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Original Research Paper

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CLINICAL EPIDEMIOLOGICAL PROFILE AND PREVALENCE OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 BY REAL-TIME POLYMERASE CHAIN REACTION

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ABSTRACT

Background: The advent of a novel coronavirus in the human population has affected the economy and prompted the mobilization of public health authorities around the world to counter the rapid spread of the virus. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assays work by detecting SARS CoV 2 RNA targets and are the cornerstone of diagnosis of COVID 19. **Aim:** The objective of this study is to evaluate and analyze the clinical-epidemiological profile and prevalence of COVID 19 from samples received in the laboratory of a tertiary care hospital. **Materials and Method:** The samples were handled in biosafety level 2 facility as per the WHO protocol. Viral Ribonucleic acid extracted and purified was reverse transcribed to cDNA and subsequently amplified by using ICMR approved qRT-PCR kits on real-time PCR instruments. **Results:** In our study, patients in the age group of ≥61years were having highest positivity rate 2254/29297 (8%) of SARS-CoV 2. In our study also SARS CoV 2 positivity was more in males 17213 (4.82%). The same trend of increase of samples in the month of September was seen in our study 9599/116803 (8%). Patients with respiratory symptoms which included Severe Acute Respiratory Illness as well as those with Symptomatic Influenza Like Illness had a good ratio of positive cases of COVID 19. **Conclusion:** The disease is spreading rapidly around the world, including India and other states, causing varying degrees of the disease. More tests, more specimens, and more methods could be considered in the diagnosis of this disease.

KEYWORDS : Epidemiological Profile , RTPCR, Real Time PCR, SARS CoV2,

INTRODUCTION

The advent of a novel coronavirus in the human population has affected the economy and prompted the mobilization of public health authorities around the world to counter the rapid spread of the virus. The earliest human coronavirus (HCoV) was isolated from the nasal discharge of patients with flu-like symptoms during 1965 and was called B814(1). Coronaviruses infect a large number of animals including mammalian and avian species(2). The Coronavirinae subfamily is divided into Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus (3). The alphacoronavirus HCoV-229E and HCoV-NL63 and betacoronavirus HCoV-OC43 and HCoV-HKU1 cause mild respiratory diseases (3). The beta-coronavirus that have emerged from animal reservoirs cause severe disease in humans: SARS-CoV(4), MERS-CoV(5), and the pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)(6)(7). The outbreak was epidemiologically linked to the seafood market where live and freshly slaughtered animals were kept and sold (8). Out of all patients hospitalized with pneumonia, two-thirds had a history of direct exposure to this market (9). Apart from the association with a seafood market that sold live animals, as the outbreak progressed, person-to-person spread became the main mode of transmission (10). Transmission is primarily via respiratory droplets or fomites and viral shedding is thought to peak on or just before the onset of symptoms, with viral loads decreasing thereafter (11).

The mean incubation period for SARS-CoV-2 is five days with the most common sign and symptoms being fever, cough, sore throat, lethargy, dysgeusia and anosmia. Organ dysfunction and death can occur in very severe cases, yet severity seems to be associated with comorbidities. Reported fatality rates vary from 0.7% to 5% (12). Detection of viral RNA in a patient even after 30 days when symptoms have resolved doesn't mean that the patient is infectious and hence transmission of the virus is thought to be unlikely based on viral culture and epidemiological studies (13)(14). The testing should be avoided in non-clinically indicated asymptomatic patients to preserve reagents and testing capacity. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assays work by detecting SARS-CoV-2 RNA targets and are the cornerstone of diagnosis of COVID-19. The analytical sensitivity of the RT-PCR tests is excellent and the clinical interpretation depends on the prevalence of SARS-CoV-2 in the population tested which is further affected by the adequacy of the sample collected and the timing of sample collection concerning the likelihood of viral shedding and stage of the illness. A single negative result is sufficient to exclude disease in most cases and the likelihood of occurrence of false positives and false negatives is very low. The chance of virus detection is increased, by using the same swab for sampling from the oropharyngeal and bilateral nasopharyngeal sites and sputum or broncho-alveolar lavage specimens (on intubated patients) are preferred in patients with lower respiratory tract specimen. The objective of this study is to evaluate and analyze the clinical-epidemiological profile and prevalence of COVID 19 after diagnosis through detection of viral nucleic acid by RT-PCR from samples received in Viral Research & Diagnostic Laboratory (VRDL), from various districts of Punjab.

