890 (7.7%) were received from Inpatient department and males 17928

(9.3%) were affected more than females table-II.

First peak of pandemic was observed from May to

Table-I: Patient characteristics.

Mean Age	38.25 \pm 16.73
Gender	
Male	138781 (71.7%)
Female	54875 (28.3%)
Age Groups	
<20 years	17720 (9.2%)
21-40 years	105363 (54.4%)
41-60 years	48203 (24.9%)
61-80 years	20339 (10.5%)
>80 years	2031 (1%)
In/Out Door Cases	
Indoor Cases	109765 (56.7%)
Outdoor Cases	83891 (43.3%)
PCR Results	
Positive for SARS CoV-2	24338 (12.6%)
Negative for SARS CoV-2	169318 (87.4%)

Table-II: Association of patient characteristics with PCR results.

Patient Characteristics	PCR Results		<i>p</i> -value
	Positive	Negative	
Gender			
Male	17928 (9.3%)	120853 (62.4%)	0.001
Female	6410 (3.3%)	48465 (25%)	
In/out door			
Indoor	14890 (7.7%)	94875 (49%)	0.001
Outdoor	9448 (4.9%)	74443 (38.4%)	
Age Groups			
<20 years	1903 (1%)	15817 (8.2%)	0.001
21-40 years	11122 (6%)	94241 (49%)	
41-60 years	8133 (4.2%)	40070 (20.1%)	
61-80 years	2893 (1.5%)	17446 (9%)	
>80 years	287 (0.1%)	1744 (0.9%)	

July 2020 while the second peak of pandemic was observed during months of October to December 2020. The highest number of cases ($n=6224$) were seen during month of June followed by 5813 cases during May and 4786 cases during November (*p*-value <0.001) (figure).

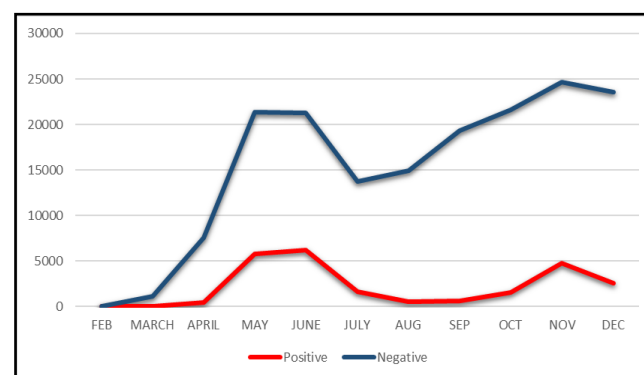


Figure-1: Monthly distribution of sars cov-2 cases.

DISCUSSION

Since, the beginning of COVID-19 pandemic diagnostics has played a central role in case identification, isolation and contact tracing. Molecular techniques especially RT-PCR has served as the backbone of SARS CoV-2 diagnosis^{11,12}. WHO recommends, RNA detection via RT-PCR on mucous collected by nasopharyngeal swab (NPS) as the reference standard for SARS CoV-2 diagnosis¹³. Three genes commonly used for detection of SARS CoV-2 in most of commercially available PCR kits include E gene, N gene and RdRp gene^{14,15}. In our study we also used the specimen obtained with nasopharyngeal swab in VTM. We have analyzed 193656 NPS for SARS CoV-2 with a positivity rate of 12.6%; 9.3% were males and 3.3% were females. A regional study was carried out at Mymensingh Medical College, Bangladesh on 14356 specimens received from four districts. 7.5% of patients were positive for SARS CoV-2 by RT-PCR with 66% males and 34% females. Their reported mean age and male to female ratio is comparable to our study, however, low positive rate might be due to small sample size as compared to our study¹⁶. In another study carried out by Liu *et al*, in Wuhan, China during the initial phase of pandemic the reported positive rate was 38% out of 4880 samples. This high rate might be due to the fact that they have included only hospitalized cases while in our study we have included samples from both in/out door cases. Males are infected more than the females which was similar to our study but they have more positive cases in patients >70 years of age while in our study highest number of cases was in age group 21-40 years¹⁷.

An Indian study on 298 symptomatic PCR positive patients also showed male dominance with the mean age of 39 years. These results are comparable to our study¹⁸. Xiao and colleagues carried out study on 301 hospitalized RT-PCR positive patients. Results of their study showed that males were effected more than females with a median age of 58 years which might be due to the fact that hospitalization rate is higher in elderly than in young patients¹⁹. The two peaks of pandemic as observed in our study were during mon-ths of May to July and during October to December, these findings were consistent with WHO data²⁰.

The main strengths of our study were large sample size and heterogeneous population while limitations include details about disease severity and outcome of patients which cannot be included due to massive workload during this period of crisis.

CONCLUSION

This is the first study from Pakistan with such a large sample size providing an insight into COVID-19 pandemic. The two peaks of pandemic in Pakistan were observed during the months of May to July and during October to December. Most positive patients were males in age group 21-40 years as this age group is more exposed to external environment. Samples received from in patient department outnumbered those from outpatient department indicating that most cases are of moderate to severe intensity requiring admission.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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