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Original Article

Microbiology Section

A Study on SARS-CoV-2 Infection and its Association with Co-morbidities: A Retrospective Cohort Study

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ABSTRACT

Introduction: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a novel coronavirus (nCoV) that causes viral pneumonia known as Coronavirus Disease 2019 (COVID-19). It can infect people of all age groups, and various clinical conditions are strong indicators of co-morbidities in COVID-19. The presence of co-morbidities is crucial in determining the clinical outcome of coronavirus infections. Therefore, it is crucial to understand the demographic profile and associated risk factors of COVID-19 in this geographical region.

Aim: To assess the association between SARS-CoV-2 infection and demographic characteristics and co-morbidities.

Materials and Methods: A retrospective cohort study was conducted at the National Accreditation Board for Testing and Calibration Laboratories (NABL)-accredited Virology Service Laboratory (VSL) of Tripura Medical College and Dr. BR Ambedkar Memorial Teaching Hospital, Agartala, Tripura, India. All the required data was collected between May 2021 and October 2022, and data analysis was carried out from February to March 2023. After obtaining Indian Council of Medical Research (ICMR) approval, real-time Reverse Transcriptase-Polymerase Chain Reaction (rRT-PCR) was used for the detection of SARS-CoV-2 RNA. A total of 10,263 samples were included in the study. RNA extraction was performed using the viral Ribonucleic Acid (RNA) extraction Miniprep Kit protocol manufactured by MDI Membrane Technologies, and RT-PCR was conducted according to the Indian Council of Medical Research (ICMR)-approved DiAGSureTM nCoV-19 Detection Assay (Multiplex, TaqMan based) protocol developed by GCC BIOTECH. The findings were based on demographic parameters such as age group and gender distribution. Results were expressed as proportions and percentages, and statistical analysis was done by Chi-square test in Statistical Package for Social Sciences (SPSS) version 20.0.

Results: Out of the 10,263 registered patients, 1471 (14.33%) were confirmed to have SARS-CoV-2 infection. The most affected age group was found to be between 21 and 40 years, with 524 (35.62%) cases. Males, 927 (63.02%) were more frequently infected than females, 544 (36.98%). Among all the SARS-CoV-2 infected patients, co-morbidities were found in 254 (17.26%) cases, and the association between co-morbidities and the rRT-PCR test results was found to be statistically significant, with a p-value=0.01.

Conclusion: The highest number of SARS-CoV-2 patients were found in the age group of 21 to 40 years, predominantly among males. Patients with co-morbidities showed a significant association with SARS-CoV-2 infection. Diabetes was found to be the most prevalent risk factor among all the patients.

Keywords: Coronavirus disease-19, Diabetes, Real time reverse transcriptase polymerase chain reaction, Severe acute respiratory syndrome coronavirus-2

INTRODUCTION

The COVID-19 have caused a healthcare emergency, annihilating healthcare facilities and emerging as a global pandemic. Coronavirus have been associated with notable disease outbreaks in East Asia and the Middle East countries over the past 20 years [1,2]. COVID-19 is a viral pneumonia caused by nCoV called SARS-CoV-2 [3]. COVID-19 was first reported in December 2019 in Wuhan, China, while in India, the first case was identified on January 30, 2020 [3]. The World Health Organisation (WHO) declared a global pandemic on March 11, 2020 [4]. According to the WHO COVID-19 dashboard, globally, 6,302,996,631 confirmed cases were reported until October 2022, with 446,605,579 cases from India [5]. Tripura, is situated in the northeastern region of India, where the first COVID-19 patient was diagnosed on April 7, 2020, at Viral Research and Diagnostic Laboratories (VRDL), Agartala Government Medical College, Tripura, India. Since then, the number of cases has been increasing steadily by local and community transmission. Until October 2022, Tripura reported a total of 108,015 confirmed cases as per COVID-19 portal, Integrated Disease Surveillance Programme (IDSP), Government of Tripura [6].