MATERIAL AND METHODS

In the present study, we analyzed data of COVID-19 samples received from various districts of Punjab over a period of seven

months i.e April to October 2020 in Viral Research & Diagnostic Laboratory (VRDL) in a tertiary care hospital. We documented and analysed demographic, epidemiological and clinical characteristics including age, gender, symptom status and category of patients. The oropharyngeal and the nasopharyngeal samples collected from patients were transported in viral transport medium under a proper cold chain to the laboratory(15). The samples were handled in biosafety level 2 facility as per the WHO protocol(16)(17). Viral Ribonucleic acid extracted and purified was reverse transcribed to cDNA and subsequently amplified by using ICMR approved qRT-PCR kits on real-time PCR instruments. The oligonucleotide primers and probe for the detection of SARS-CoV-2 were selected from the N genome, orf1b genome region and also targeting RNA-dependent RNA polymerase –RdRp. The results were analysed by reading the cycle threshold values and the graphs of amplification for E, N, RdRp & ORF 1b genes. The extracted RNA was stored at −70C in a freezer for long-term storage. To ensure the integrity of RT-PCR assay results, internal control was analysed for each patient samples, as well as testing of the positive and negative control in each batch. Continuous variables were expressed as mean and standard deviation or simple range. Classification variables were summarized as counts and percentages. There is no provision for missing data. Since this group of patients in our study was not randomly selected, all statistics are considered descriptive only. This analysis was performed with SPSS16.

RESULTS

In the present study total prevalence of COVID 19 amongst samples received in the lab was 4% (Figure 1).

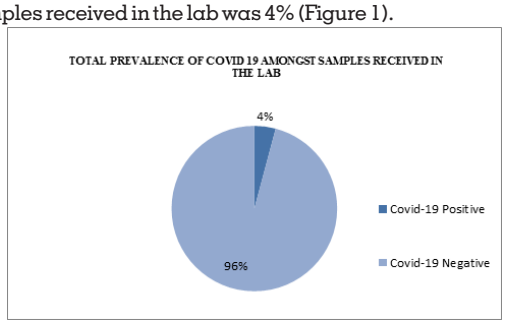

Figure 1 : Prevalence Of Covid 19 In The Study Population.

Samples were received from six districts of Punjab and of all the districts Amritsar was having maximum COVID 19 positivity 9552/139597 (7%) followed by other districts (Table I)

Table I : District Wise Distribution Of Covid 19 Patients

Districts	Covid-19 Positive	Covid-19 Negative	Total Samples received
Amritsar	9552 (7%)	130045 (93%)	139597
Gurdaspur	3382 (3%)	110628 (97%)	114010
Kapurthala	1897 (3%)	62786 (97%)	64683
Pathankot	2868 (5%)	54032 (95%)	56900
Tarn Taran	1389 (3%)	49975 (97%)	51364
Hoshiarpur	2550 (3%)	88708 (97%)	91258
Others*	242 (2%)	9574 (98%)	9816
Total	**21880**	**505748**	**527628**

*Others in this table include the districts from which we were receiving the samples in initial months but later on, the other labs started doing the testing and those districts were shifted for testing to some other labs. SARS-CoV 2 positivity was seen mostly from the elderly group (>61 years) 2254/29303 (8%) followed by 41-60 years 7278/157757 (5%) as shown in Table 2. Out of 21880 SARS-CoV 2 RT-PCR positive, 17213 (4.82%) were males while 4667 (2.7%) were females as in Table 2.

Table II : Age & Gender Wise Distribution Of Covid 19 Patients

Variable	Covid-19 Positive	Covid-19 Negative	Total
Age			
0 to 20	2221 (4%)	56256 (96%)	58477
21 to 40	10127 (4%)	271954 (96%)	282081
41 to 60	7278 (5%)	150489 (95%)	157767
≥ 61	2254 (8%)	27049 (92%)	29303
Total	21880	505748	527628
Sex			
Male	17213 (4.8%)	337682 (95.2%)	354895
Female	4667 (2.7%)	168066 (97.3%)	172733
Total	21880	507894	527628

September month experienced maximum number of COVID 19 positive cases 9599/116803 (8%) and minimum cases were observed during the month of June 874/60287 (1%) (Figure 2).

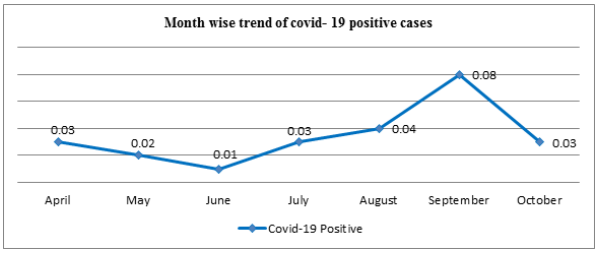

Figure 2: Month Wise Distribution Of Covid19 Samples

Patients with comorbidities and those having Severe Acute Respiratory Illness (SARI) were seen to have more positivity 789/3782 (21%) and 642/3486 (18%) respectively. Symptomatic patients were seen to have more COVID 19 positivity 3350/26644 (13%) than Asymptomatic patients 18530/ 500984 (4%). The prevalence of COVID 19 in High-risk contacts (patients in direct contact with the laboratory-confirmed patient) was found to be 6063/80262 (8%) in our study (Table 3).

Table III : Distribution Of Covid 19 In Different Categories Of The Patients

Category	Covid-19 Positive	Covid-19 Negative	Total
Healthcare worker	1913 (5%)	37767 (95%)	39680
High Risk Contacts*	6063 (8%)	74199 (92%)	80262
International traveller	461(2%)	26672 (98%)	27133
Severe Acute Respiratory Infection (SARI)	642 (18%)	2844 (82%)	3486
Patient with co-morbidities	789 (21%)	2993 (79%)	3782
Patients with Atypical manifestations	77 (11%)	640 (89%)	717
Pregnant Women	277 (2%)	12231 (98%)	12508
Symptomatic ILI	1255 (7%)	15748 (93%)	17003
Others	10403 (3%)	332654 (97%)	343057
Total	**21880**	**505748**	**527628**

*High Risk Contacts: Patients in direct contact with the laboratory-confirmed patient

DISCUSSION

Our study is systematically evaluating the prevalence of COVID19, impact of age and gender severity of the disease in patients with Covid-19. Although the mortality caused by COVID– 19 is lower than that by SARS and MERS, the infectivity and transmissibility of the virus are higher(18). A total of 526728 samples were received in the lab from April 2020 till October 2020, out of which 21880 (4%) were SARS CoV 2 positive by real-time PCR. Samples were received from six districts of Punjab and among all of the districts Amritsar was having maximum COVID 19 positivity 9552/139597 (7%) which can be attributed to the city being a tourist place, and

the international airport. And apart from these reasons overall maximum number of sampling as compared to other districts.

In our study, patients in the age group of ≥61years were having highest positivity rate 2254/29303 (8%) of SARS-CoV 2 as shown in Table 2**.** There is a significant difference ($p<0.05$), between ages in positive cases, which indicates that age is one of the effective parameters in Covid-19 which is in concordance with the study done by Khan et. al.(19). The median age of the patients was 36 years old (SD=14.70), the minimum age was less than one year and the maximum age was 119 years old. The reason for the higher incidence of COVID 19 in elderly could be due to weakened immune responses and underlying diseases such as cardiovascular diseases, diabetes, lung diseases, hypertension etc.. An age-related decline in the clearance of inhaled particles in the small airway region (20), could be responsible for the high prevalence of COVID like symptoms among the elderly. Several existing literature reports have documented the escalated prevalence among male patients. In our study also SARS CoV 2 positivity was The advent of a novel coronavirus in the human population has affected the economy and prompted the mobilization of public health authorities around the world to counter the rapid spread of the virus. The earliest human coronavirus (HCoV) was isolated from the nasal discharge of patients with flu-like symptoms during 1965 and was called B814(1). Coronaviruses infect a large number of animals including mammalian and avian species(2). The Coronavirinae subfamily is divided into Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus summarized in (3). The alphacoronavirus HCoV-229E and HCoV-NL63 and betacoronavirus HCoV-OC43 and HCoV-HKU1 cause mild respiratory diseases (3). The beta-coronavirus that have emerged from animal reservoirs cause severe disease in humans: SARS-CoV(4), MERS-CoV(5), and the pandemic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)(6)(7). The outbreak was epidemiologically linked to the seafood market where live and freshly slaughtered animals were kept and sold (8). Out of all patients hospitalized with pneumonia, two-thirds had a history of direct exposure to this market (9). Apart from the association with a seafood market that sold live animals, as the outbreak progressed, person-to-person spread became the main mode of transmission more in males 17213 (4.82%) as compared to females 4667 (2.7%) Table 2. When we analysed the positive rate between the two genders a statistically significant difference ($p<0.05$) was observed which indicates that there is a correlation between gender and COVID19. In the study done by Khan et al (19) predominantly male (70.25%) population were infected by COVID 19. Another meta-analysis by Yang et al (21) showed the same results. Our results are also in concordance with studies done by Owusu et al, Qun Li et al in which males had higher infection rate compared to females (22)(23). The male preponderance to infection has been explained by some studies that mast cells in females can trigger a more active immune response, which may help them fight infectious diseases better than the males. Furthermore, a genetic component such as X chromosome and sex hormones especially estrogens, both predominantly found in females have been linked to providing some significant level of protection against SARS-CoV2 by playing an important role in innate and adaptive immunity (24). The males generate less robust immune responses and are more susceptible to infectious agents (25).