The SARS-CoV-2 virus infects people of all ages, with evidence of two groups who are at higher risk of developing COVID-19. One group consists of elderly people, and the other group consists of adolescents and young adults [7,8]. SARS-CoV-2 has led to millions of deaths worldwide, with mortality being greatly associated with pre-existing co-morbidities. Various clinical conditions like hypertension, diabetes, coronary heart disease, chronic kidney disease, chronic obstructive pulmonary disease, and immunocompromised states serve as strong indicators of co-morbidities in coronavirus disease. It has been observed that most hospitalised patients had atleast one pre-existing comorbidity [9]. Therefore, in this geographical region, there is a need to generate evidence on the proportion of SARS-CoV-2 infections and its age group and gender distribution. This will help to identify the predominantly vulnerable age group and gender, enabling the formulation of proper precautionary measures to prevent further spread of the virus. The hypothesis of the study is that co-morbid patients are more susceptible to SARS-CoV-2 infection than non co-morbid patients. Hence, the aim of the study was to determine the demographic characteristics and co-morbidities associated with SARS-CoV-2 infection.

MATERIALS AND METHODS

This retrospective cohort study was conducted at the NABL-accredited virology service laboratory of Tripura Medical College and Dr. BR Ambedkar Memorial (BRAM) Teaching Hospital, Agartala, Tripura, India. After obtaining approval from ICMR, rRT-PCR for SARS-CoV-2 confirmation began in May 2021. Institutional Ethical Clearance (Ref-IEC/SFTMC/2023/1/002) was obtained before data collection and analysis for the present study. A total of 10,263 patients from May 2021 to October 2022 were included in the present study, using a sampling technique based on the whole census that fulfilled the inclusion and exclusion criteria. Data analysis was conducted between February 2023 and March 2023.

Inclusion criteria: All patients whose nasopharyngeal swabs were collected from May 2021 to October 2022 and sent to Virology Service Laboratory (VSL), Tripura Medical College for SARS-CoV-2 rRTPCR testing were included in the study.

Exclusion criteria: Patients whose clinical history and pre-existing illness were not filled up in the Specimen Referral Form (SRF) were excluded from the study.

Study Procedure

All required data, such as the patient's name, age, sex, address, contact number, and test results, were collected from the ICMR COVID-19 portal. Information about co-morbidities like diabetes, heart disease, chronic lung disease, chronic kidney disease, overweight, hypertension, and cancer were also collected from the ICMR portal and Senior Research Fellowship (SRF).

Viral RNA extraction: After collecting the sample following the standard ICMR protocol, it was transported to the laboratory in Viral Transport Medium (VTM), maintaining a cold chain [10]. Extraction of viral RNA was performed according to the Viral RNA extraction Miniprep Kit protocol manufactured by mdi membrane technologies [11].

rRT-PCR: The rRT-PCR was conducted using the ICMR-approved DiAGSureTM nCoV-19 Detection Assay (Multiplex, TaqMan-based) protocol developed by GCC BIOTECH [12]. This assay protocol was designed to target the 'ORF1ab' and 'E' genes along with the Internal Control (IC) Ribonuclease P (RNAse P), Positive, and Negative controls. The Cycle threshold (Ct) value cut-off for each targeting gene was set at ≤36, and for the IC, it was set at ≤35 as per the kit literature. Positive SARS-CoV-2 results were interpreted when the 'ORF1ab' gene was positive, 'E' gene positive/negative, and IC positive. Negative SARS-CoV-2 results were determined when the 'ORF1ab' gene was negative, 'E' gene positive/negative, and IC positive.

STATISTICAL ANALYSIS

The collected data was entered into Microsoft Excel 2007 and analysed using SPSS version 20.0. All results were expressed in frequency (n) and percentage (%). The Chi-square test was applied to determine the association between SARS-CoV-2 infection and co-morbidities. A p-value <0.05 was considered statistically significant.

RESULTS

Among a total of 10,263 patients, the majority of patients were in the age group of 21 to 40 years, with 4,204 (40.96%) patients, followed by 41 to 60 years with 2,998 (29.21%) patients, 61 to 80 years with 1,838 (17.91%) patients, and 1 to 20 years with 950 (9.26%) patients. Among the total patients (N=10,263), there were 6,400 (62.36%) male patients and 3,863 (37.64%) female patients. In the present study, out of all registered patients, 1,551 (15.11%) had one or more co-morbidities, while 8,712 (84.88%) patients had no associated co-morbidities. It was found that a total of 1,471 (14.33%) patients were confirmed to have SARS-CoV-2 infection [Table/Fig-1].