In India the maximum number of COVID 19 positive cases were repoted in the month September (26) The graph in Figure 3 shows India's Coronavirus (COVID-19) statistical data which was identified by public health officials and used criteria developed by government health authorities.

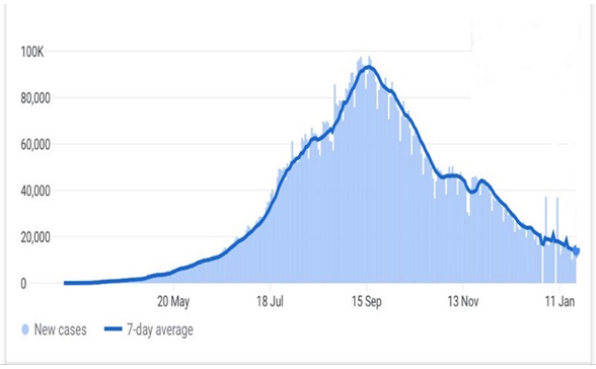

Figure 3 : India's Coronavirus (covid-19) Statistical Data.

The same trend of the increase was seen in our study 9599/116803 (8%). Similar results were reported by Owusu et al(22). The impact of a suppressive intervention on the rate of infections was noticed in the initial months due to imposition of lockdown which was announced on 22nd of March, 2020. This was followed by a period of some easing of restrictions and an increase in the number of cases was seen thereafter till the month of September. Cases have been consistently decreasing since October which may be due to the controlled transmission of SARS CoV 2 virus among the people because of their awareness of the safety measures, hygiene practices and following social distancing. The other reason for the downward trend can be attributed to localised herd immunity playing its role against the virus. Herd immunity develops when sufficient number of people have been infected with the virus within the community(27). The virus which is transmitted from person to person fails to find an adequate number of individual to infect, so the growth rate of the virus faulters. Various risk factors have been seen to be associated with COVID19. Patients with underlying risk factors were seen to have more positivity 789/3782 (21%) which could be due to the lowered immune status because of impaired macrophage and lymphocyte function, (28)(29). Therefore, patients with underlying diseases are more prone to have poorer baseline well-being. The same trend of increased prevalence of COVID 19 in patients with other comorbid conditions was also seen in the study done by Guan et al (25). Our results are coherent with the study done by Liu Y et al (30)and Owusu et al(22) in which symptomatic patients were having more positivity than asymptomatic patients.

CONCLUSION

COVID-19 is a global health emergency that has changed the world in an unprecedented way. The disease is spreading rapidly around the world, including India and other states, causing varying degrees of the disease. Patients with respiratory symptoms which included Severe Acute Respiratory Illness (SARI) as well as those with Symptomatic Influenza Like Illness (ILI) had a good ratio of positive cases of symptomatic symptoms. The disease has shown a wide range of severity in various studies published so far. Close monitoring, quarantine and groups strategy will be required to prevent extensive transmission within the community. More tests, more specimens, and more methods could be considered in the diagnosis of this disease.

Conflict of interest: None to declare

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