Variables		Total n (%)
Age (in years)	<1	9 (0.09)
	1 to 20	950 (9.26)
	21 to 40	4204 (40.96)
	41 to 60	2998 (29.21)
	61 to 80	1838 (17.91)
	81 and above	264 (2.57)
Gender	Male	6400 (62.36)
	Female	3863 (37.64)
Co-morbidities	Present	1551 (15.11)
	Absent	8712 (84.88)
rRT-PCR (SARS-CoV-2)	Positive	1471 (14.33)
	Negative	8792 (85.67)

[Table/Fig-1]: Frequency and percentage of different variables.
RT-PCR: Real-time reverse transcriptase-polymerase chain reaction; SARS-CoV2: Severe acute respiratory syndrome coronavirus 2

Furthermore, the most prevalent co-morbidities were diabetes, with 542 (34.94%) cases, followed by hypertension with 348 (22.43%) cases, chronic lung disease with 301 (19.40%) cases, chronic renal disease with 168 (10.83%) cases, and chronic heart disease with 120 (7.73%) cases [Table/Fig-2].

Co-morbidities	Frequency (n)	Percentage (%)
Diabetes	542	34.94
Hypertension	348	22.43
Chronic lung disease	301	19.40
Malignancy	36	2.32
Chronic renal disease	168	10.83
Chronic heart disease	120	7.73
Chronic liver disease	12	0.77
Others	24	1.54

[Table/Fig-2]: Frequency and percentage of different co-morbidities (n=1551).
#Chi-square; p-value <0.05 taken as a statistically significant

Among all SARS-CoV-2 infected patients, 524 (35.62%) were in the 21 to 40 years age group, 445 (30.25%) were in the 41 to 60 years age group, 354 (24.07%) were in the 61 to 80 years age group, 88 (5.98%) were in the 1 to 20 years age group, and 59 (4.01%) were in the 81 years and above age group. One case was found in the less than one year age group. A statistical association between age group and test results was determined and found to be significant (p-value=0.001) [Table/Fig-3].

Age group (in years)	rRT-PCR results		p-value
	Negative (n=8792) n (%)	Positive (n=1471) n (%)	
<1	8 (0.09)	1 (0.07)	0.001
1-20	862 (9.80)	88 (5.98)	
21-40	3680 (41.86)	524 (35.62)	
41-60	2553 (29.04)	445 (30.25)	
61-80	1484 (16.88)	354 (24.07)	
>81	205 (2.33)	59 (4.01)	

[Table/Fig-3]: Age group distribution among positive and negative patients with p-value.
Chi-square test; p-value <0.05 taken as a statistically significant

On the other hand, out of all SARS-CoV-2 infected patients, 927 (63.02%) were male and 544 (36.98%) were female. However, no significant statistical association was noted between gender and test results (p-value=0.57) [Table/Fig-4]. Co-morbidities were found among 254 (17.26%) of all SARS-CoV-2 infected patients. A significant statistical association was observed between co-morbidities and the rRT-PCR test results (p-value=0.01) [Table/Fig-5].

Gender	Results		p-value
	Negative (n=8792) n (%)	Positive (n=1471) n (%)	
Female	3319 (37.75)	544 (36.98)	0.57
Male	5473 (62.25)	927 (63.02)	

[Table/Fig-4]: Gender distribution among positive and negative patients with p-value.
#Chi-square test; p-value <0.05 taken as a statistically significant

Co-morbidities	Results		p-value
	Negative (n=8792) n (%)	Positive (n=1471) n (%)	
Present	1297 (14.75)	254 (17.26)	0.01
Absent	7495 (85.24)	1217 (82.73)	

[Table/Fig-5]: Association of co-morbidities and SARS-COV-2 infection.
#Chi-square test; p-value <0.05 taken as a statistically significant

DISCUSSION

The dedicated VSL of Tripura Medical College reported 1,471 positive cases out of 10,263 cases tested, resulting in an overall positivity rate of 14.33%. This rate is similar to the study conducted by Farhana A et al., who observed an 11% positivity rate [2]. The highest positivity rate was observed in the age group of 21 to 40 years (35.62%), followed by 41 to 60 years (30.25%), 61 to 80 years (24.07%), 1 to 20 years (5.98%), and 81 years and above (4.01%). These findings align with the observations made by Yengkhom BS et al., [13]. The study also revealed a significant association between age group distribution and SARS-CoV-2 infection (p=0.001), which is consistent with the findings of Sadeghi F et al., [14].

Environmental and socioeconomic factors can contribute to the vulnerability to COVID-19 [15]. Sociological factors such as occupation, daily activities, economy, and literacy can expose individuals to a higher risk of infection [16]. Susceptibility to SARS-CoV-2 infection also varies due to biological differences among different age groups and between genders (male and female) [17,18]. The present study showed a lower positivity rate among older age group patients, which may be attributed to their increased awareness and adherence to staying at home to minimise the risk of infection. Conversely, according to Lu Q and Shi Y, SARS-CoV-2 infection was milder among neonates, which explains the low number of cases observed in this age group, aligning with the findings of the present study [19]. However, young adults were identified as a more vulnerable age group in the present study, possibly due to increased exposure and lower adherence to preventive measures such as maintaining safe distances, wearing masks, and practicing hand hygiene. The hypothesis of the study was thus accepted.

It was observed that out of all SARS-CoV-2 infected patients, 63.02% were male and 36.98% were female, which is consistent with Indian studies conducted by Gupta N et al., and Kushwaha S et al., [20,21]. However, on statistical analysis, there was no significant association with respect to test results (p=0.57), which was also observed by Allameh SF et al., and Goshayeshi L et al., [22,23]. The male predominance can be attributed to their roles as the primary working members of the family, thereby being more exposed in workplaces.

Of the total SARS-CoV-2 infected patients, 17.26% had pre-existing diseases or co-morbidities, whereas a study by Farhana A et al., reported a rate of 23.2% [2]. Conversely, only 14.75% of co-morbid patients had no SARS-CoV-2 infection, which suggests that co-morbidities may be predisposing factors for SARS-CoV-2 infection. However, the high number of patients among young adults in the present study may explain the lower prevalence of co-morbidities. Further analysis revealed that diabetes (34.94%) was the most common comorbidity, followed by hypertension (22.43%), chronic lung disease (19.40%), chronic renal disease (10.83%), and chronic heart disease (7.73%). Similar findings were also reported by Soni SL et al., which included diabetes (14.9%), hypertension (16.6%), and chronic renal disease (2.6%) [1]. Similarly, hypertension was found to be the most common comorbidity in a study by Gupta N et al., and Choudary MK et al., reported diabetes as the most common comorbidity [20,24]. In the present study, co-morbidities were significantly associated with SARS-CoV-2 infected patients (p=0.01), which is in concordance with the study conducted by Sadeghi F et al., [14]. Therefore, such pre-existing diseases contribute to a higher mortality and morbidity rate among SARS-CoV-2 infected patients. The vulnerable age group and gender of SARS-CoV-2 infection guide us in formulating proper precautionary measures to prevent further spread of the infection. Due to the significant association, patients with co-morbidities need to take utmost care to reduce mortality and morbidity. Whole Genome Sequencing (WGS) of all positive SARS-CoV-2 RNA should be conducted to gather information about the strain type for molecular epidemiology and vaccine development purposes.

Limitation(s)

The demographic profile of patients during the first wave of the pandemic was not studied in this research as rRT-PCR testing began in May 2021. Additionally, there was no follow-up of patients with co-morbidities, which prevented an understanding of the severity, progression, and outcome of the disease in these individuals. Furthermore, there is a need to conduct community-based studies that can provide a more accurate distribution of the demographic and sociological characteristics of patients during such a pandemic.

CONCLUSION(S)

The present study found that the majority of SARS-CoV-2 infected patients were young individuals, with a predominance of males. While the patients from the first wave of the pandemic were not included in current study, the subsequent waves showed a significant proportion of SARS-CoV-2 infected patients at Tripura Medical College. Patients with co-morbidities are particularly vulnerable to infection and may experience complications. The present study revealed that a substantial number of patients had pre-existing diseases or co-morbidities, and there was a statistically significant relationship between these conditions and SARS-CoV-2 infection. Upon further analysis, it was determined that diabetes, hypertension, chronic lung disease, and chronic renal disease are strong risk factors associated with COVID-19. Therefore, early diagnosis and treatment of these susceptible patients are crucial in order to reduce mortality and improve outcomes.

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